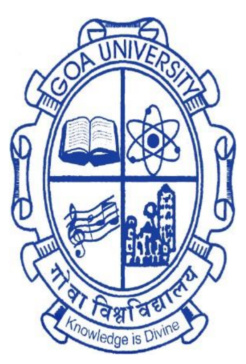
EVALUATING THE POTENTIAL OF HYPERSALINE BACTERIA AS A BIOCONTROL AGENT AGAINST MICROBIAL PATHOGENS AFFECTING CHILLI (*Capsicum annuum* L.) PLANT

A Thesis submitted in partial fulfillment for the Degree of

DOCTOR OF PHILOSOPHY

In Biotechnology (School of Biological Sciences and Biotechnology)

Goa University



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March *2023*

DECLARATION

I, Miss. Manasi Mahesh Pawaskar hereby declare that the thesis entitled "Evaluating the potential of hypersaline bacteria as a biocontrol agent against microbial pathogens affecting chilli (*Capsicum annuum* L.) plant" represents work which has been carried out by me and that it has not been submitted, either in part or full, to any other University or Institution for the award of any research degree.

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CERTIFICATE

I hereby certify that the work entitled "Evaluating the potential of hypersaline bacteria as a biocontrol agent against microbial pathogens affecting chilli (*Capsicum annuum* L.) plant" was carried out under my supervision and may be placed for evaluation.

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Dedicated to my parents…

… a father who encouraged me to dream big
and
a mother who made sure I am not hungry
while achieving those dreams

Contents

ABBREVIATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1: INTRODUCTION		**1-11**
1.1 Biological Control and Biocontrol agents (BCAs)		**1**
1.2 *Capsicum annuum* L.		**4**
1.3 Disease-causing mycopathogens of chilli plants		**6**
1.4 Potential of hypersaline bacteria as BCAs		**8**
OBJECTIVES AND SIGNIFICANCE OF THESIS		**10**
CHAPTER 2: REVIEW OF LITERATURE		**12-27**
2.1	Salt pan microbes and their antimicrobial potential	**12**
2.2	Management of phytopathogens	**15**
2.3	Disease causing fungal pathogens in chilli plants	**16**
2.4	Biological control of mycopathogens infecting chilli using microorganisms	**17**
CHAPTER 3: MATERIALS AND METHODS		**28-63**
3.1	Sampling sites for the isolation of hypersaline bacteria	**28**
3.2	Isolation of bacteria from salt pan water and sediment	**28**
3.3	Sample collection for the isolation of fungus from diseased chilli plants	**29**
3.4	Isolation of the fungus from diseased plant samples	**29**
3.5	Identification of the fungal isolates	**29**
3.6	Screening of salt pan bacteria for antifungal activity	**30**
3.7	Screening for growth of salt pan bacteria in soil extract	**30**
3.8	Evaluating type of antagonism by salt pan bacteria	**31**
	3.8.1 Qualitative analysis of mixed path antagonism and indirect antagonism tests	**31**
	3.8.2. Quantitative estimation of mixed path antagonism and indirect antagonism tests	**37**
	3.8.3. Screening for genes involved in the lipopeptide synthesis	**41**
	3.8.4. Testing for direct antagonism	**41**
3.9	Identification of salt pan bacteria by classical and molecular approach	**42**

3.9.1 Morphological characterization **42**
3.9.2 Biochemical characterization **45**
3.9.3 Molecular characterization **47**
3.10 Safety evaluation of salt pan bacteria **48**
3.10.1 Blood hemolysis test **48**
3.10.2 Antibiotic sensitivity test **48**
3.11 Influence of the selected salt pan bacteria on the *in-vitro* germination of chilli seeds **49**
3.11.1 Bioagent - Biopolymer compatibility assessment **49**
3.11.1 Coating efficacy of biopolymer in seed biopriming **50**
3.11.2 Effect of bioagent-biopolymer on seed germination and growth **50**
3.12 Effect of carbon and nitrogen sources, salinity, pH and temperature on growth of the salt pan bacterium MPSK 23 **51**
3.12.1 Effect of carbon sources **51**
3.12.2 Effect of nitrogen sources **51**
3.12.3 Effect of salinity **52**
3.12.4 Effect of pH **52**
3.12.5 Effect of temperature **52**
3.13 Continuous assessment of growth and antifungal activity of MPSK 23 **52**
3.14 Growth and viability of MPSK 23 in soil **53**
3.15 *In-vitro* assessment of the growth of *F. solani* in presence of MPSK 23 **53**
3.15.1 Preparation of cell suspension of MPSK 23 **53**
3.15.2 Co-inoculation of *F. solani* and MPSK 23 **53**
3.16 *In-vivo* pathogenicity testing of *F. solani* in Khola and Sitara chilli cultivar **54**
3.16.1 Preparation of sick soil **54**
3.16.2 Inoculation and re-isolation of *F. solani* from chilli plants **54**
3.17 *In-vivo* testing of MPSK 23 as a BCA against *Fusarium* wilt in chilli plants **55**
3.17.1 Experiment I: Assessment of the efficacy of MPSK 23 in regulation of defense enzymes and phenols in chilli plants against *F. solani* **55**
3.17.2 Experiment II: Efficacy of MPSK 23 in suppressing the wilt and boosting the growth of chilli plants against *F. solani* **60**

3.18 Assessing the shelf-life of MPSK 23 **62**
3.19 Statistical analysis of data **63**
CHAPTER 4: RESULTS **64-128**
4.1 Location and physico-chemical parameters of the sampling site **64**
4.2 Bacteria from salt pan water and sediment **65**
4.3 Critical examination of the diseased chilli plants for isolation of fungi **66**
4.4 Fungal isolates obtained from the diseased chilli plant samples **66**
4.5 Characterization of the fungal isolates obtained from diseased chilli plants **68**
4.6 Antifungal activity of salt pan bacteria **70**
4.7 Growth assessment of salt pan bacteria in soil extract **74**
4.8 Antagonism type exhibited by selected salt pan bacteria **75**
4.8.1 Analysing salt pan bacteria qualitatively for mixed path antagonism and indirect antagonism tests **75**
4.8.2 Quantitative estimation of mixed path antagonism and indirect antagonism tests **81**
4.8.3 PCR amplification of genes of lipopeptide synthesis **83**
4.8.4 Direct antagonism by interaction between fungal mycelia and salt pan bacteria **84**
4.9 Identification of salt pan bacteria MPSK 22, MPSK 23 and MPSK 186 **86**
4.10 Blood haemolysis and antibiotic sensitivity tests of MPSK22, MPSK 23 and MPSK 186 **91**
4.11 Effect of bioagents MPSK 22, MPSK 23 and MPSK 186 on the *in-vitro* germination of chilli seeds **93**
4.11.1 Bioagent - Biopolymer compatibility assessment **93**
4.11.2 Coating efficacy of 1 % gum acacia in seed biopriming **94**
4.11.3 Germination and growth of chilli seeds exposed to *F. solani* spores after coating with bioagent-biopolymer **95**
4.12 Growth of MPSK 23 in different carbon and nitrogen sources at varying salinity, pH and temperature **100**
4.13 Growth profile of MPSK 23 and the antifungal activity **101**
4.14 The stability and viability of MPSK 23 in soil **101**
4.15 Inhibition of *F. solani* by MPSK 23 *in-vitro* **102**
4.16 Pathogenicity of *F. solani* in Khola and Sitara chilli cultivars **104**

4.17 Establishing the potential of MPSK 23 as a biocontrol agent by *in-vivo* pot trials **104**

4.17.1 Experiment I: Regulation of defense enzymes and phenols by MPSK 23 in chilli plants after *F. solani* exposure **105**

4.17.2 Experiment II: Suppression of the disease incidence in chilli plants infected with *F. solani* and growth enhancement by MPSK 23 **115**

4.18 Shelf life and viability of MPSK 23 **128**

CHAPTER 5: DISCUSSION **129-144**

5.1 Wilt and Rot causing fungal pathogens of Chilli plants and their control **129**

5.2 Mode of antagonism exhibited by the bacteria against fungal pathogens **131**

5.3 *Bacillus* spp. as biocontrol agents and its safety evaluation **139**

5.4 *In-vitro* germination tests with the salt pan *Bacillus* spp. **141**

5.5 *In-vivo* trials with *Bacillus* strain MPSK 23 as a biocontrol agent against *F. solani* **142**

SUMMARY **145-146**

CONCLUSION **147**

BIBLIOGRAPHY **148-178**

APPENDIX **179-194**

PUBLICATIONS **195**

Abbreviations

%	Percentage
>	More than
°C	Degrees centigrade
α	Alpha
β	Beta
γ	Gamma
μg	Microgram
μmole	Micromole
μg/mL	Microgram per milliliter
μL	Microliter
μm	Micrometer
μM	Micromolar
ACC	1-aminocyclopropane-1-carboxylate
ANOVA	Analysis of variance
bp	Base pair
CAS	Chrome azurol S
CFU/mL	Colony forming units per milli liter
cm	Centimeter(s)
cm^2	Square centimeter
CMC	Carboxymethylcellulose
CRD	Completely randomized design
DI	Disease incidence
DNA	Deoxyribonucleic acid
DNSA	DiNitro Salicylic Acid
DSI	Disease severity index
DW	Distilled water
FW	Fresh weight
g	Gram
g/L	Grams per liter
GA	Gum acacia
GE	Germination energy
GP	Germination percentage

h	Hour(s)
H_2O_2	Hydrogen peroxide
IAA	Indole acetic acid
ISR	Induced systemic resistance
ITCC	Indian type culture collection
ITS	Internal Transcribed Spacer
kb	Kilo basepair
kg	Kilo gram
kHz	Kilohertz
L	Liter
LAF	Laminar air flow
Log	Logarithm
M	Molar
mg	Milligram
mg/mL	Milligram per milliliter
MGT	Mean germination time
min	Minute(s)
mL	Milliliter
mm	Millimeter(s)
mM	Millimolar
mM/L	Millimolar per litre
N_2	Nitrogen
NA	Nutrient agar
NaCl	Sodium chloride
nm	Nanometre
OD	Optical Density
PAL	Phenylammonia lyase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PGP	Plant growth promotion
pH	Potential of hydrogen
PO	Peroxidase
ppm	Parts per million

PPO	Polyphenol oxidase
ppt	Parts per thousand
Psi	Pound-force per square inch
psu	Percentile salinity units
rpm	Rotations per minute
rRNA	Ribosomal ribonucleic acid
SD	Standard deviation
SE	Soil extract
sec	Second(s)
SEM	Scanning electron microscope(y)
TPC	Total phenolic compounds
TW	Tap water
U	Unit
v:v	Volume per volume
w:v	Weight per volume
ZMA	Zobell marine agar

List of Tables

Table 1: BCAs against plant pathogens affecting chilli crops and their suggested mode of action

Table 2: Specific PCR primer pairs for the identification of lipopeptide biosynthetic genes

Table 3: PCR amplification conditions for the screening of individual lipopeptide biosynthetic genes

Table 4: Description of the treatments used for pot trial (Experiment I)

Table 5: Description of the treatments used for pot trial (Experiment II)

Table 6: Geographical co-ordinates and physico-chemical parameters of the sampling sites

Table 7: Morphological identification of the fungal isolates with the deposition number

Table 8: Percentage inhibition of the fungal mycelia by the hypersaline bacteria

Table 9: Percentage inhibition of the fungal mycelia by volatile metabolites produced by the hypersaline bacteria

Table 10: Zone of inhibition of the fungal pathogens by the diffusible metabolites produced by the hypersaline bacteria

Table 11: Production of extracellular lytic enzymes by the selected hypersaline bacteria

Table 12: *In-vitro* plant growth promoting attributes of the hypersaline bacteria

Table 13: Quantitative estimation of the extracellular lytic enzymes secreted by the hypersaline bacteria

Table 14: Quantitative estimation of the ammonia, IAA and solubilised zinc, silicon and phosphate in the culture supernatant of the hypersaline bacteria

Table 15: Biochemical characteristics of the selected hypersaline bacteria

Table 16: Carbohydrate utilization pattern of the selected hypersaline bacteria

Table 17: Percent inhibition of the fungal pathogens, antagonism test results and the identification of the three shortlisted hypersaline bacteria

Table 18: Antibiotic sensitivity of the hypersaline bacteria

Table 19: Coating efficacy (E %) of 1 % Gum acacia to coat chilli seeds with bacterial cells

Table 20: Characteristics of the agricultural soil used in the pot trials

Table 21: Correlation matrix of defense enzymes, total phenolic content, disease severity and the plant total length of Khola (A) and Sitara (B) chilli plants in pot trials

Table 22: Correlation matrix of disease incidence (DI), disease suppression, disease severity, number of leaves and the plant height of Khola (A) and Sitara (B) chilli plants in pot trials

List of Figures

Figure 1: Flow chart of the mode of antagonism exhibited by a BCA against pathogens

Figure 2: Abundance of bacteria in the salt pans

Figure 3: The phylogenetic tree obtained by sequence analysis of the ITS region of fungal isolates with 1000 bootstrap replicates

Figure 4: Hypersaline bacteria showing antifungal activity by Dual culture assay

Figure 5: Hypersaline bacteria showing inhibition of the fungal pathogens

Figure 6: Phylogenetic tree depicting the sequence similarity of the three hypersaline bacteria with other bacteria based on 16S rRNA analysis

Figure 7: Initial and final viable count of MPSK 22, MPSK 23 and MPSK 186 after incubating with different biopolymers as compared to control

Figure 8: Graph of Germination Percentage (GP) of Khola and Sitara seeds

Figure 9: Graph of Germination Energy of Khola and Sitara seeds

Figure 10: Graph of Mean Germination Time of Khola and Sitara seeds

Figure 11: Graph of Hypocotyl and Root length of germinated Khola and Sitara seedlings

Figure 12: Graph of Vigour Index of Khola and Sitara seeds

Figure 13: Growth of the hypersaline bacterium MPSK 23 at varying carbon sources (A), nitrogen sources (B), pH (C), NaCl concentrations (D) and temperatures (E)

Figure 14: Growth profile and antifungal activity exhibited by the bacterium MPSK 23

Figure 15: Viable count of the hypersaline bacterium MPSK 23 in the soil

Figure 16: Weight of the *F. solani* measured as dry weight (A) and wet weight (B) in presence of MPSK 23 in liquid broth

Figure 17: Progression of disease severity (wilting) in Khola (A) and Sitara (B) chilli plant varieties over a period of 15 days

Figure 18: Length of chilli plants after 15 days of pathogen exposure to *F. solani*

Figure 19: Phenylalanine ammonia lyase activity in Khola (A) and Sitara (B) plants over a period of 15 days after pathogen exposure

Figure 20: Peroxidase activity in Khola (A) and Sitara (B) plants over a period of 15 days after pathogen exposure

Figure 21: Polyphenol oxidase activity in Khola (A) and Sitara (B) plants over a period of 15 days after pathogen exposure

Figure 22: Total phenolic content in Khola (A) and Sitara (B) plants over a period of 15 days after pathogen exposure

Figure 23: Graph of Disease Incidence and Disease Suppression among different treatments in Khola and Sitara chilli plants

Figure 24: Graph of Disease Severity in Sitara (A) and Khola (B) plants caused by *F. solani* over a period of 98 days

Figure 25: Graph of Protective Value of different treatments against the *F. solani* infection in Sitara (A) and Khola (B) chilli plants over a period of 98 days

Figure 26: Graph of change in Khola (A) and Sitara (B) chilli plant height over a period of 98 days

Figure 27: Graph of change in number of leaves of Khola (A) and Sitara (B) chilli plants over a period of 98 days

Figure 28: Graph of total plant length of Khola (A) and Sitara (B) chilli plants over a period of 98 days

Figure 29: Graph of total wet and dry weight of Khola (A) and Sitara (B) chilli plants over a period of 98 days

Figure 30: Viability of MPSK 23 stored at room temperature (RT, 28 ± 2 °C) and refrigerator temperature (FT, 4 ± 2 °C) after resuspending in tap water (TW) and 1 % Gum acacia (GA)

List of Plates

Plate 1: Ribandar salt pan

Plate 2: Plant samples uprooted from fields showing symptoms of root rotting (A) and wilting (B)

Plate 3: Isolation of fungus from the root and the stem sections

Plate 4: Morphology and microphotographs of stained fungi on PDA isolated from diseased chilli plants

Plate 5: Bacteria MPSK 109 showing inhibition of *R. solani* (A), *P. aphanidermatum* (B), *F. oxysporum* (C), *F. pallidoroseum* (D), *F. solani* (E) and *M. phaseolina* (F)

Plate 6: Growth of hypersaline bacteria on the SE agar plates

Plate 7: Antifungal activity exhibited by BGUM 14B by the volatile metabolite production against *R. solani* (A), *M. phaseolina* (B) and *F. solani* (C) by inverted plate method

Plate 8: Antifungal activity exhibited by MPSK 23 against *M. phaseolina* (A), *F. solani* (B) and *R. solani* (C) by well diffusion method (carried out in triplicates)

Plate 9: Production of extracellular enzymes amylase (A), protease (B), lipase (C), chitinase (D), glucanase (E) and cellulase (F) by hypersaline bacteria

Plate 10: Ammonia production test of the hypersaline bacteria

Plate 11: *In-vitro* plant growth promoting attributes IAA production (A), AAC deaminase production (B), N_2 fixation (C), Silicate solubilisation (D), Zinc solubilisation (E) and Phosphate solubilisation (F) by hypersaline bacteria

Plate 12: Production of siderophore (A) and exopolysaccharide (B) by hypersaline bacteria

Plate 13: PCR amplification products of MPSK 22 (A), MPSK 23 (B) and MPSK 186 (C)

Plate 14: Fungal mycelia interacting with hypersaline bacteria (red boxes) and the control non-inhibited mycelia (green boxes) of *F.solani* (A), *R. solani* (B) and *M. phaseolina* (C)

Plate 15: Light Microscopy images of test (highlighted in red boxes) and control mycelia (highlighted in green boxes) of *F.solani* (A), *R. solani* (B) and *M. phaseolina* (C)

Plate 16: SEM Microscopy microphotographs of test (highlighted in red boxes) and control mycelia (highlighted in green boxes) of *F.solani* (A), *R. solani* (B) and *M. phaseolina* (C)

Plate 17: Colony morphology and Gram staining of MPSK 22 (A), MPSK 23 (B) and MPSK 186 (C)

Plate 18: Scanning electron microphotograph of MPSK 22 (A), MPSK 23 (B) and MPSK 186 (C)

Plate 19: Primary safety evaluation by Blood agar hemolysis (A) and Antibiotic sensitivity test (B)

Plate 20: Khola and Sitara seeds coated with the cells of MPSK 23 using 1 % Gum acacia

Plate 21: Growth chamber containing the wet paper towel for germination of seeds coated with cells of MPSK 23 (A), MPSK 22 (B), MPSK 186 (C) and control PBS coated seeds (D)

Plate 22: Pathogenesis of *F. solani* exhibiting wilting symptoms in chilli plant (A), re-isolation of the fungus (B) and lactophenol cotton blue staining of the fungus (C)

Plate 23: The polyhouse used for the pot trials (A) and the arrangement of the pots in polyhouse (B)

Plate 24: Growth of plants at day 0 (A), 7 (B), 15 (C) and 63 (D) days after transplantation

Plate 25: Plants of Khola (A) and Sitara (B) variety at day 84 after transplantation

Chapter 1

Introduction

From past several decades, chemical agents have been used indiscriminately for reducing the incidence or for the inhibition of plant diseases caused by the pathogens. The extensive and rapid use of synthetic chemicals to cover up the yield loss has elevated a number of ecological problems of residual toxicity, resistance, environmental pollution, altered biological balance in the soil and formation of carcinogenic products, thus leading to gradual loss of protection efficiency with increasing pesticide dosage (Rani et al. 2021, Pawaskar & Kerkar 2021). Thus, the uncontrolled use of chemical pesticides in agricultural fields is now a major concern. There is a need to develop self-maintaining, long-lasting and more effective measures for controlling plant pathogens which will be safe not only for humans but also for the environment and other beneficial microorganisms.

1.1 Biological Control and Biocontrol agents (BCAs)

The world is now moving towards the biological control of pests and pathogens, wherein no or a limited usage of chemicals in the environment is recommended. Given the increased global demand for crop production; researchers and industries are seeking new, more sustainable and biological approaches to control the pathogens and boost the plant growth. In 2001, Eilenberg et al. defined biological control or biocontrol as “the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be”. These antagonistic living organisms which are used in biocontrol are termed as biological control agent(s) (BCA) which are naturally efficient or genetically modified strains that reduce the incidence or disease severity caused by pests and pathogens.

Biological control can be a naturally occurring phenomenon involving the resident natural enemies of pests and pathogens without human involvement or it can be a management practice implemented for the inhibition of pests and pathogens. The latter is categorised into three different types: classical, conservation, and augmentation; which can be used separately or in combination with each other. The introduction of an indigenous co-evolved enemy from a pest origin habitat to the new diseased crop area with an intention of a long term establishment of the enemy for the control of the pest population is known as Classical

biological control. The time scale for this can be in years but the results are long-lasting. Conservation involves the practices that protect, maintain, and enhance the existing natural enemies by modifying the environment and practices which will in turn reduce the pest population. Conservation practices include either reducing or eliminating the factors which interfere with or destroy the natural enemies. This process might also require longer period but has a long-lasting effect. Augmentation involves the mass culture and intentional release of natural enemies either in limited amounts with an expectation that it will multiply and control the pest's seasonal outbreak (Inoculative biological control) or in enormous amounts to outcompete the increasing pest population completely by the released organisms themselves (Inundative biological control). Thus the result from augmentation is quick but not long-lasting (Landis & Orr 1996; Eilenberg et al. 2001; Eilenberg 2006).

Microorganisms represent the most common candidate as BCAs because of their broad biochemical diversity (source of lytic enzymes and metabolites), the feasibility of mass culture and ease of genetic manipulation. The inhibition exhibited by the microorganisms is based on "antagonism" wherein there is a reduction in severed diseases of plants due to the physiological interaction between the microbial BCA(s) and the disease causing pathogen (Dahiya et al. 2020; Gupta et al. 2021). This antagonism is classified into three modes *viz.* direct antagonism, indirect antagonism and mixed path antagonism (Figure 1) (Singh & Faull 2020). In direct antagonism, the interaction can lead to either 1) predation, wherein the predator (in this case a BCA) kills the prey (a phytopathogen) for its survival or 2) hyperparasitism, wherein the parasite (in this case a BCA) lives on or inside a host (a phytopathogen, which is also a parasite), thus harming the later. This kind of inhibition is achieved due to the virtue of cell wall-degrading enzymes or metabolites which assist a BCA in the lysis of the host (Heydari & Pessarakli 2010). Indirect biological control is based on the principle of competition between a BCA and the pathogen or on the induction of the host plant resistance by a BCA. A BCA may limit the growth of the pathogen by competing for host supplied nutrients (exudates, leachates, or senescent tissue), essential soluble nutrients (iron sequestering by producing siderophore) or for colonising root and plant tissues (by forming biofilms around the roots through exopolysaccharide release), thus depriving access of the pathogen at the infection site (Vurukonda et al. 2018). Siderophores are small organic molecules produced by microorganisms under iron-limiting conditions which enhance the uptake of iron to the microorganisms (Crowley 2006). Exopolysaccharide (EPS) are organic macromolecules synthesized using different carbon sources which permits the adhesion of BCA to the root surfaces of the host and helps in biofilm formation, thus making the surface

unavailable to pathogens (Arfarita et al. 2016). Application of BCA to a plant can also stimulate the systemic resistance of the hosts (boosting its defense mechanisms) thus making the whole plant more resistant towards infections. Such kind of resistance of the plants towards the pathogens is termed Induced systemic resistance (ISR). ISR involves the production of reactive oxygen species, phytoalexins, phenolic compounds, or enormous number of enzymes involved in plant defense, like polyphenol oxidase, β-1, 3-glucanase, chitinase, phenylalanine ammonia lyase, peroxidase (Kaur et al. 2022). In mixed path antagonism, a BCA can produce bioactive compounds or enzymes which will either inhibit the pathogen or boost the growth of the host plant. Antimicrobial metabolites (volatile organic compounds (VOCs) or diffusible antibiotics) are often considered as the most potent mode of action of microorganisms allowing antibiotic producing microorganisms competitive advantages (Köhl et al. 2019). The bacterial VOCs are small molecules biosynthesized by primary and secondary metabolic pathways and include chemical classes such as alcohols, esters, aliphatic and aromatic hydrocarbons, terpenes, nitrogen, and sulphur compounds. It contributes in the intra- or inter-communication between microorganisms leading to the inhibition of target pathogens (Garrido et al. 2020). Among the various antibiotics produced by a BCA; Lipopeptides (LPs) are low-molecular-weight amphiphilic cyclic oligopeptides synthesised by non-ribosomal peptide synthetases (Romero et al., 2007; Gond et al., 2015). The amphipathic nature of these lipopeptides helps them to induce the formation of pore and ion channels in lipid bilayer membranes of pathogens, thus lysing the target pathogen. They cause less resistance compared to the traditional antibiotics or fungicides and possess high stability towards extreme pH, temperature and salinity (Cochrane & Vederas 2016; Wu et al. 2019a, 2019b). Similarly unregulated waste products such as ammonia, hydrogen cyanide and carbon-di-oxide are also known to be toxic in nature and hence contribute in the disease suppression by BCA (Sehrawat et al. 2022). Many BCA also do secrete lytic enzymes into their environment to generate food sources from polysaccharides, resulting in the cell wall lysis of several pathogens (Klock et al. 2002). Apart from all these antagonistic activities, a BCA can facilitate nutrient uptake by a host or increase nutrient availability to a host by converting the atmospheric nitrogen to assimilative form, solubilizing insoluble forms of mineral nutrients from the soil or by producing phytohormones and enzymes which will boost the growth and development of the host plant (Gupta et al. 2015).

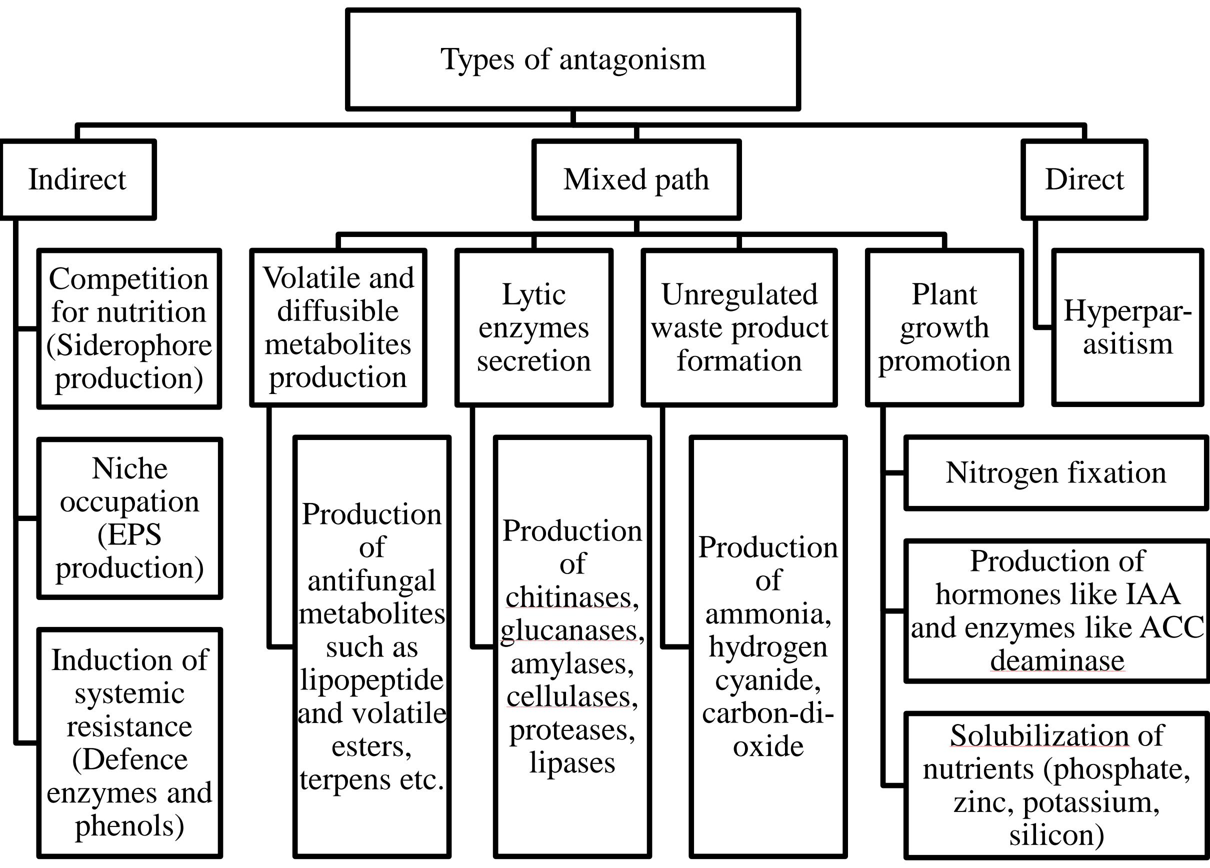


Figure 1: Flow chart of the mode of antagonism exhibited by a BCA against pathogens

Replacing agrochemicals with the application of microbial BCAs have both economic and environmental impacts with relevant benefits. The major advantages that microbial BCA confer over synthetic counterparts are less taxing to environment, an increase in crop yield, reduction or elimination of chemical residues, limited or no development of resistance and a low risk to non-target organisms. Also, some of the microbial BCAs might as well enhance the physical and biological properties of soil, thus boosting the growth of plants. Thus BCAs have been applied in agriculture on its own or as a part of integrated management system to protect various crops in India.

1.2 *Capsicum annuum* L.

Chilli (*Capsicum annuum* L.) is a major vegetable and spice crop, cultivated globally for its commercial value. It is also known as chilli peppers or “mirchi” and belongs to the Solanaceae family and genus *Capsicum*. This genus is common in temperate, tropical and sub-tropical regions and grows in warm, humid yet dry weather. It is known as "miracle spice" since it is the most extensively used universal spice. Production of the chilli crop

globally is estimated to be over 7 million tonnes annually and it is cultivated on 1.5 million hectares of land (Olatunji & Afolayan, 2018). It is an herbaceous annual that reaches a maximum height of one meter, has glabrous or pubescent lanceolate leaves, a highly branched root system with a tap root at centre, white flowers and fruits that vary in length, colour and pungency depending on the cultivar (Echave et al. 2020). The whole growth cycle of the plant consists of a vegetative phase (germination and seedling development) and a reproductive phase (stem elongation, branching, flowering and fruiting) which is completed in duration of 150 - 180 days depending on the variety, season and climate. The flowering of the crop is mostly observed on 80 - 85 day old crop on average with fruits appearing 90 days after transplantation (Agrifarming, 2014). Chillies can be grown both as Kharif and Rabi crops. Sowing months are May to June for Kharif crop with cropping done from June-September and September to October for Rabi crops with cropping done from October-May. If they are grown as summer crops as practiced in the state of Goa, then January-February months are chosen for sowing and cropping is done from February – June (FAIFA, 2016). Chillies made their appearance in the mid-15th century in Goa being introduced by the Portuguese from Brazil (Peter & Hazra 2012). The important indigenous red chilli cultivars of Goa are Aldona, Button/ Bootaon, Canacona/ Khola, Masouri, PiriPiri/ Portugal/ Tarvoti. Khola chilli of Goa has been registered as a GI tag under agricultural products for its unique brilliant red colour coupled with a medium-pungent flavour (Kumar 2020). Apart from these various commercial varieties of green chillies such as Nisha, Sitara, G-4, Arka Meghana, and Pusa Jwala are also cultivated here.

The carotenoid responsible for colour is "Capxanthin" which is known to have nutritional value. The pigment content increases (yellow to dark orange) as the fruit ripens and continues even after maturity. Compound Capsaicin (8-methyl-N-Vanillyl-6-enamide) is responsible for the pungency of chilli pepper, imparting the acrid and burning taste and is expressed in Scoville Heat Units (SHU) (Singh et al. 2009). Apart from being appreciated for its colour and pungency, it is also an incredible antioxidant, anti-neuropathic, counter irritant and cancer defensive agent and its extracts are used in a lot of pharmaceutical and cosmetic products. They are rich in Vitamin C (ascorbic acid), E (tocopherols and tocotrienols), P (citrin), B1 (thiamine), B2 (riboflavin), B3 (niacin) and provitamin A (β-carotene). They are also known to be a good source of flavonoids, carotenoids and xanthophylls (Gopalakrishnan 2007; Bosland et al. 2012). Capsaicin is used in allopathic creams to alleviate pain. It also demonstrates beneficial effects on metabolic and arterial function and helps reduce the long term inflammation and itching. Oleoresin is another high heat compound obtained from

chillies which is used in sprays for self-defence application (Carmichael 1991; Howard et al., 2000; Marin et al., 2004; Matsufuji et al., 2007; Perucka & Materska, 2007). Because of the irreplaceable taste and the array of interesting phytochemicals, chilli pepper has become an economically important crop in the agricultural sector across the world.

1.3 Disease-causing mycopathogens of chilli plants

Asia shows the maximum chilli production worldwide, of which India is the largest producer of this spice (Surepeddi & Giridhar, 2015). Chilli now is the essential condiment in food and is indispensable in Indian cuisine. The colour and pungent levels of Indian chillies are regarded to be world famous commercial attributes. Though a leading producer, the yield of the chilli crop in the country is highly hampered due to the disease causing phytopathogens. A heavy loss is chilli pepper is observed due to various soil-borne rot and wilt pathogens which affect germination of seeds, growth of seedling and plant and the number and quality of the fruits (Güney & Güldür 2018). Disease symptoms have been noticed in seedlings, mature plants, fruit or leaves depending on the phytopathogen that attacks the plant. However root rot and wilt diseases are the most devastating plant diseases for pepper plants (Alaa Fathalla & El-Sharkawy 2020). The most obvious symptom of root rots is wilting and death of plants even when the soil has enough moisture. As disease progresses, the stem dries up and withers, die back occurs, leaves defoliate and the whole plant finally die (Mmbaga et al. 2018). Wilt symptoms are characterized initially by slight yellowing of the older leaves followed by younger leaves; the leaves became chlorotic and desiccated and the whole plant withers and dies slowly (Khan et al. 2018). Living microbial pathogens that cause these diseases mostly include bacteria and fungi; of which fungal pathogens are most dreaded due to the significant loss of quantity and quality traits (Gachomo et al. 2003).

Rotting and wilting symptoms are manifested in the mature plants by soil borne fungal pathogens belonging to the genera *Macrophomina, Phytophthora, Rhizoctonia, Sclerotinia, Fusarium, Sclerotium* and *Verticillium*. *Rhizoctonia solani* can cause several types of damage at multiple growth stages of chilli such as seed decay, pre and post emergence seedling damping off, wire stem, root rot and necrotic spots on hypocotyl or root (Mannai et al. 2018). *R. solani* induces disease by penetrating and tainting the roots and stem from which it attains nutrients. It is an asexually reproducing fungus which spreads in the host system by forming a network of its hyphae. In the soil, it colonizes organic material and produces sclerotia (compact form of hyphae) for its survival (Keijer 1996). Another fungus *Macrophomina*

phaseolina is a harmful seed and soil borne pathogen and cause many diseases like damping off, seedling blight, collar rot, stem rot, charcoal rot, wilt, root rot and reddish-brown discoloration and black streaks that can form in the pith and vascular tissues of the root and stem (Babu et al., 2007; Güney & Güldur, 2018). Hyphae of this pathogen penetrate the host epidermis by forming germ tubes in root cells through natural openings and grow intra-cellular in the vascular tissue. Here it forms a network of dark mycelia and microsclerotia which leads to obstruction of the xylem. Eventually, the plant defoliates and wilts. The microsclerotia formed by the pathogen can survive for up to 15 years in the soil, which allows the fungus survive after the plants rot (Abawi & Corrales, 1990). It is difficult to control these soil-borne pathogens because of the wide host range and specialized structures of resistance produced by these fungi that can survive in soil for long periods in the absence of their hosts. Thus, many efforts are made for the control of these pathogens to reduce or avoid the loss of agricultural yields and the consequent economic damage. Apart from these, soil borne fungal pathogens belonging to genus *Fusarium* predominantly hamper a number of significant agricultural crops. *Fusarium* wilt disease caused by different species such as *F. oxysporum, F. pallidoroseum, and F. solani* produces morphological appearance such as wilting, chlorosis, necrosis, leaves fall, stunting and seedling crumbling in chilli plant (Okungbowa & Shittu 2012). This fungus enters the plant via roots and grows from inside of the roots towards the cortex. Here it produces microconidia which enter the sap stream and travel upwards. Germination of these spores leads to the clogging of the vascular vessels resulting in wilting and eventually plant death. The fungus also produces multiseptate macroconidia for infecting neighbouring plants. It lives in the soil around the infested plants and survives in the form of chlamydospores (Bowers & Locke, 2000; Smith 2007). Genus *Pythium* previously classified as fungus is now separated as is a genus of parasitic oomycetes as its cell wall is made of cellulose instead of chitin. It is known to infect the host and spread by forming sexual oospores and asexual zoospores. This pathogen show damping off symptoms in seeds or young seedlings wherein the infected seeds fail to germinate and the damaged seedlings generally rot, causing the seedling to wilt and eventually die, or collapse from the ground line (Mishra et al. 2013).

1.4 Potential of hypersaline bacteria as BCAs

An ideal BCA must confer high resistance in harsh conditions, display ubiquity in diverse habitats and maintain stability in formulated products. Extremophiles are known to satisfy all the above needs. Extremophilic microorganisms are known to grow and proliferate in extreme conditions like pressure, temperature, salinity, and depletion of micronutrients; with survival and proliferation often depending on the ability to produce biologically active compounds and enzymes which are highly stable in nature (Coker 2016). Since broad research has prompted to the revelation of a great many bioactive metabolites from conventional environments, new habitats must be screened with new methodologies keeping in mind the end goal to decrease the likelihood of rediscovering known (Arul Jose et al., 2011). Also marine derived microorganisms show more competence in fighting microbial infections because the terrestrial bacteria have not developed any resistance against them (Donia & Hamman 2003).

Hypersaline niches are an example of extremophilic environments which have 3.5% (w/v) to 35% dissolved salts and exist either as natural water bodies such as permanent saline lakes, ephemeral salt marshes or as artificial solar salt pans (Oren 2015, Oren 2016). Marine salt pans are man-made interconnected multi-pond systems originating from seawater. For commercial applications, the seawater from these ponds is evaporated for the production of crude salt (Kamat & Kerkar, 2011). A saltpan is one of the extreme environments characterized by high salinity, continuous harsh ionizing radiations and low oxygen concentration. Microorganisms inhabiting this niche have developed various methods to adapt to these harsh conditions and are collectively termed as hypersaline microorganisms (Rothschild & Mancinelli 2001; Voica et al. 2016; Jones & Baxter 2017; DasSarma et al. 2020). They are further characterized as halophilic microorganisms which require salts for their viability and halotolerant microorganisms which are able to grow in both the presence and absence of the salts (Ventosa et al., 1998). Based on Kushner's classification, halophiles are classified into two physiological groups, depending on their optimal growth with respect to the salt content: extreme halophile (can grow in 2.5–5.2 M NaCl) and moderate halophile (can grow in 0.5–2.5 M NaCl) (Kushner & Kamekura 1988).

Bacteria from salt pans have shown tremendous potential for numerous biotechnological and environmental processes such as the production of industrially important enzymes, probiotic potential, bioremediation of heavy metals, degradation of hydrocarbon and many more. A large number of hypersaline bacteria accumulate organic solutes (osmolytes) such as glycine,

betaine, sugar and ectoine under high salt concentration conditions. Among these solutes, ectoine is a stabilizing agent commercialized in the cosmeceutical industry with its ability to protect the skin from UVA rays (Nyyssölä et al. 2000). Several industrial enzymes such as hydrolase, amylase, alkaline metalloprotease, cellulase and lipase can also be obtained from these bacteria (Coronado et al. 2000; Karbalaei-Heidari et al. 2013). Bacteriorhodopsin, a photochemical material for bioelectronics and other applications is produced by halophiles (Oren 2010). Moreover, halophile organisms accumulate a large number of carotenoids under high salinity and high light intensity conditions, to protect the cell against UV-related damage and oxidative stress. Because carotenoids show beneficial effects on human health, the cultivation of halophilic microorganisms to harvest their bioactive pigments is of increasing interest to the Blue economy (Giani et al. 2019). These bacteria can also be used in the treatment of saline wastewaters, in aquaculture for boosting the yield and disease suppression, in biodegradation of heavy metals and organic pollutants and as agents for preventing post-harvest diseases of various fruits and vegetables (Nicholson & Fathepure 2004; Le Borgne et al. 2008; Sadfi-Zouaoui et al. 2008; Hao & Lu 2009; Blum et al. 2012; Fernandes et al. 2021).

To conclude, hypersaline bacteria represent a wide source of yet undiscovered compounds that, besides unprecedented chemical structures, often possess interesting biological activities. Hence the application of halophilic and halotolerant marine bacteria in agriculture, to control plant pathogens for increasing the yield hold up as a promising application. Additionally, they have been tested for their ability of root colonisation, promotion of plant growth and induction of systemic resistance mechanism in plants which can add to their advantages. Soil salinization is also a detrimental and significant limiting factor of agricultural productivity and food security; hence the application of hypersaline bacteria in agriculture makes a perfect sense in this scenario (Daliakopoulos et al., 2016). Halophilic and halotolerant microorganisms from the Goan salt pans have been reported to be a source of several hydrolytic enzymes and bioactive metabolites having antimicrobial action against various clinical pathogens (Kamat & Kerkar 2011; Ballav et al. 2015). A study Fernandes et al. (2019) have revealed the anti-*Vibrio* activity of halotolerant bacteria isolated from Goan salt pan in aquaculture. Recently, the application of halotolerant *Bacillus* spp. from a Goan saltpan as antifungal agent against mushroom pathogens has also been reported (Fernandes et al. 2021). However there have been no studies reporting the application of salt pan hypersaline bacteria against the fungal pathogens affecting chilli plant. Therefore this study aims at exploring the potential of salt pan bacteria as a biocontrol agent against the fungal pathogens that cause wilt and rot in chilli plants.

The following objectives were thus formulated for the current work:

1. Isolation of antagonist bacteria from water and sediment samples from saltpans and isolation of pathogen from chilli plants from fields of Goa.
2. *In-vitro* screening of the isolates for antagonistic activity against pathogens causing root rot and wilt in chilli (*Capsicum annuum* L.).
3. Evaluating the type of antagonism exhibited by the candidate bacteria.
4. *In-vivo* testing of the antagonistic bacteria as a biocontrol agent.

Significance of the thesis:

India is amongst the top countries in the world to producing chilli (*Capsicum annuum* L.) spice. Rising demand for chillies across the world combined with a higher value acknowledgement in the domestic market has spurred farmers to expand areas under chilli cultivation for consumption as well as export. However, heavy loss in the yield of chillies is observed across the country due to diseases caused by microbial pathogens. Among the various diseases causing microbial agents, fungal pathogens are the most problematic which hamper the growth and fruit bearing capacity of the crop to a great extent. Synthetic fungicides have been used for quite some time in lessening the occurrence of plant infections; however, overutilization of these chemicals has raised various biological and ecological issues. Along these lines, late endeavours have been centred on utilizing sustainable biological remedies for inhibiting phyto-pathogens. As defined by Eilenberg et al. (2001), "biological control or biocontrol is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be". This phenomenon is based on the antagonism between the pathogen and the antagonistic microorganism which is known as biocontrol agent (BCA). As biocontrol agents are usually isolated from natural habitats, the risk posed by them to the environment is minimal.

The potential of several rhizospheric and endophytic bacteria as a BCA has been previously studied; however inadequate resistance to fluctuating conditions and the inability in working up a stable formulation have limited their commercial use. In view of these restrictions, there is a need to divert attention to the isolation and characterization of extremophilic organisms which can be used as stable and effective BCAs in frequently altering the dynamics of the soil. Halophilic and halotolerant bacteria from hypersaline environments such as salt pans are known reservoirs of hydrolytic enzymes and have also been reported to produce potent antifungal and antibacterial bioactive metabolites. Some of the salt pan strains have also

shown plant growth promoting attributes and have been used in the formulation of bio-fertilizers. Despite all these traits, the efficiency of these microorganisms as biocontrol agents remains unexplored in the agricultural sector. Our study exemplifies the use of such hypersaline microorganisms from the salt pan of Goa as BCAs against disease causing fungal pathogens of chilli crops.

Chapter 2

Review of Literature

Hypersaline bacteria generally thrive in ecosystems with a high concentration of salts, which are referred to as “Hypersaline environments”. These environments contain salt concentrations higher than seawater and their physico-chemical properties vary depending on the type of salts present in this ecosystem. Primarily it can be divided into two main types, thalassohaline and athalassohaline environments, based on whether they originated from Seawater or not, respectively. Salt pans are man-made thalassohaline environments that inhabit a rich biodiversity of halophilic microorganisms that require salt for their growth and viability and halotolerant microorganisms that can grow in high salinity but are not dependent on it. These microorganisms have received considerable recognition owing to their potential as whole cells and as biomolecules such as enzymes, antibiotics, pigments, bioplastics, biosurfactants etc. in various fields (Margesin & Schinner 2001; Setati 2010; Oren 2010; de Lourdes Moreno et al. 2013; Hamedi et al. 2013; Couto et al. 2015; Waditee-Sirisattha et al. 2016; Enache et al. 2017; Gontia-Mishra et al. 2017; Dutta & Bandopadhyay 2022).

2.1 Salt pan microbes and their antimicrobial potential

Halophilic and halotolerant microorganisms from the salt pans have been reported to be a source of several hydrolytic enzymes and bioactive metabolites possessing antimicrobial properties (Kamat & Kerkar 2011; Ballav et al. 2015). A major application has been in aquaculture wherein hypersaline microorganisms have shown activity against *Vibrio* pathogens. A study reported the suppression of *Vibrio harveyi*, *V. parahaemolyticus*, *V. anguillarum*, *V. alginolyticus* and *V. vulnificus* by an alkaline protease producing strain of *Bacillus cereus* which was isolated from a salt pan in Tamilnadu, India (Donio et al. 2012). Similarly Sekar & Packyam (2014) proved the effectiveness of *Bacillus* sp. isolated from a salt pan, in inhibiting the shrimp pathogens *V. paraheamolyticus* and *V. harveyi* both *in-vitro* and *in-vivo*. Apart from these, a study by Fernandes and Kerkar, have revealed the anti-vibrio activity and production of lytic enzymes amylase, cellulase, protease and lipase of halotolerant bacteria *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis* and *Pseudomonas* sp. isolated from Goan salt pans (Fernandes et al. 2019). Furthermore John et al. (2020) isolated lipase and protease producing strains of *Chromohalobacter* and *Halovibrio* from Marakkanam salt pan in South India, showing antagonistic activity against pathogen *V. parahaemolyticus*.

Apart from its implementation in aquaculture, potential application of salt pan microorganisms and their metabolites in health sector has also been identified. Reports show that soil samples from the Ennore saltpan region of Bay of Bengal, Chennai, inhabited actinomycetes belonging to Genus *Streptomyces* exhibiting potential anti-dermatophytic activities against fungus *T. rubrum* and *M. gypseum* (Lakshmipathy & Kannabiran et al. 2009). Yet another research indicated the strong antibiosis against fungal and Gram negative pathogens of *Saccharopolyspora salina* isolated from a salt pan (Suthindhiran & Kannabiran, 2009). Similarly, a Gram-positive, moderately halophilic *Streptomyces* strain, isolated from salt pan soil collected at Tuticorin, India, showed inhibition of *Candida albicans, Klebsiella pneumoniae,* Methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus vulgaris* (Arul Jose et al. 2011). Different actinomycetes isolated from the salt pan soils of Vedaranyam, Tamilnadu, India were tested for their antimicrobial activity against various pathogens such as *Escherichia coli, Vibrio cholera, Salmonella typhi, Proteus vulgaris, Staphylococcus epidermidis, Cryptococcus neoformans, Salmonella typhimurium, Klebsiella pneumonia, Salmonella paratyphi B, Proteus mirabilis, Staphylococcus aureus* and *Candida albicans* (Vijayakumar et al. 2012). Isolated from the same salt pan, a halophilic *Streptomyces diastaticus* showed promising activity against Gram positive pathogens *Stapylocoocus aureus, Streptococcus pneumoniae, Vibrio parahaemolyticus, Enterococus faecalis* and Gram negative pathogens *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Vigneshwari et al. 2022). Further, an isolate obtained from a salt pan in Tamilnadu, India, *Streptomyces noursei* exhibited inhibition of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus* and *Vibrio cholera* via production of a methyl substituted β-lactum compound (Dharumadurai et al. 2013). Another study showed potential of metabolites extracted from salt pan actinomycetes isolated from Marakanam district (Tamil Nadu, India) against fungal pathogens *Aspergillus niger* and *Aspergillus fumigates* and *Staphylococcus aureus* (Roshan et al. 2013). A compound isolated from halophilic *Bacillus amyloliquefaciens* isolated from Tuticorin coastal salt pan, Tamil Nadu, India showed activity against methicillin resistant *Staphylococcus aureus* (Jeyanthi & Velusamy, 2016). In addition, a study by Murugan and Murugan, identified the antibacterial potential of biosurfactants produced by *Pseudomonas* sp. isolated from soil samples collected from a salt pan of Puthalam, Tamil Nadu, India (Murugan and Murugan, 2018). Further, a report revealed a potent metabolite against drug-resistant strains of *E. coli* and *K. quasivariicola*, purified from halophilic strain

Pseudomonas aeruginosa isolated from the Tuticorin coastal salt pan, Tamil Nadu, India. This metabolite proved to be a promising antibacterial agent especially for diabetic foot infections (Henciya et al. 2020). The *Streptomyces matensis* and *Streptomyces chilikensis* isolated from salt pan environment of Thondi, Ramanathapuram showed effective antibacterial activity against six human pathogens, *Staphylococcus aureus, Streptococcus pneumoniae*, *Serrtia sp., Pseudomonas sp., Vibrio cholera* and *Escherichia coli* (Priya et al. 2020).

A brownish-yellow pigmented salt pan bacterium *Aquimarina Spongiae*, from Southern Taiwan, showed potent activity against the toxic cyanobacterium *Microcystis Aeruginosa* and controlled its harmful blooms thus exhibiting a potent environmental application (Chen et al., 2011). Halocin, produced by an extremely halophilic archeon *Natrinema* sp. BTSH10 isolated from salt pan of Kanyakumari, Tamilnadu, India showed antibacterial activity against food spoilage-causing bacteria and hence, acted as a potential preservative in food industry. It is also used successfully as a potential preservative in the leather industry (Karthikeyan et al., 2013). In addition to all these applications, salt pan bacteria have also been used in the agricultural sector as either plant growth promoters or as biocontrol agents. Chen et al. (2010) showed the antagonistic ability of moderately halophilic bacteria isolated from the Weihai Solar Saltern (China) against the phytopathogens *Verticillium dahliae, Fusarium oxysporum f.sp. cucumerinum, Colletotrichum gloeosporioides* and *Alternaria solani*. A study has demonstrated the plant growth promoting ability of salt tolerant microorganisms isolated from a Goan saltpan which significantly improve rice plant growth parameters, soil chemical properties and also the biological activity (Bhambure et al. 2018). *Bacillus* spp. isolated from Algeria exhibited the inhibition of *B. cinerea, F. oxyporum, F. verticillioides* and *Phytophthora capsici* by *in-vitro* studies (Menasria et al. 2019). *Bacillus* strains used in another study by Petrillo et al. (2021) were isolated from sand samples collected in the proximity of *J. sabina* plants growing in the salt pans of Formentera (Spain). Whole-genome analysis on these strains confirmed the presence of numerous gene clusters with plant growth promoting and biocontrol functions and of novel secondary-metabolite biosynthetic genes, which could have beneficial impact on plant growth and protection. From the same source another study carried out by Castaldi et al. (2021) has revealed the biocontrol of *M. phaseolina* by a *Bacillus* spp.

2.2 Management of phytopathogens

The term ‘pesticide’ in agriculture refers to a component or mixture of components used to prevent, control, reduce or inhibit a pest (bacteria, fungi, virus, nematodes, vectors and even unwanted species of plants or animals) interfering with the production, processing, storage or marketing of any agricultural commodity. Fungicides are pesticides that kill or control the growth of fungi and molds and their spores that damage plants, including rusts, mildews and blights (Brauer et al. 2019). Synthetic pesticides are those which are formulated or manufactured using chemical processes, using chemically synthesized components or by chemically changing any component derived from natural sources (Stoytcheva 2011). For any pest problem, chemical agents are usually preferred over organic ones as they are cheaper, readily available and have a longer shelf life than the later. They are mostly broad-spectrum agents and thus can be applied against various pests. They are even known to be more persistent in nature thus reducing the frequency of application in the field, which in turn saves time and economics (McCoy et al. 2020). However due to such properties of persistency and broad-spectrum activity, the indiscriminate use of synthetic pesticides has resulted in serious biological and ecological problems. As reported by Pimentel (1995), only < 0.3% of chemical pesticides showed interaction and inhibition only of target pathogens. Most chemical pesticides affect the beneficial organisms in the soil which assist in natural processes of mycorrhizal colonization, transformation or fixation of nitrogen, improvement of soil porosity and fertility (Aktar et al. 2009). Overuse of pesticides has also resulted in acquisition of resistance by the target pathogens, thus making its control further taxing. Some of the chemical agents are also seen to be converted to toxic by-products in the soil, thus hampering other plants, animals and even humans (Smith & Perfetti 2020). Considering these risks associated with synthetic pesticides, there is need to shift to biological ways of controlling phytopathogens.

Biological control is a method of restricting harmful effects of pathogens using other antagonistic organisms that inhibit or suppress the population of harmful organisms. The method takes advantage of basic ecological interactions between organisms, such as predation, parasitism, pathogenicity and competition (Lahlali et al. 2022). Biocontrol agent (BCA) refers to the antagonist used for suppression of the pathogen (or pest) in the cropping system (Pal & Gardener 2006). A BCA should be easily available, easy to use, inexpensive and effective on even high densities of pests in any season and condition. It can be “permanent” for those BCAs that can survive in soil and become self-perpetuating. However

it should be disrupted by other pest management tactics in case of any emergency. And most important of all it should be pest-specific, not broad-spectrum (Bale et al. 2008). Single BCA may show different modes of action against a phytopathogen which may be expressed sequentially, concurrently or synergistically. There are three main modes of antagonism exhibited by a candidate BCA: direct antagonism, indirect antagonism and mixed path antagonism (Koul et al. 2022). Direct antagonism, involves the principle of parasitism or predation wherein a BCA kills the phytopathogen for its survival or lives on or inside the phytopathogen, thus harming the later (Köhl et al. 2019). Indirect modes of antagonism exhibited by BCAs can be either competition for space and nutrition with the pathogen or induction of systemic resistance (ISR) in the host against the pathogen. In mixed path antagonism BCAs produce secondary metabolites such as antibiotics, lytic enzymes, unregulated waste products and volatile compounds which are toxic to the target pathogens (Nega 2014). A BCA can also exhibit attributes that help in plant growth promotion (PGP) which makes the host more resistant towards the diseases caused by the pathogens (Beneduzi et al. 2012).

2.3 Disease causing fungal pathogens in chilli plants

India has been amongst the major countries producing, consuming and exporting chillies for a long time. A rising demand for chillies because of its colour, pungency and flavour combined with higher value acknowledgment in the domestic market has spurred farmers to expand areas under chilli cultivation for export (Mohanty et al. 2019). However fungal pathogens are known to cause a severe loss of the chilli yield. Fungi are responsible for damping off, fruit rot, die-back, anthracnose, leaf spot/ blight, powdery mildew, wilt and rot (soft rot, fruit rot, white, root rot and charcoal rot) in chilli plants (Pawaskar & Kerkar, 2021). The damping off caused by the *Pythium aphanidermatum* affects up to 90 % of the plantation and thus is one of the most destructive pathogen (Arora et al. 2021). In pre-emergence damping off, the growing points of the seeds are damaged in the initial stages of germination before they come out through the soil. In post-emergence damping off, the seedlings topple over the ground due to collar rotting and rapid shrinkage, followed by spreading to the cortical tissue of the hypocotyls, basal stem and developing taproot, thus resulting in death of plants (Majeed et al. 2018). Next the wilt causing *Fusarium* species such as *F. oxysporum*, *F. solani* and *F. pallidoroseum*, not only reduces the growth but also affects the fruit quality and causes about 10 – 80 % yield loss in the total global production (Bashir et al. 2018). Along with the wilting of the plant, leaf chlorosis and vascular discoloration is observed as result of the infection.

Symptoms may appear at two stages *viz*. seedling and adult plant stage. At the seedling stage, foliage turns yellow and then dries off. In an adult plant, initially slight drooping of leaves is seen which leads to the drying of leaves starting from lower ones and then extending to the apex region followed by wilting symptoms (Sanogo 2003, Suryanto et al. 2010). Apart from these diseases, a saprophytic pathogen *Rhizoctonia solani* causing rot shows up to 33.2 % disease incidence of the seedling in greenhouse conditions itself and 40.2 % in the main field (Rini & Sulochana 2007). This pathogen affects the seedling and mature plants and induces dry rot of the collar region and root rot which leads to wilting and death of chilli plants. It is easily identified by the presence of white mycelia in the infected plant. It also shows symptoms of damping off (Mannai et al. 2018). Another pathogen *Macrophomina phaseolina* causing charcoal rot have been posing challenging task for its management. This pathogen remains viable in the soil for longer period without showing any symptoms of disease and eventually when the environment is conducive, can destroy the whole field. In this disease, blackening of lower stem and taproot is observed under the epidermis due to the numerous microsclerotia formed by the fungus. This gives the plant tissue a charcoal-sprinkled appearance and hence the name of the disease. As the disease advances, leaves turn yellow, then wilt, turning brown and eventually the plant dies off (Shahid & Khan 2016). Due to the infection of these disease causing pathogens, huge loss in crop yield is observed which increases the economics of the sold fruit in the market.

2.4 Biological control of mycopathogens infecting chilli using microorganisms

Among the different types of BCAs, microbial BCAs have become an alternative to chemical agents with increased target specificity and ecological safety so that they are used either directly or in combination with other pest management programmes. Microbial BCAs are perceived as being less demanding to the environment and their generally complex mode of action makes it unlikely that resistance will develop (Usta 2013). Majorly BCAs belonging to *Pseudomonas, Bacillus* and *Trichoderma* spp. have been applied for controlling the phytopathogens in chilli plants. Dating back to 1998, Perveen et al. has reported the reduction of infection of *Meloidogyne javanica* root knot nematode and root infecting fungi *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *Fusarium oxysporum* by *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* used alone or together. Later the observations made by Khan et al. (2003) demonstrated that *Pseudomonas chlororaphis* is able to suppress the development of *Pythium aphanidermatum* and root browning caused by the

pathogen, in chilli peppers grown in hydroponic trough units. A combined strategy to control chilli fruit rot caused by *Colletotrichum capsici* and powdery mildew caused by *Leveillula taurica* consisting of reduced fungicide dose and biological control with antagonistic *Pseudomonas fluorescens* was studied by Anand et al. (2010). In another study, Mojica-Marin et al. (2008) determined, *in vitro,* the antagonistic effectiveness of *Bacillus thuringiensis* against damping-off and root and stem rot caused by *Rhizoctonia solani* in chili pepper. Paul et al. (2013) pointed out the inhibition of *Alternaria panax, Botrytis cinerea, Colletrotichum acutatum, Fusarium oxysporum* and *Phytophthora capsici* by *Bacillus tequilensis, Burkholderia cepacia* and *Pseudomonas aeruginosa* isolated from healthy tissues of leaves, stems and roots of chili pepper plants. The severity index of *Cercospora* leaf spot on pepper plants caused by *Cercospora capsici* was significantly lower on *Lactobacillus plantarum* treated plant than on pepper without seed treatment (Adedire et al. 2019). Three root colonizing *Bacillus* spp. namely *B. amyloliquefaciens, B. subtilis* and *B. tequilensis*, exhibited broad-spectrum *in-vitro* antifungal activities against *Aspergillus niger, A. flavus, Fusarium oxysporum, F. moniliforme, F. solani, Colletotrichum falcatum, Curvularia* sp. and *Rhizopus* sp. (Shahid et al. 2021). Further another study by Zhou et al. (2021) revealed 34 *Bacillus* strains with antimicrobial activity against *Rhizoctonia solani; Botrytis cinerea; Verticillium dahliae* and *Phytophthora infestans*. Application of *Trichoderma harzianum* spores on chilli seeds led to a reduction of stem necrosis caused by *Phytophthora capsici*, which got reduced to nearly half as compared to the values observed in plants grown from non-treated seeds (Sid Ahmed et al. 2000). Ekefan et al. (2009) determined the potential of *Trichoderma harzianum* isolates as biocontrol agents of *Colletotrichum capsici*, causing anthracnose of pepper. This was supported by Brand et al. (2009) which pointed out the inhibition of *Leveillula taurica* by *Trichoderma harzianum* strain. Wani & Najar (2012) showed the antifungal potential of *Trichoderma viride* against *F. pallidoroseum* in chilli plants. Also Dar et al. (2015) evaluated *Trichoderma*, *Pseudomonas fluorescens* and *Bacillus* sp. against the pathogens responsible for wilt/root rot and damping off (*Sclerotium rolfsii, Rhizotonia solani, F. pallidoroseum, Fusarium solani, F. oxysporium* and *Pythium* sp.) *in vitro* and reported effective inhibition of the mycelia by these BCAs. Supporting this in another study, fungal antagonists *Trichoderma harzianum* and *Trichoderma viride* isolates completely overgrew *F. solani* and inhibited mycelia growth by 40–50 % *in vitro* (Bhat et al. 2016). Study by Lopez et al. (2019), investigated the effectiveness of *Trichoderma asperellum* and *Metarhizium anisopliae* in controlling pepper powdery mildew. The authors concluded saying that *T. asperellum* showed higher and persistent activity against *Leveillula taurica* in pepper. Apart from these various

scientific publications in the past have reflected the application of microorganisms as BCAs to inhibit various mycopathogens known to infect chilli crops which shows the interest of the community in the subject (Table 1).

One of the fundamental constraints in the utilization of BCAs in the commercial market is its restricted resistance to fluctuating ecological conditions and the troubles in building up a stable formulated product. In view of these restrictions, there is need to divert attention to the isolation and characterization of extremophilic organisms (organisms thriving in extreme conditions such as temperature, pressure, radiation, salinity and pH) which can be used as stable and effective biocontrol agents. Also, massive urbanization is putting pressure on agricultural lands, resulting in the shrinking of land holdings. Hence there is the utmost need to restore the degraded lands for agricultural applications. The government of India has fixed a target of restoring 26 million ha of degraded lands, including salt-affected soils, by the year 2030 to ensure food security for the people (Kumar & Sharma, 2020). Also Among the abiotic factors, soil salinization is the most detrimental and is considered one of the significant limiting factors of agricultural productivity and food security (Daliakopoulos et al. 2016). Worldwide, about 20 % of the agricultural land is inundated with salt water, and this is continuously increasing (Gupta & Huang 2014). In this view, the application of hypersaline microorganisms from niches as BCA stands out as a promising option.

Hypersaline bacteria isolated from different sources have been reported to be exhibiting antifungal activity against various plant pathogens. Sadfi et al. (2001) evaluated the potential of *Bacillus* spp. isolates from salty soils for the biocontrol of *Fusarium* dry rot of potato tubers. Similar study was undertaken by Sadfi-Zouaoui et al. (2008) who demonstrated the ability of moderately halophilic bacteria isolated from salt lakes to control grey mould disease caused by *Botrytis cinerea* on tomato fruits. Later in 2009, Essghaier et al. proved the biological control of *Botrytis cinerea* in strawberry fruits by halophilic bacteria isolated from the same source. The antagonistic activity of the hypersaline bacteria against *Botrytis cinerea* on tomato fruits was further supported by Berrada et al. (2012). Exploration of antifungal activity of halophilic bacterial communities from saline soils of Iran against *Aspergillus parasiticus* affecting Pistachios nuts was done by Jafari et al. (2018). All these studies dealt with the post-harvest disease control.

Table 1: BCAs against plant pathogens affecting chilli crops and their suggested mode of action

Sr. No.	Pathogen	Diseases	Biocontrol agents	Source	Suggested mode of action	Reference
1	*Alternaria alternata*	Leaf spot/ blight	*Bacillus* spp.	Sweet pepper rhizosphere	Hyperparasitism	Sid et al. 2003
			Bacillus atrophaeus *Brevibacterium frigoritolerans*	Soil	Antibiosis	Chacón-López et al. 2021
2	*Colletotrichum acutatum*	Fruit rot/Die-back/Anthracnose	*Bacillus vallismortis*	Chili pepper rhizosphere	ISR	Park et al. 2013
3	*Colletotrichum capsici*	Fruit rot/Die-back/Anthracnose	*Pseudomonas aeruginosa*	Rice rhizosphere	Antibiosis	Kumar et al. 2005
			Pichia guilliermondii	Thai fruits and vegetables	ISR	Chanchaichaovivat et al. 2007
			Ophiocordyceps sobolifera	Dead cicada nymphs	Antibiosis	Nantawanit et al. 2010
			Bacillus sp.	Soil	Antibiosis	Jaihan et al. 2016
			Pseudomonas aeruginosa	Chilli	ISR/ Volatile compounds/	Srikhong et al. 2018

					Hydrolytic enzymes	Jisha et al. 2019
4	*Colletotrichum gloeosporioides*	Fruit rot/Die-back/Anthracnose	*Bacillus subtilis*	Soil, Chilli rhizosphere	Antibiosis	Kim et al. 2010 Ashwini & Srividya 2014
			Pseudomonas aeruginosa	Soil	Competition	Sasirekha & Srividya 2016
			Streptomyces philanthi	Wheat seed	Antibiosis	Boukaew et al. 2018
			Trichoderma koningiopsis	Soil	Antibiosis	Ruangwong et al. 2021
5	*Colletotrichum scovillei*	Fruit rot/Die-back/Anthracnose	*Paenibacillus polymyxa*	Soil	Antibiosis	Suprapta et al. 2020
6	*Colletotrichum truncatum*	Fruit rot/Die-back/Anthracnose	*Burkholderia rinojensis*	Soil	Hyperparasitism	Sandani et al. 2019
			Trichoderma harzianum *Trichoderma asperellum* *Paenibacillus dendritiformis*	Chilli rhizosphere	ISR	Yadav et al. 2021
7	*Fusarium*	Wilt	*Pseudomonas aureofaciens*	Rice rhizosphere	Competition	Chaiharn et al.

	oxysporum					2009
			Bacillus subtilis	Soil	Competition	Yu et al. 2011
			Bacillus sp.	Bean rhizosphere	Lytic enzymes	Kumar et al. 2012
			Burkholderia tropica	Plants	Volatile compounds	Tenorio-Salgado et al. 2013
			Bacillus amyloliquefaciens	Millet seeds endophyte	Antibiosis	Verma & White 2018
			Bacillus cereus	Root nodules of *Prosopis laevigata*	Volatile compounds	Ramírez et al. 2022
8	*Fusarium pallidoroseum*	Wilt	*Trichoderma viride* *Chaetomium indicum* *Aspergillus flavus* *Gliocladium roseum* *Trichothecium roseum* *Paecilomyces variotic* *Trichoderma harzianum*	Apple rhizosphere Mushroom compost Tulip rhizosphere Mulberry rhizosphere Mulberry twigs	Hyperparasitism/ Competition/ Antibiosis	Munshi & Dar 2004
9	*Fusarium solani*	Wilt	*Bacillus subtilis* *Pseudomonas fluorescens*	Coconut and Cotton	ISR	Sundaramoorthy et al. 2012

				rhizosohere		
			Bacillus sp.	Bean rhizosphere	Lytic enzymes	Kumar et al. 2012
			Pseudomonas fluorescens *Bacillus subtilis* *Trichoderma viride*	Soil	Competition/ Volatile compounds	Narayanan et al. 2015
			Paecilomyces sp.	Aloe vera and Pomegranate rhizosphere	Antibiosis	Hussain et al. 2016
			Bacillus subtilis *Pseudomonas fluorescence*	Cucumber rhizosphere	Lytic enzymes/ Antibiosis	Al-Fadhal et al. 2019
			Bacillus spp.	Soil and air samples	Volatile compounds	Gutiérrez-Santa Ana et al. 2020
10	*Macrophomina phaseolina*	Charcoal Rot	*Pseudomonas cepacia*	Caryopses of gamma grass	Volatile compounds	Jayaswal et al. 1993
			Pseudomonas plecoglossicida *Brevibacterium antiquum* *Bacillus altitudinis,* *Enterobacter ludwigii*	Rice rhizosphere	Competition/ Unregulated waste product formation/ PGP	Gopalakrishnan et al. 2011

			Acinetobacter tandoii *Pseudomonas monteilii* *Bacillus* sp.	Bean rhizosphere	Lytic enzymes	Kumar et al. 2012
			Streptomyces sp.	Soil	Lytic enzymes and waste products/ PGP	Alaa Fathalla & El-Sharkawy 2020
11	*Phytophthora capsici*	Phytophthora blight/Fruit rot/root rot/leaf blight	*Bacillus subtilis* *Bacillus licheniformis*	Pepper rhizosphere	Hyperparasitism	Sid et al. 2003
			Streptomyces halstedii	Soil	Hyperparasitism/ Mycophagy	Joo 2005
			Bacillus megaterium	Chilli rhizosphere	Antibiosis	Akgül & Mirik 2008
			Bacillus subtilis	Pepper rhizosphere	Antibiosis	Lee et al. 2008
			Lysobacter antibioticus	Soil	Lytic enzymes/ Antibiosis	Ko et al. 2009
			Streptomyces griseus	Soil	Hyperparasitism/ Lytic enzymes	Nguyen et al. 2012

			Bacillus vallismortis	Chilli rhizosphere	ISR	Park et al. 2013
			Pseudomonas fluorescens	Endophyte of strawberry	ISR	Kim et al. 2014
			Trichoderma hamatum *Pseudomonas aeruginosa*	Chilli rhizosphere	Competition/ Antibiosis	Chemeltorit et al. 2017
			Trichoderma virens	Chilli rhizosphere	Hyperparasitism	Tomah et al. 2020
12	*Pythium aphanidermatum*	Damping off	*Pseudomonas* sp.	Chickpea and green gram rhizosphere	Lytic enzymes	Sindhu & Dadarwal 2001
			Bacillus subtilis *Pseudomonas chlororaphis*	Soil	ISR	Kavitha et al. 2005
			Bacillus subtilis	Vegetable rhizosphere	ISR	Nakkeeran et al. 2006
			Calothrix elenkenii	Rice fields	Antibiosis	Manjunath et al. 2010
13	*Rhizoctonia solani*	Root rot	*Pseudomonas* sp.	Chickpea and green gram rhizosphere	Lytic enzymes	Sindhu & Dadarwal 2001

			Pseudomonas fluorescens *Pseudomonas putida* *Bacillus subtillis*	Chilli rhizosphere	Competition/ Antibiosis	Abeysinghe 2009
			Bacillus sp.	Bean rhizosphere	Lytic enzymes	Kumar et al. 2012
			Pseudomonas aeruginosa	Soil	Lytic enzymes	Suryadi et al. 2014
			Bacillus subtilis	Almond rhizosphere	Hyperparasitism	Khedher et al. 2015
			Bacillus subtilis	Tomato rhizosphere	ISR/ Antibiosis	Wu et al. 2019b
			Pseudomonas fluorescens	Oilseed rape roots	Competition	Chlebek et al. 2020
			Streptomyces sp.	Soil	Lytic enzymes and waste products/ PGP	Alaa Fathalla & El-Sharkawy 2020
14	*Sclerotium rolfsii*	Wilt	*Pseudomonas fluorescens* *Pseudomonas putida* *Bacillus subtillis*	Chilli rhizosphere	Competition/ Antibiosis	Abeysinghe 2009
15	*Verticillium dahliae*	Wilt	*Pythium oligandrum*	Soil	ISR	Al-Rawahi et al. 1998

As mentioned earlier, salt pans are a rich ecosystem with vast potential. Countrywide there are a handful of states with functional salt pans which have not been destroyed due to urbanization and industrialization. Goa is one such state with active research being carried out on the potential applications of hypersaline bacteria inhabiting these salt pans. Halophilic and halotolerant microorganisms from the salt pans of Goa have been reported to be a source of several hydrolytic enzymes and bioactive metabolites having antimicrobial action. Kamat & Kerkar (2011) demonstrated the inhibition of clinical pathogens namely *Acinetobacter baumanii, Aeromonas hydrophila, Citrobacter diversus, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis, Pseudomonas spp., Salmonella paratyphi A, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella flexneri, Vibrio cholerae,* Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Sensitive *Staphylococcus aureus* (MSSA) and *Staphylococcus citreus* by bacteria isolated from Goan salt pans. Halophilic and halotolerant actinomycetes from Goan salt pans have been also studied as a potential source of anti-bacterial compounds against *Staphylococcus aureus, Staphylococcus citreus*, and *Vibrio cholerae* (Ballav et al. 2015). Bhambure et al. (2018) demonstrated the potential of salt tolerant *Pseudomonas multiresinivorans, Microbacterium esteraromaticum* and *Bacillus subtilis* individually and their consortium with amended farmyard manure (FYM) as promoters of rice growth and microbial activity in coastal saline soil. A study by Fernandes et al. (2019) have revealed the anti-*Vibrio* activity of halotolerant bacteria *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis* and *Pseudomonas* sp. isolated from Goan salt pan. Recently, the application of halotolerant *Bacillus* spp. from a Goan saltpan as antifungal agent against mushroom pathogens has also been reported (Fernandes et al. 2021). However there have been no studies reporting the application of hypersaline bacteria from Goan salt pans against the fungal pathogens affecting the chilli plant.

Chapter 3

Materials and Methods

3.1 Sampling sites for the isolation of hypersaline bacteria

Water and sediment samples were collected from three salt pans *viz.* Batim, Agarwado and Ribandar, in the month of February 2017. Two ponds were chosen from each salt pan for the sample collection. The locations of the study site were recorded by a hand held Global Positioning System (GPS) [eTrexVistaTM personal navigator®, Garmin International, Inc., USA]. The physicochemical parameters of the overlying saltpan water *viz.* temperature, salinity, dissolved oxygen, pH and total dissolved solids were measured on-site. Temperature was measured using a field thermometer (76 mm immersion, ZEAL, England), salinity was measured using a hand held refractometer (S/Mill-E, ATAGO Co. Ltd., Japan) and pH was measured using a digital pH meter (pH 700, Eutech instruments, Thermo Fisher Scientific, India). Dissolved oxygen and total dissolved solids were measured using a hand held multi-parameter device (CyberScan PC 650, Eutech Instruments, Thermo Fisher Scientific, India).

3.2 Isolation of bacteria from salt pan water and sediment

Water samples overlying the salt pans from two different ponds were collected in sterile disposable bottles. Sediment samples were collected using a 5.5 cm diameter corer and were sealed in sterile plastic bags. All the samples were transported to the laboratory for processing within 24 h (Kamat & Kerkar 2011). For the isolation of hypersaline bacteria, serial dilutions were carried out with sterile 0.85 % saline (Sodium chloride) and 0.1 mL of each sample was spread plated, in triplicates on Nutrient Agar (NA, Appendix I) and Zobell Marine Agar (ZMA, Appendix I) of different strengths (25 %, 50 % and 100 %). All the media components and chemicals used were of analytical grade. The plates were incubated at room temperature (28 ± 2 °C) for 72 h. Morphologically different colonies were selected and their detailed colony characteristics were noted. Each isolate was assigned a culture code in the MPSK series. The strains isolated were then stored at 4 °C until further use. Glycerol (50 % w:v) stocks of each isolate were also prepared in their respective media and stored at –80 °C. Apart from these, additional bacterial cultures previously isolated by

Prof. Savita Kerkar's research team from the salt pan water, sediments and associated biofilms samples were also used in the study. These isolates were previously coded as SK, BGUM and ABSK.

3.3 Sample collection for the isolation of fungus from diseased chilli plants

Chilli plant samples were collected from the fields of Bicholim, Saligao, Valpoi, Margao and Khola region of Goa in the months of April and May of 2017. Plants showing wilt and rot symptoms were uprooted from these fields and transported to the laboratory within 24 h. Three plant samples from each field were selected. Stems and roots of these plants were used as sample sources for the isolation of fungus.

3.4 Isolation of fungus from diseased plant samples

The lower stem region along with roots of the collected diseased chilli plants were first washed with tap water followed by a detergent (teepol) wash for 10-15 min to remove dirt and soil. Finally it was rinsed in distilled water (DW) to remove any traces of the wetting agent. Explants were then transferred to a laminar air flow (LAF, Medlab Biosafety Class II B2, Medlab Scientific Equipments, India) and dipped in 70 % ethanol for 60 sec. These were then surface sterilized using 0.1 % mercuric chloride for 10 - 15 min and finally washed thoroughly with sterile DW. Vertical slices of the internal tissue were cut using a sterile scalpel and were placed on Potato dextrose Agar (PDA, Appendix I) amended with 1 mg/mL of chloramphenicol (De, 2013). These plates were then incubated at 25 ℃ till the appearance of the fungal mycelia. The fungal strains isolated, were then purified and screened out on the basis of their growth pattern on PDA plates incubated at 25 °C for 3 - 5 days. The fungal isolates with different morphological patterns were then identified.

3.5 Identification of the fungal isolates

Preliminary identification of the fungal isolates (5) based on their morphology was done at Indian type culture collection, ICAR- Indian Agricultural Research Institute (ITCC, New Delhi). These cultures were then deposited at their repository to obtain the registration number. Out of these five fungal isolates, three fungi which were chilli plant pathogens were then characterized by PCR amplification of ITS region at ITCC,

New Delhi. Maximum likelihood tree was constructed from the data obtained using MEGA 11 software (Molecular Evolutionary Genetics Analysis Version 11, 2021) with 1000 bootstrap replicates.

3.6 Screening of salt pan bacteria for antifungal activity

The preliminary screening of hypersaline bacteria (197) against the fungal isolates was done by dual culture technique (Karimi et al., 2016). The antifungal activity of the bacterial isolates was checked against three fungal cultures isolated in this study *viz. Fusarium solani*, *Fusarium oxysporum* and *Macrophomina phaseolina*. Besides these, three standard fungal chilli plant pathogens *viz. Fusarium pallidoroseum* (ITCC 7890), *Rhizoctonia solani* (ITCC 5338) and *Pythium aphanidermatum* (ITCC 4746) were procured from ITCC, New Delhi. The hypersaline bacteria were inoculated separately in Zobell Marine Broth (ZMB, HiMedia M385) (10 mL) and incubated at 28 ± 2 °C for 48 h on a shaker incubator (Remi CIS-24 plus, Remi Elektrotechnik Ltd., India) at 120 rpm. An 8 mm plug (3 mm thick) from the leading edge of a fungal culture growing on PDA was removed with the help of a sterile cork borer. This plug was then placed at the center of a new PDA plate and the cell suspension of the hypersaline bacteria was streaked around the plug at a 2.5 cm distance on all four sides. Plates streaked with uninoculated broth around the fungal plug were used as a control. After the incubation period (48 h in case of *M. phaseolina, R. solani* and *P. aphanidermatum* and 120 h in case of *F. solani*, *F. oxysporum* and *F. pallidoroseum*) percent inhibition of the fungal mycelia by the hypersaline bacteria as compared to that of control, was calculated using the following formula:

$$L\ (\%) = [(C - T)/C] \times 100;$$

Where, L is inhibition percentage; C is colony radius in the control plate and T is radial growth of the fungi in the presence of hypersaline bacteria.

3.7 Screening for growth of salt pan bacteria in soil extract

Soil extract (SE) was prepared by mixing 17.75 g of soil in 1 L of DW and kept on a shaker incubator at 150 rpm at 40 °C for 1 h (Liebeke et al., 2009). This was then filtered and the extract obtained was utilized for preparing SE agar (Appendix I). Bacterial cultures were streaked on these plates and incubated at 28 ± 2 °C and checked for growth within 5 days of incubation.

3.8 Evaluating type of antagonism by salt pan bacteria

Hypersaline bacteria were assessed for various tests under the three modes of antagonism: Mixed path, Indirect and Direct. For antagonism tests, the antifungal activity of the hypersaline bacteria was checked against three fungal cultures *viz. F. solani, M. phaseolina* and *R. solani*, as these pathogens are responsible for the major yield loss of chillies and are predominant in Goan soils.

3.8.1 Qualitative analysis of mixed path antagonism and indirect antagonism tests

Ten hypersaline bacteria BGUM 14B, SK 473, MPSK 9, MPSK 14, MPSK 20, MPSK 22, MPSK 23, MPSK 109, MPSK 186 and ABSK 9 which showed > 40 % inhibition of all the six fungal cultures and which were able to grow on SE agar were tested for mixed path antagonism and indirect antagonism tests. Under mixed path antagonism tests, the bacteria were screened for the production of volatile and diffusible metabolites, lytic enzymes and unregulated waste products and for plant growth promoting attributes. The production of siderophore and exopolysaccharide by the hypersaline bacteria were evaluated as a part of indirect antagonism.

3.8.1.1 Production of volatile and diffusible metabolites

The hypersaline bacteria were first tested for the production of antifungal metabolites of both volatile and diffusible nature; against *F. solani, M. phaseolina* and *R. solani* by inverted plate and well diffusion method respectively as described by Lim et al. (2017).

a) Production of volatile metabolites

Hypersaline bacteria were inoculated individually in 10 mL of ZMB and were incubated on a shaker incubator at 120 rpm at 28 ± 2 °C. After 48 h incubation, cell suspension of each bacterium was swabbed on individual ZMA plates (A). The fungi to be tested were previously grown on PDA plates, incubated at 25 ± 2 °C (for 48 h in case of *M. phaseolina* and *R. solani* and for 120 h in case of *F. solani*). After the incubation period, a mycelial plug was removed using a sterile cork borer and was placed in the center of a sterile PDA plate (B). This was repeated for all the three fungi separately. Each plate A was then inverted on each of the plates B, sealed with parafilm and incubated at 25 ± 2 °C for the respective incubation period. For control, ZMA plates were swabbed with

uninoculated broth, inverted on the PDA plates containing the respective fungal plugs and sealed with parafilm and incubated under the same conditions as the test plates. Radial growth (R) of each fungal culture was recorded within 2-5 days and the inhibition percentage of mycelial growth was calculated using the formula:

$$R\ (\%) = (r_l - r_2/r_l) \times 100$$

Where, r_l is radial growth of the fungus in control and r_2 is the radial growth of the fungus in sets inoculated with the bacterium.

b) Production of diffusible metabolites

The production of diffusible metabolites was tested by the well diffusion method. Hyperasline bacteria were inoculated individually in ZMB (10 mL) and incubated at 28 ± 2 °C on a shaker incubator at 120 rpm. After 48 h incubation, culture broth of the bacteria was sonicated (VIA sonicator, Sonics & Materials Inc., USA) at a frequency of 20 kHz for 40 sec with pulse and 40 sec without pulse. The sonicated sample was then centrifuged (in Eppendorf 5804 R centrifuge, Eppendorf, Germany) at 10,000 rpm for 15 min at 28 ± 2 °C and the supernatant was assessed for antifungal activity. *F.solani*, *R. solani* and *M. phaseolina* were separately grown in 100 mL flasks with 50 mL Potato Dextrose Broth (PDB, HiMedia M403). The flasks were placed on an orbital shaker at 120 rpm, 25 ± 2 °C for an incubation period of 48 h in case of *M. phaseolina, R. solani* and 120 h in case of *F. solani*. Subsequently, the fungal cultured were spread plated with sterile cotton swabs (HiMedia) on PDA. On these plates, 8 mm agar wells were bored using a sterile cork borer. A total of 100 µL of each of the hypersline bacterial cell free supernatant was added to the wells and incubated at 25 ± 2 °C for 48 / 120 h depending on the fungal culture. Antifungal activity was recorded from the diameter of the zone of inhibition (mm) of the mycelial mat around the wells. For the control, 100 µL of sterile ZMB was inoculated in the wells.

3.8.1.2 Production of lytic enzymes

The qualitative estimation of cell wall degrading enzymes chitinase, glucanase, amylase, cellulase, protease and lipase production was carried out by plate assays on 1X M9 Minimal Medium Salts (Appendix I) containing specific substrates. All

the results were recorded as: Solubilization index = Total zone diameter (mm) / Colony diameter (mm) (Shet & Garg, 2022).

a) Chitinase

Colloidal chitin was prepared by vigorously stirring 5 g of chitin flakes (HiMedia GRM1356) in 60 mL concentrated hydrochloric acid overnight at 4 °C on a magnetic stirrer (Remi Q-19, Remi Elektrotechnik Ltd., India). Colloidal form of chitin was then precipitated using 95 % ice cold ethanol (2 L) at 4 °C overnight. This suspension was then centrifuged at 10,000 rpm for 10 min at 4 °C and the pellet obtained was washed with DW repeatedly until its pH became neutral. This colloidal chitin was used to make 0.2 % colloidal chitin agar plates (Appendix I). To check the chitinase activity, individual hypersaline bacteria were streaked on these agar plates and incubated at 28 ± 2 °C. After 7 days, the plates were flooded and incubated with 1 % congo red solution for 20 min and washed with 1 M NaCl solution. The results were recorded when a zone of clearance against a pink-red background was seen.

b) Glucanase

Individual hypersaline bacteria were spot inoculated on 0.2 % laminarin agar plates (Appendix I) and incubated at 28 ± 2 °C for 7 days. Subsequently the plates were flooded with 1 % congo red solution and incubated for 20 min. After washing with 1 M NaCl solution, the observations were recorded when a zone of clearance against a pink-red background was seen.

c) Amylase

Individual hypersaline bacteria were spot inoculated on 0.2 % starch agar plates (Appendix I) and incubated at 28 ± 2 °C for 2 days. The plates were flooded with Lugol's iodine solution (Appendix II), decanted and incubated in 1 M NaCl solution overnight at 4 °C. The observations were recorded when a zone of clearance against a blue-black background was seen.

d) Cellulase

Individual hypersaline bacteria were spot inoculated on 1 % Carboxymethyl cellulose agar plates (Appendix I) and incubated at 28 ± 2 °C for 2 days. The plates were flooded and incubated with 1 % congo red solution for 20 min,

subsequently washed with 1 M NaCl solution and the observations were recorded when a zone of clearance against a pink-red background was seen.

e) Protease

Individual hypersaline bacteria were spot inoculated on 1 % casein agar plates (Appendix I) and incubated at 28 ± 2 °C for 2 days. The plates were flooded with 20 % Trichloroacetic acid for 2 min and observed for a zone of clearance against a white opaque background.

f) Lipase

Individual hypersaline bacteria were spot inoculated on 1 % tributyrin agar plates (Appendix I) and incubated at 28 ± 2 °C until the appearance of growth. The plates were held against natural light and observed for opaque zones against a clear background.

3.8.1.3 Formation of unregulated waste products

Next the hypersaline bacteria were evaluated for their ability to form toxic waste products *viz.* ammonia and hydrogen cyanide according to the protocol given by Sahu et al. (2020).

a) Ammonia

The hypersaline bacteria were inoculated in 10 mL peptone water broth (Appendix I) and incubated at 28 ± 2 °C on a shaker incubator at 120 rpm. After 48 h incubation, 0.1 mL of culture supernatant was obtained by centrifuging the culture broth at 10,000 rpm for 10 min. The supernatant obtained was further diluted with DW to make the final volume to 5 mL. To this 0.5 mL of Rochelle's salt solution (Appendix II) was added followed by 0.5 mL of Nessler's reagent (Appendix II). Orange-brown coloration or precipitate formation was an indicator of a positive ammonia test.

b) Hydrogen cyanide (HCN)

Sterile glass petri-plates (40 mm) with 15 mL ZMA media supplemented with 4.4 g/L glycine were prepared, where the inner side of the lid of the petri-plates was lined with sterilized Whatman No. 1 paper. Hypersaline bacteria were streaked individually onto these plates, followed by the impregnation of the

Whatman paper with a solution containing 2 % sodium carbonate in 0.5 % picric acid. The plates were then sealed with parafilm and incubated in an upright position at 28 ± 2 °C for 48 h. A colour change in the Whatman paper to orange-red indicated HCN production.

3.8.1.4 Assessing plant growth promoting attributes

Growth promoting attributes were analyzed as part of mixed path antagonism, wherein a BCA impart the host plant with traits which boost the plant's growth and help in disease suppression. The hypersaline bacteria were tested for their ability to solubilize nutrients, fix atmospheric nitrogen and produce indole-3-acetic acid and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Kayasth et al., 2014; Pirhadi et al., 2016; Chandrakala et al., 2019; Sahu et al., 2020; Shet & Garg, 2022).

a) Solubilization of nutrients (phosphate, zinc, potassium and silicate)

The qualitative estimation of nutrient solubilisation was carried out by plate assays on specific media containing insoluble forms of mineral nutrients. For phosphate solubilisation, hypersaline bacteria were spot inoculated onto Pikovskaya's agar plates (Appendix I) and incubated at 28 ± 2 °C for 7 days. Similarly for Zinc, Potassium and Silicate solubilisation, hypersaline bacteria were spot inoculated on Zinc Solubilizing medium (Appendix I), Aleksandrow medium (Appendix I) and Modified Bunt and Rovira medium (Appendix I) agar plates respectively. After the incubation period, the zone of clearance was measured and the results were recorded as the solubilization index.

b) Production of indole-3-acetic acid (IAA)

Each hypersaline bacteria was inoculated separately in 40 mL ZMB supplemented with 0.1 % tryptophan and incubated on a shaker incubator (120 rpm) at 28 ± 2 °C for 48 h. The culture suspension was then centrifuged at 5000 rpm, 4 °C for 10 min to obtain supernatant. To 2 mL of the supernatant in a test tube, 2 drops of 85 % orthophosphoric acid and 4 mL Salkowski reagent (Appendix II) was added and the tubes were incubated at room temperature in dark for 30 min. After the incubation period, a color change from colourless to light pink indicated a positive test.

c) Production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase

The ability of hypersaline bacteria to use ACC as a sole nitrogen source was assessed by spot inoculating each isolate on Dworkin-Foster salts medium with 2.0 % agar (Appendix I) supplemented with 3 mM ACC or with 2 g/L ammonium sulphate as in case of growth control. These plates were then incubated at 28 ± 2 °C for 48 h and the growth was observed.

d) Fixation of atmospheric nitrogen

Hypersaline bacteria were spot inoculated on Jensen's (N free) medium agar plates (Appendix I) and incubated at 28 ± 2 °C for 10 days and the growth was observed.

3.8.1.5 Production of siderophores

Universal chrome azurol S (CAS) agar plates were prepared to test the ability of the hypersaline bacteria to produce iron-binding siderophores. To 25 mL of CAS solution prepared by adding 30.25 mg CAS in DW, 5 mL of iron (III) solution (1 mM $FeCl_3.6H_20$, 10 mM HCl) was added. To this mixture, 20 mL of cetrimonium bromide (CTAB) solution (prepared by adding 36.45 mg CTAB in DW) was slowly added with constant stirring to avoid any froth formation. This CAS reagent mixture was filter sterilized and 10 mL of it was added to 90 mL of sterile M9 Minimal Medium Salts (Appendix I) supplemented with 0.5 % glucose. The pH of this minimal salt mixture was maintained at 6.8 using PIPES buffer (3.24 g PIPES in the same solution). The media was solidified using 1.5 % agar and hypersaline bacteria were spot inoculated. After 7 days of incubation at 28 ± 2 °C, the plates were observed for the formation of orange halo around the colonies which indicated siderophore production (Chaiharn et al., 2009).

3.8.1.6 Production of exopolysaccharide (EPS)

To determine the EPS secretion, hypersaline bacteria were streaked on ZMA plates supplemented with 10 % sucrose and 0.01 % congo red dye. The plates were incubated at 28 ± 2 °C for 48 h and were observed for black coloration of the colonies which marked the EPS production by the bacteria (Chaudhari et al., 2017).

3.8.2. Quantitative estimation of mixed path antagonism and indirect antagonism tests

As part of mixed path antagonism, all the hypersaline bacteria which showed positive results for chitinase, glucanase, amylase, cellulase, protease and lipase were tested quantitatively to determine the enzyme activity of each of the above mentioned enzymes. Similarly quantitative estimation of ammonia, siderophore and IAA was also carried out by spectrophotometric analysis. Finally the amount of solubilized phosphate, zinc and silicate in the media was determined.

3.8.2.1 Estimation of lytic enzyme activity

Quantitative estimation of amylase, cellulase, protease and lipase produced by hypersaline bacteria MPSK 14, MPSK 20, MPSK 22, MPSK 23, MPSK 109, MPSK 186 and SK 473 was carried out using the protocol given by Fernandes et al. (2019). Additionally chitinase and glucanase produced by MPSK 22, MPSK 23 and MPSK 186 was also estimated (Suryadi et al. 2014).

a) Chitinase

One unit of chitinase activity was defined as the amount of enzyme that released 1 µmole of N-Acetylglucosamine (NAGA) equivalent per min (Appendix III). Measurement of chitinase activity was carried out using 0.5 mL of 0.2 % colloidal chitin (dissolved in 0.05 M Phosphate buffer (pH 5.2) (Appendix II)) and 0.5 mL of crude enzyme. This reaction mixture was incubated at 50 °C for 20 min. The resulting NAGA was analyzed using the DNSA (DiNitro Salicylic Acid) method wherein to the reaction mixture 3 mL DNSA solution (Appendix II) was added and the reaction was terminated by placing the tubes in a boiling water bath (100 °C) for 10 min. After boiling, the tubes were cooled and absorbance was read at 575 nm in a spectrophotometer (Schimadzu UV spectrophotometer UV-1800, Shimadzu Corporation, Japan).

b) Glucanase

One unit of glucanase activity was defined as the amount of enzyme that released 1 µmole of glucose equivalent per minute under assay conditions (Appendix III). Cell free supernatant (0.5 mL) was added to 0.5 mL of 0.2 % laminarin prepared in 1 M acetate buffer (pH 5.0) (Appendix II), mixed and

incubated at 50 °C for 20 min. The resultant glucose was measured using the DNSA method and the absorbance recorded at 550 nm.

c) Amylase

One unit of amylase activity was defined as the amount of enzyme that released 1 µmole of maltose equivalent per minute under assay conditions (Appendix III). Cell free supernatant (0.5 mL) was added to 0.5 mL of 1 % starch prepared in 0.05 M phosphate buffer (pH 7.0) (Appendix II), mixed and incubated at 50 °C for 20 min. The resultant glucose was measured using the DNSA method and the absorbance noted at 540 nm.

d) Cellulase

One unit of cellulase activity was defined as the amount of enzyme that released 1µmole of glucose equivalent per minute under assay conditions (Appendix III). Cell free supernatant (0.5 mL) was added to 0.5 mL of 1 % carboxymethyl cellulose prepared in 0.5 M citrate buffer (pH 4.8) (Appendix II), mixed and incubated at 50 °C for 20 min. The resultant glucose was measured using the DNSA method and the absorbance noted at 540 nm.

e) Protease

One unit of protease activity was defined as the number of micromoles of tyrosine released per min. A calibration curve was prepared using Bovine serum albumin as a standard solution (Appendix III) to measure the concentration of tyrosine released according to Bradford (1976). Protease activity was measured by using 1 % casein as the substrate. The cell free supernatant solution (0.5 mL) was incubated with 0.5 mL of 1 % casein at 50 °C for 20 min. To 0.5 mL of the above substrate-crude enzyme mixture, 5.5 mL Coomassie Brilliant Blue G-250 solution (Appendix II) was added and after 10 min of incubation at room temperature the absorbance was read at 595 nm.

f) Lipase

One unit was defined as the amount of enzyme liberating 1 µmole of p-nitrophenyl per minute under the standard assay conditions (Appendix III).

Lipase activity was determined using p-nitrophenyl palmitate (p-NPP) as a substrate according to Winkler & Stuckmann (1979), with some modifications. The substrate p-NPP dissolved in 2-propanol was mixed with 0.05 M Tris-HCl buffer (pH 8.0) (Appendix II) containing gum acacia (0.1 %), Triton X-100 (0.6 %) to give final concentration of 1 mM. The reaction was carried out at 70 °C by adding 100 µL of cell free supernatant solution to 1200 µL of substrate solution after pre-incubation for 5 min. Incubation was continued for 10 min after adding 500 µL of Sodium carbonate solution (0.1 M) to stop the reaction. The amount of p-nitrophenol (p-NP) released was measured at 410 nm against a blank and comparing to the standard calibration curve of P-NP.

3.8.2.2 Estimation of ammonia

Ammonia estimation test was carried out as mentioned in section 3.8.1.3 a. The absorbance of the orange/brown-coloured complex formed was measured at 450 nm using a spectrophotometer. The concentration of ammonia produced was estimated using the ammonium chloride standard curve (Appendix III) (Roychowdhury et al., 2019).

3.8.2.3 Quantifying plant growth-promoting attributes

Solubilisation of nutrients and production of IAA was measured as part of plant growth-promoting attributes following the protocols of Kamran et al. 2017; Roychowdhury et al. 2019 and Chandrakala et al. 2019.

a) Solubilization of nutrients (phosphate, zinc and silicate)

Hypersaline bacteria with the positive qualitative phosphate solubilisation test were grown in Pikovskaya's broth for 5 days at 28 ± 2 °C on a shaker incubator at 120 rpm. The culture supernatant obtained (0.1 mL) by centrifuging the broth at 10,000 rpm for 5 min, was diluted with DW to make the final volume of 25 mL. To this 0.5 mL of acidified ascorbic acid (Appendix II) and 0.5 mL of mixed reagent (Appendix II) were added. The reaction mixture was incubated for 20 min and the absorbance was recorded at 810 nm. A standard graph of Potassium dihydrogen phosphate (Appendix III) was prepared following the same protocol to determine the amount of phosphate solubilized by the bacteria.

For estimation of silicon, hypersaline bacteria were grown in Modified Bunt and Rovira broth for 5 days at 28 ± 2 °C on a shaker incubator at 120 rpm and 0.1 mL of culture supernatant obtained in the manner as mentioned above, was diluted with DW to make the final volume to 25 mL. To this 1 mL of mixed reagent (Appendix II) was added and kept for reacting for 15 min after which 1 mL of 10 % oxalic acid and 0.5 mL of 2.8 % ascorbic acid was added and absorbance was measured at 810 nm after 30 min. A standard graph of Sodium hexafluorosilicate (Appendix III) was prepared following the same protocol to determine the amount of silicate solubilized by the bacteria.

For estimation of zinc solubilisation, the hypersaline bacteria were grown in Zinc Solubilizing medium at 28 ± 2 °C on a shaker incubator at 120 rpm. After 7 days of incubation, the culture supernatant obtained by centrifuging the broth at 10,000 rpm for 5 min was diluted 100 times. Total soluble zinc content was estimated in the culture supernatant using atomic absorption spectrophotometry (4210- MP AES, Agilent Technologies, Australia) and was expressed in ppm.

b) Production of indole-1-acetic acid (IAA)

The IAA estimation was carried out as mentioned in section 3.8.1.4 b and the absorbance of the pink-coloured reaction mixture was recorded at 530 nm after the 30 min incubation in the dark. The amount of IAA produced by each hypersaline bacteria were then determined from the standard graph of pure IAA prepared in acetone (Appendix III).

3.8.2.4 Percentile siderophore production

Bacteria were inoculated in the minimal salt mixture (section 3.8.1.5) and were incubated at 28 ± 2 °C for 7 days on a shaker incubator at 120 rpm. Cell-free supernatant (0.5 mL) obtained by centrifuging the culture broth at 10,000 rpm for 10 min was mixed and incubated with 0.5 mL of CAS reagent mixture (section 3.8.1.5) for 5 min. A mixture of the uninoculated minimal salt mixture and CAS reagent was kept as control (Kumar et al., 2017). After the change in colour of the reaction mixture to orange, the absorbance of the mixture was measured at 630 nm and percent of siderophore production was calculated using the formula:

$$\% \text{ Siderophore units} = (Ar-As)/Ar;$$

Where, Ar = Absorbance of control and As = Absorbance of sample

3.8.3. Screening for genes involved in the lipopeptide synthesis

Antibiosis through the production of antifungal compounds is a part of mixed path antagonism exhibited by a BCA. The class of antifungal lipopeptides produced by the hypersaline bacteria was determined by the PCR analysis. Based on the results of the previous tests (section 3.8.1 and 3.8.2), three hypersaline bacteria (MPSK 22, MPSK 23 and MPSK 186) were selected for the screening of the lipopeptide synthesis genes through Polymerase chain reaction (PCR) analysis. Genomic DNA of the three bacteria MPSK 22, MPSK 23 and MPSK 186 was isolated using the HiPurA^{TM} Bacterial Genomic DNA Purification Kit (HiMedia, India). This DNA was then used as a template for PCR amplification of biosynthetic genes responsible for the production of fengycin A, B, C, D; lichenysin A, B, C; iturin A, C, D and surfactin. Specific PCR primer pairs were selected based on literature study for the detection of the genes mentioned in Table 2. PCR reaction mixture (25 µL) used contained 12.5 µL of 2X PCR Taq mix (BioLit™ Taq Mix (2X)), 10 µL of nuclease-free water, 1 µL of template DNA and 0.5 µL of each forward and reverse primer (0.5 µM each). PCR amplifications were carried out in a PCR thermal cycler (Sure cycler 8800, Agilent Technologies, Singapore) as per the reaction conditions given in Table 3. A total of 30 cycles of amplification were run for each gene after which, 10 µL of each sample along with a 50 kb DNA ladder (HiMedia, MBT084) was loaded onto 2 % Agarose gel and visualized with an ultraviolet illuminator (UV transilluminator, Fotodyne incorporated, USA).

3.8.4. Testing for direct antagonism

Direct antagonism was studied through microscopic analysis of the interaction between the bacterial cells and fungal hyphae. Change in morphology of the mycelia of fungal cultures *F.solani*, *R.solani* and *M.phaseolina*, after interacting with the hypersaline bacteria MPSK 22, MPSK 23 and MPSK 186 were studied by both light microscopy and scanning electron microscopy (SEM). Hypersaline bacterial and fungal cultures were co-inoculated as in the dual culture assay protocol (section 3.6) with coverslips in the interaction zone (in case of test) or at the growing edge of the fungal cultures (in case of the control). After the growth of the fungus, the coverslips were picked and studied via microscopy techniques. For light microscopy, the mycelia were stained by Lactophenol cotton blue (Appendix II) and was observed at 100 X magnification under a light microscope (Lynx X52-N107T, Lawrance & Mayo, India).

For SEM analysis, the coverslips with the mycelium were fixed overnight at 4 °C in 2.5 % glutaraldehyde followed by washing with sterile phosphate buffered saline (PBS, Appendix II) till the glutaraldehyde was washed off. This was followed by dehydration through a series of ethanol (10, 30, 50, 70, 80, 90 and 100 % (v:v)) with 10 min for each concentration. Finally, the samples were air-dried and mounted onto stubs, coated with gold (SC7620 Mini sputter coater, Quorum technologies, UK) and the photomicrographs were captured at 1-5 K magnification under SEM (Evo 18, Carl Zeiss, Germany) (Mallikarjunaswamy & Noushad 2021).

3.9 Identification of salt pan bacteria by classical and molecular approach

The three hypersaline bacteria MPSK 22, MPSK 23 AND MPSK 186 were further characterized morphologically and biochemically and identified tentatively as described in Bergey's Manual of Systematic Bacteriology. Further the molecular analysis of the bacteria was undertaken to confirm its identity Claus 1986; Holt et al. 1994).

3.9.1 Morphological characterization

For preliminary identification: colony morphology, Gram character and SEM analysis of the hypersaline bacteria were carried out. Bacteria were streaked individually on ZMA and the characteristics of the individual colonies were noted. Further the bacterial cells were Gram stained according to Hans Christian Gram (Coico 1997) and cell morphology was determined. To determine the size of the bacterial cells by scanning electron microscopy, freshly grown bacterial culture (10 µL) was spread onto a clean glass slide (1 cm^2), air dried and fixed with 2.5 % glutaraldehyde overnight at 4 °C. The next day, the glutaraldehyde was washed off with sterile PBS followed by successive dehydration with 10, 30, 50, 70, 80, 90 and 100 % acetone (v:v) for 10 min each. The slides were subsequently attached to a stub, sputter coated with gold and viewed under a SEM with magnification from 15 K to 40 K (Bratbak 2018).

Table 2: Specific PCR primer pairs for the identification of lipopeptide biosynthetic genes

Sr. No.	Compound	Gene	Primer Sequence	Product length	Reference
1.	Surfactin	*Sfp*-f *Sfp*-r	5'-ATG AAG ATT TAC GGA ATT TA-3' 5'-TTA TAA AAG CTC TTC GTA CG-3'	675	Hsieh et al., 2004
2.	Iturin	*ItuD*-f *ItuD*-r	5'-ATG AAC AAT CTT GCC TTT TT-3' 5'-TTA TTT TAA AAT CCG CAA TT-3'	1203	Hsieh et al., 2004
		ItuC-f *ItuC*-r	5'-GGC TGC TGC AGA TGC TTT AT-3' 5'-TCG CAG ATA ATC GCA GTG AG-3'	423	Hsieh et al., 2004
		ItuA-f *ItuA*-r	5'-GAT GCG ATC TCC TTG GAT GT-3' 5'-ATC GTC ATG TGC TGC TTG AG-3'	647	Kumar et al., 2017
3.	Fengycin	*FenA*-f *FenA*-r	5'-GAC AGT GCT GCC TGA TGA AA-3' 5'-GTC GGT GCA TGA AAT GTA CG-3'	964	Athukorala et al., 2009
		FenB-f *FenB*-r	5'-CAA GAT ATG CTG GAC GCT GA-3' 5'-ACA CGA CAT TGC GAT TGG TA-3'	670	Płaza et al., 2015
		FenC-f *FenC*-r	5'-CCG CAA GAC TGA GAA ATA-3' 5'-CGA CGA CCA AAT GAT GAA TG-3'	964	Ramarathn-am et al., 2007
		FenD-f	5'- GGC CCG TTC TCT	964	Ramarathn-

		FenD-r	AAA TCC AT-3’ 5’-GTC ATG CTG ACG AGA GCA AA-3’		am et al., 2007
4.	Lichenysin	*LicA*-f *LicA*-r	5'-GTG CCT GAT GTA ACG AAT-3' 5'- CAC TTC CTG CCA TAT ACC-3'	735	Madslein et al., 2013
		LicB-f *LicB*-r	5'- TGA TCA GCC GGC CGT TGT CT-3' 5'- GGC GAA TTG TCC GAT CAT GT-3'	904	Madslein et al., 2013
		LicC-f *LicC*-r	5'- GCC TAT CTG CCG ATT GAC-3' 5'- TAT ATG CAT CCG GCA CCA-3'	1195	Madslein et al., 2013

Table 3: PCR amplification conditions for the screening of individual lipopeptide biosynthetic genes

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
	(Temperature °C / Time sec)				
Sfp	94 / 240	94 / 60	46 / 30	72 / 60	72 / 300
ItuA	95 / 300	94 / 60	50 / 60	72 / 60	72 / 300
ItuC	94 / 240	94 / 60	58 / 60	70 / 60	70 / 300
ItuD	94 / 240	94 / 60	50 / 60	72 / 90	72 / 300
FenA	94 / 240	94 / 60	60 / 30	72 / 105	72 / 300
FenB	94 / 240	94 / 60	60 / 30	72 / 105	72 / 360
FenC	94 / 240	94 / 60	62 / 30	72 / 105	72 / 360
FenD	95 / 240	94 / 60	58 / 60	70 / 60	70 / 300
LicA	94 / 240	94 / 60	60 / 30	72 / 50	72 / 300
LicB	94 / 240	94 / 60	60 / 30	72 / 50	72 / 300
LicC	94 / 240	94 / 60	57 / 30	72 / 50	72 / 300

3.9.2 Biochemical characterization

For biochemical analysis, sulphur and nitrate reduction, indole and enzymes (oxidase, catalase, gelatinase, and urease) production, citrate and O-nitrophenylbeta-D-galactopyranoside (ONPG) utilization and Methyl Red-Voges Proskauer (MR-VP) tests were conducted. In addition, the oxygen requirement of the hypersaline bacteria was also assessed. Further the utilisation of an array of carbohydrates by the bacteria was checked using KB009 HiCarbo™ kits.

a) Sulfur reduction, Indole Production and Motility (SIM) Test

SIM Media (Appendix I) was used to test the motility of the hypersaline bacteria along with the sulfide reduction and Indole formation. Sterile SIM media was poured in a test tube and allowed to solidify in a vertical position to obtain a butt. This media was then inoculated individually using a sterile stab and incubated at 28 ± 2 °C. After 48 h, the tubes were checked for bacterial growth or black precipitate formation. Additionally, ring formation was observed after the addition of Kovac's Reagent (Appendix II) to test indole production.

b) Nitrate Reduction Test

Nitrate broth (Appendix I) was inoculated with bacterial cell suspension (50 µL) and incubated for 48 h at 28 ± 2 °C on a shaker incubator at 120 rpm. After the incubation period, a few drops of 0.8 % Sulphanilic acid (Appendix II) and α-Naphthylamine Reagent (Appendix II) were added and was the broth medium was observed for color change to pink.

c) Oxidase Test

To a sterile Oxidase disc (HiMedia DD018), pure colony of the hypersaline bacteria was applied individually with a help of a sterile toothpick. Upon the contact with the disc, immediate change in color of disc from white to purple was observed as an indicator of positive oxidase test.

d) Catalase Test

Using a sterile toothpick, purified colonies of hypersaline bacteria were picked and suspended into a drop of 3 % Hydrogen Peroxide placed on a clean glass slide. Effervescence formation was looked for immediately as an indicator of the positive result.

e) Urease Test

Sterile 40 % urea solution was added to the autoclaved Urea Agar Base (Appendix I) and was solidified in a slanting position. These slants were inoculated with hypersaline bacteria and incubated for 48 h at 28 ± 2 °C. Color change of the medium from yellow-orange to magenta (pink) was observed after incubation period.

f) Gelatinase Test

Sterile gelatin media (Appendix I) was inoculated with 50 µL of bacterial cell suspension in a test tube and incubated at 28 ± 2 °C. After 48 h of incubation, the media tubes were kept at 4 °C for 30 min and checked for solidification or liquefaction of the media.

g) Citrate Utilization Test

Hypersaline bacteria were streaked onto Simmon Citrate Agar (Appendix I) slants and incubated at 28 ± 2 °C. After 72 h of incubation, the color change of the medium from green to blue was observed.

h) ONPG Test

Emulsion of the pure colony of hypersaline bacteria was prepared in a clean glass vial containing 0.85 % sterile saline and an ONPG disc (HiMedia DD008). After incubation of the vial at 28 ± 2 °C for 48 h, color change of the saline from colorless to yellow was noted.

i) Methyl Red - Voges Proskauer (MR-VP) Test

Bacterial cell suspension (50 µL) was inoculated individually in 5 mL of Methyl-Red Voges- Proskauer Broth (Glucose Phosphate Broth, Appendix I) and incubated on a shaker incubator at 120 rpm. After 48 h of incubation at 28 ± 2 °C, the broth was divided equally and poured into two different sterile vials. In one vial, few drops of Methyl red indicator (Appendix II) were added and the change of colour from colorless to red or yellow was noted for Methyl Red Test. To the second vial, a few drops of Barrit's Reagent A (Appendix II) and Barrit's Reagent B (Appendix II) were added and the formation of stable pink colour was observed as an indicative of positive Voges Proskauer test reaction.

j) Oxygen requirement

Two thirds of a sterile screw-capped glass test tube was filled with autoclaved Anaerobic agar (Appendix I) and mixed with 0.5 mL of the bacterial culture suspension. The tubes were then tightly capped and incubated at 28 ± 2 °C for 5 days and growth pattern of the hypersaline bacteria was studied in the tube.

k) Carbohydrate Utilization

Carbohydrate utilization pattern was studied using KB009 HiCarbo™ kits (HiMedia KB009A/ KB009B1/ KB009C) which contained a total of 35 carbohydrates. In each tubule of the kit strip, individual bacterial cell suspension (50 µL) was inoculated and incubated at 28 ± 2 °C. After 48 h the change in colour of the media in each tubule was noted and the utilization profile was determined as per the manufacturer's instruction manual (HiMedia, India).

3.9.3 Molecular characterization

The genomic DNA of the hypersaline bacteria was extracted using HiPurA Bacterial Genomic DNA purification kit (HiMedia, India) according to the instruction manual. The 16S rRNA of each bacterium was amplified using the isolated DNA as the template by PCR using universal bacterial primers 27F (Forward): AGAGTTTGATCCTGGCTCCAG and 1492R (Reverse): TACGGTTACCTTGTTACGACTT. The PCR reaction mixture contained 20 µL nuclease-free water, 3 µL template, 1 µL forward primer, 1 µL reverse primer and 25 µL PCR Taq mix (BioLit™ Taq Mix (2X)). PCR was carried out with an initial denaturation of 5 min at 95 °C followed by 30 cycles of 95 °C for 1 min; 54 °C for 1 min and 72 °C for 1 min. A final extension was performed at 72 °C for 10 min. The PCR product of around 1500 bp thus obtained was sequenced (G3500 Genetic Analyzer, Applied Biosystem, Hitachi Ltd., Japan) and the obtained raw sequences were used to assemble the contigs using DNA baser software. These contigs were then matched with the GenBank database using NCBI-BLAST and the phylogenetic tree (Maximum likelihood tree) was constructed using MEGA11 software with 1000 bootstrap replicates. The sequences of the three hypersaline bacteria were deposited in GenBank and accession number was obtained.

3.10 Safety evaluation of salt pan bacteria

A primary evaluation of the safety of the hypersaline bacteria MPSK 22, MPSK 23 and MPSK 186 was assessed by blood hemolysis test wherein the hemolytic activity of the bacteria on human erythrocytes was analyzed. The individual hypersaline bacteria were also screened for their susceptibility to various antibiotics.

3.10.1 Blood hemolysis test

The above mentioned hypersaline bacteria were streaked on plates containing Blood agar base (Appendix I) supplemented with 6 % human sterile blood. The plates were incubated at 28 ± 2 °C for 48 h and zone of clearance around the colonies were noted. Slight destruction of erythrocytes producing a green zone around the bacterial colony indicated α- hemolysis. A clear hemolysis zone around the bacterial colony indicated β – hemolysis, while no change in agar around the colony was recorded as γ – hemolysis (Chang et al., 2000). *Vibrio* sp. and *Streptococcus* sp. cultures were used as positive control which shows β and α hemolysis respectively.

3.10.2 Antibiotic sensitivity test

The susceptibility of the hypersaline bacteria was assayed according to the Kirby-Bauer disk diffusion method (Vivekanandhan et al. 2002). The antibiotic containing discs (HiMedia) were placed on Muller Hinton agar (Appendix I) plates previously swabbed with the individual bacterial culture suspension. The antibiotics tested were Amikacin (30 µg), Amoxyclav (30 µg), Ampicillin:Sulbactam (20 µg), Cephalexin (30 µg), Cephalothin (30 µg), Cephotaxime (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Co-trimoxazole (25 µg), Clindamycin (2 µg), Doxycycline hydrochloride (30 µg), Erythromycin (15 µg), Gentamycin (10 µg), Kanamycin (30 µg), Levofloxacin (5 µg), Lincomycin (10 µg), Nalidixic acid (30 µg), Neomycin (30 µg), Nitrofurantoin (300 µg), Ofloxacin (5 µg), Streptomycin (10 µg), Tetracyclin (30 µg), Tobramycin (10 µg) and Vancomycin (30 µg). The diameters of the zone of inhibition were measured after incubating the plates at 28 ± 2 °C for 24 h. The strains were then categorized as sensitive (S), intermediate (I) or resistant (R) as recommended by the Clinical and Laboratory Standards Institute (CLSI, USA, 2013).

3.11 Influence of the selected salt pan bacteria on *in-vitro* germination of chilli seeds

Chilli seeds of two different cultivars *viz.* Khola and Sitara were used in this study which was procured from Don Bosco College of Agriculture - Sulcorna, Goa. The effectiveness of the bioagents (MPSK 22, MPSK 23 and MPSK 186) was assessed against *F. solani*, as this pathogen contributes majorly to the yield loss of the chilli plant. For the preparation of fungal spore suspension, *F. solani* culture was inoculated onto a PDA slant and was incubated at 25 ± 2 ºC. After 5 days of incubation, the slant was flooded with sterile 0.01 % tween 20 solution and spores were dislodged in this solution by scraping the mycelia with help of a nichrome loop. The obtained solution was then filtered through muslin cloth and individual spores were counted using a hemocytometer (Kuske et al. 1998). The spore suspension was adjusted to the count of 10^6 spore/mL and was used for further experiments.

3.11.1 Bioagent - Biopolymer compatibility assessment

Three polysaccharides i.e. sodium alginate (SA), carboxymethylcellulose (CMC) and gum acacia (GA) were selected based on the literature as adjuvants for coating the chilli seeds with the cells of the bioagents MPSK 22, MPSK 23 and MPSK 186. These biopolymers were prepared in concentrations of 0.5, 1.0, and 1.5 % (w:v) in DW and were sterilized for 20 min. Hypersaline bacteria MPSK 22, MPSK 23 and MPSK 186 were inoculated individually in ZMB and incubated at 28 ± 2 ºC. After 48 h, the culture broth was centrifuged at 10,000 rpm for 10 min and the bacterial pellet collected was washed twice with sterile PBS. Subsequently, the cell pellet obtained was re-suspended in a biopolymer solutions and vortexed vigorously to obtain a uniform suspension. Cell pellet re-suspended in PBS was used as control. The initial viable count of the bioagent cells in this suspension was determined by serial dilution in 0.85 % saline and spread plating on ZMA and was expressed as colony forming units (CFU/mL). These mixed suspensions were then incubated for 24 h at 28 ± 2 ºC and the change in CFU/mL was recorded (Chin et al., 2022). The concentration of biopolymer that enabled the highest increase in the viable count of the bioagent was considered as the most compatible biopolymer and chosen for subsequent assessments.

3.11.2 Coating efficacy of biopolymer in seed biopriming

After determining the compatible type and concentration of biopolymer with the bioagent cells, the coating efficacy of that particular biopolymer was calculated in accordance with Chin et al. (2022). To achieve this, chilli seeds (Khola and Sitara) of uniform size and shape which did not show any visible deformity were handpicked. These were then dipped in tap water and seeds which floated on top were discarded. Seeds which settled down were transferred to the LAF and dipped in sterile DW followed by dipping in 70 % ethanol for 60 sec. Seeds were then surface sterilized using 1 % sodium hypochlorite solution for 1 min and were finally rinsed thoroughly with sterile DW. Bioagent–biopolymer suspension was prepared and its viable count was enumerated as mentioned in the above section (3.11.1). The air-dried seeds were then bioprimed by imbibing the seeds in this suspension for 1 h. The coated seeds were then removed out, air-dried in the LAF for 15 min and then vigorously vortexed in 1 mL sterile PBS to release the coated bacterial cells. The viable count of the bacterial cells in this suspension was determined and enumerated as CFU/mL. The coating efficacy of the biopolymer was calculated using the formula:

$$\text{Percentage of bacteria trapped (E \%)} = \log (C_i\text{-}C_v/C_i) \times 100;$$

Where, C_i: initial CFU; C_v: CFU after 1 h

3.11.3 Effect of bioagent - biopolymer on seed germination and growth

To check the effect of bioagent – biopolymer suspension on seed germination and growth, the chilli seeds of both the cultivars were first surface sterilized (as mentioned in section 3.11.2). Seeds were now bioprimed with the bioagent – biopolymer suspension for 1 h and allowed to air dry. Seeds coated with sterile PBS and 1 % GA were kept as control. After air drying, the seeds were then exposed to *F. solani* spore suspension prepared earlier (3.11) for 1 h. The exposed bioprimed seeds of each set (total of 15 seeds each of MSPK 22 + 1 % GA, MPSK 23 + 1 % GA, MPSK 186 + 1 % GA and PBS + 1 % GA) were then allowed to germinate using the paper towel method in a growth chamber. The chamber was moistened every day with sterilized DW and germination and growth of the seedlings were studied over a period of 30 days (Kumar et al., 2011). The Number of seeds germinated every day was noted down and Germination Percentage, Germination Energy and Mean Germination Time were calculated using the given formulas. Additionally, the shoot and root length of

the germinated Khola and Sitara seedlings was also measured with the help of a scale (expressed in cm) after 30 days and Vigour Index was calculated using this data.

Germination Percentage (GP %) = (Number of seeds germinated / total number of seeds) × 100

Germination Energy (GE %) = (Number of seeds germinated on day 15 / total number of seeds) × 100

Mean Germination Time (MGT day) = $\sum(n \times d) / N$

Where, n = number of seeds germinated on each day, d = day of germination from the beginning of the test, and N = Sum number germinated

Vigour Index (VI) = Seedling length × Germination percentage

3.12 Effect of carbon and nitrogen sources, salinity, pH and temperature on growth of the salt pan bacterium MPSK 23

Based on the results of the germination tests, hypersaline bacterium MPSK 23 was selected for further studies. Bacterial cell suspension (100 µL of 10^{10} CFU/mL) of MPSK 23 was inoculated in the media with varying carbon and nitrogen sources, concentrations of sodium chloride (NaCl) and pH and was incubated on an orbital shaker at 120 rpm at different temperatures. The growth of the bacterium was assessed after 48 h of incubation by recording the absorbance at 600 nm and uninoculated media was used as blank (Vasanthakumari, 2009). The carbon and nitrogen sources were selected based on literature and availability.

3.12.1 Effect of carbon sources

The effect of different carbon sources (Trehalose, Dextrose, Galactose, Sucrose, Mannitol and Arabinose) on the growth of MPSK 23 were studied at 1 % (w:v) concentration. Each carbon source (1 %) was added to the basal media containing 0.5 % Peptone and 2 % NaCl concentration with the pH of the media maintained at 7.6 ± 0.2. MPSK 23 was inoculated and incubated at 28 ± 2 ºC for 48 h.

3.12.2 Effect of nitrogen sources

The effect of different nitrogen sources (Casein, Peptone, Tryptone, Potassium nitrate, Ammonium sulphate and Ammonium chloride) on the growth of MPSK 23 was

studied at 1 % (w:v) concentration. Each nitrogen source (1 %) was added to the basal media containing 0.5 % Sucrose and 2 % NaCl concentration with the pH of the media maintained at 7.6 ± 0.2. MPSK 23 was inoculated and incubated at 28 ± 2 ℃ for 48 h.

3.12.3 Effect of salinity

The effect of the NaCl on the growth of MPSK 23 was assessed at varying concentrations of NaCl in the basal media (containing 1 % peptone and 1 % sucrose, pH 7.6 ± 0.2) ranging from 0 to 20 % (w:v) with increments of 4 units. MPSK 23 was inoculated and incubated at 28 ± 2 ℃ for 48 h.

3.12.4 Effect of pH

The tolerance of MPSK 23 towards pH was studied by varying the pH of the basal medium (containing 1 % peptone, 1 % sucrose and 2 % NaCl). The pH of the medium was adjusted from 2.0 to 14.0 with increments of 2 units. MPSK 23 was inoculated and incubated at 28 ± 2 ℃ for 48 h.

3.12.5 Effect of temperature

The tolerance of MPSK 23 towards various temperatures was studied by varying the incubation temperature after inoculation of MPSK 23 in the basal medium (containing 1 % peptone, 1 % sucrose and 2 % NaCl, pH 7.6 ± 0.2). The temperatures tested were 5, 10, 20, 30, 40, 50 and 60 ℃.

3.13 Continuous assessment of growth and antifungal activity of MPSK 23

The growth profile of the hypersaline bacterium MPSK 23 was studied over a period of 48 h. The bacterium was inoculated in ZMB and incubated at 37 ± 2 °C on an orbital shaker at 120 rpm and the initial absorbance was noted at 600 nm. The culture broth (2 mL) was withdrawn aseptically every 2 h and absorbance was measured at 600 nm (Zwietering et al., 1990). Simultaneously, the antifungal activity against *F. solani* was studied every 2 h by the well diffusion assay (previously described in 3.8.1.1 b).

3.14 Growth and viability of MPSK 23 in soil

For studying the growth of the MPSK 23 bacterium in the soil, agricultural soil was obtained from Green Essentials, Socorro, Goa and was autoclaved at 15 Psi for 1 h, consecutively for three days. Portions (100 g) of sterile soil were then placed in sterile 150 mL milk dilution bottles and inoculated with 1.0 mL of 10^{10} CFU/mL cell suspension of MPSK 23. The soil was moistened everyday with sterile DW and the bottles were held at 37 ± 2 °C (Acea et al, 1988). Viable count of MPSK 23 in the soil was estimated by the spread plating technique for a period of 9 weeks.

3.15 *In-vitro* assessment of the growth of *F. solani* in presence of MPSK 23

To determine the inhibitory effect of varying cell concentrations and volumes of the bacterial cell suspension of MPSK 23 on the growth of *F. solani*, co-inoculation of the bacterium and the fungi was carried out in the liquid media (Swain et al., 2008).

3.15.1 Preparation of cell suspension of MPSK 23

Cell suspensions of MPSK 23 were prepared by inoculating the bacterial isolate in ZMB and incubating at 37 ± 2 °C on a shaker incubator at 120 rpm. After 48 h, the culture broth was centrifuged at 10,000 rpm for 10 min and cell pellet was collected. This pellet was washed twice with sterile PBS and the viable count was determined by serial dilution. A standard graph of CFU/mL v/s Optical density at 600 nm of the bacterial suspension was prepared. The final count was adjusted to 10^4 to 10^{10} CFU/mL as required, by diluting with PBS in accordance with the standard graph.

3.15.2 Co-inoculation of *F. solani* and MPSK 23

In Erlenmeyer flasks containing 50 mL of PDB; one plug (8 mm) of *F. solani* from the growing edge of PDA containing the fungi was inoculated and incubated at 25 ± 2 °C at 120 rpm on a shaker incubator. After 24 h of incubation; 5 mL, 10 mL, 15 mL, 20 mL and 25 mL of cell suspension of MPSK 23 of viable count 10^4, 10^6, 10^8 and 10^{10} were added individually to these flasks and incubated at 37 ± 2 °C for more 4 days. After incubation, the broth was filtered through the pre-weighed Whatman filter paper No. 1 and the fungal mass was collected. The fresh weight of this mass was measured using an electronic weighing balance (Quintix 224-101N, Sartorius Lab Instruments,

Germany) and was expressed in grams. Further, the dry weight (g) of the fungal mass was determined after drying the filter paper with the fungal mass in a preheated oven at 60 ºC for 6 h. Weight of sterile PDB and inoculated only with fungus were kept as controls. The weight of the bacterial inoculum was also determined and subtracted from the final fungal mass weight. From these weights, the percentage inhibition of *F.solani* by MPSK 23 cell suspension was determined using the formula:

$$\text{Inhibition (\%)} = [(\text{Weight in control} - \text{Weight in test}) / \text{Weight in control}] \times 100$$

3.16 *In-vivo* pathogenicity testing of *F. solani* in Khola and Sitara chilli cultivar

To prove the pathogenicity of the isolated *F. solani* fungi to the chilli cultivars Khola and Sitara, an experiment was set up in accordance with the protocol given by Nelson & Toussoun (1989) with some modifications.

3.16.1 Preparation of sick soil

Sick soil was prepared by mixing the agricultural soil with sorghum flour (25 g/ kg soil) followed by autoclaving at 15 psi for 1 h consecutively for 3 days. To this amended sterile soil, spore suspension of *F. solani* was added (5 mL of 10^6 spores/mL suspension per kg of soil) and the soil was kept covered with a plastic sheet for 5 days. The soil was moistened everyday with sterile DW for boosting the growth of the pathogen.

3.16.2 Inoculation and re-isolation of *F. solani* from chilli plants

Chilli seeds of both varieties were surface sterilized as mentioned in section 3.11.2 and germinated. After germination, 15 days old seedlings were uprooted and incisions were made on the roots of the seedlings using a scalpel. These seedlings were then dipped in spore suspension of *F. solani* (prepared as mentioned in section 3.11) for 30 min and were transplanted in plastic pots (8 inches) filled with 2 kg sick soil with two drainage holes at the bottom. Plants dipped in sterile DW were kept as control. These plants were then transplanted in sterilized agricultural soil and allowed to grow. Continuous assessment of the growth of the plants and disease (wilt) appearance was done over a period of 15 days. On the appearance of the wilt symptoms, the pathogen was re-isolated from diseased plants as mentioned in Section 3.4. Further to prove

Koch's postulate the isolated fungus from the diseased plants was identified morphologically by lactophenol cotton blue staining. The experiment was repeated thrice, each time with 5 seeds of both varieties to ensure the pathogenicity of *F. solani* to Khola and Sitara varieties of chilli.

3.17 *In-vivo* testing of MPSK 23 as a BCA against *Fusarium* wilt in chilli plants

In-vivo pot studies were conducted at Don Bosco College of Agriculture-Sulcorna, Goa, to evaluate the potential of the hypersaline bacterium MPSK 23 in controlling the *Fusarium* wilt and boosting the growth of chilli plants. Experiments were carried out with two varieties of chilli (*Capsicum annuum* L.): a local dry red chilli variety, Khola and a commercial fresh green chilli variety, Sitara. Two separate pot culture experiments were conducted; 1st set in the months of February – March 2021 and the 2nd was in January – June 2022. In the first trial, defense enzymes expressed in plants after pathogen exposure and treatment application was estimated and second the trial focused on monitoring the growth of the plant. In both the experiments, disease assessment was carried out throughout the period. The agricultural soil used for both the experiments was procured from Green Essentials, Socorro, Goa and its properties were checked at the soil testing laboratory of Don Bosco College of Agriculture-Sulcorna, Goa. The soil was then thoroughly sieved and mixed with sand and farmyard manure to give a final ratio of 2:1:1 (soil: sand: manure). For exposing the chilli plants to *F. solani*, sick soil was prepared as mentioned in section 3.16.1 and then mixed with the soil and manure. Plastic pots (size-8 inch diameter) with two drainage holes at the bottom were filled with 2 kg of this soil mixture each and were used for the experiments.

3.17.1 Experiment I: Assessment of the efficacy of MPSK 23 in regulation of defense enzymes and phenols in chilli plants against *F. solani*

This experiment was carried out for a period of 15 days and the disease severity, total length and expression of defense enzymes in the leaves of the plants were assessed (Sundaramoorthy et al., 2012; Zagade et al., 2012, Rais et al., 2017).

3.17.1.1 Experimental details

Crop plant: *Capsicum annuum* L.

Variety: Khola and Sitara

Treatments: Four

Replications: Five

Design: Completely Randomized Design (CRD)

3.17.1.2 Treatment details and preparation

A total of four treatments were used in this experiment. First treatment (T1) was a negative control wherein sterile 1X PBS was used as treatment. The second treatment, a commercially available powdered form of biocide *Trichoderma* (T2) along with the third treatment, a commercial fungicide Trophy (T3); were used as positive controls. These were prepared in sterile DW to give the final concentration of 2 %. Final treatment (T4) was the 10^{10} CFU/mL cell suspension of the bacterial isolate MPSK 23 which was used as the test bioagent. Cell suspension of MPSK 23 was prepared as mentioned in section 3.15.1 to get the desired final viable count.

Table 4: Description of the treatments used for pot trial (Experiment I)

Treatment Code	Treatment Detail
T1	Buffer Control (PBS)
T2	Biological Control (*Trichoderma*)
T3	Chemical Control (Carbendazim 12 % + Mancozeb 63 %)
T4	Test culture (MPSK 23)

3.17.1.3 Pathogen exposure and treatment application

Trials were carried out on a 15 day old seedling of both the varieties. The seedlings were exposed to the *F. solani* spores as mentioned in section 3.16.2 prior to treatment application, followed by the treatment application by root dip and soil drenching method. Roots of the seedlings were dipped in 10 mL of treatments for 1 h followed by pouring the 10 mL of same treatments in each pot at the time of transplant as soil drenching application. One seedling was transplanted in each pot with sick soil mixture and five pots were kept under each

treatment. Each pot was watered daily in the evening to maintain desired moisture in the soil and was kept under natural conditions of light.

3.17.1.4 Observations recorded

After carrying out the experiment, the following observations were recorded. Also the correlation between the observations recorded was also determined using the statistical software.

a) Disease severity in chilli plants

For assessing the wilt caused by *F. solani* in the chilli plants, disease severity was studied over a period of 15 days in the leaves of the plants under the four treatment groups. Wilting was assessed on 0 - 5 scale with zero representing no sign of wilting and 5 representing complete wilting and death of the plant. Disease severity ratings were categorized as follows:

0 No symptom
1 <25 % leaves show symptom
2 26-50 % leaves show symptom
3 51-75 % leaves show symptom
4 76-100 % leaves show symptom
5 Dead plant

On day 0, each and every plant of the four treatment groups were categorized in accordance with the above ratings and number of plants falling under each rating were counted. This was repeated on day 3, day 6, day 9 and day 15. From this data, Disease Severity Index (DSI) was calculated using the formula:

$$DSI = \{[(n\times0) + (n\times1) + (n\times2) + (n\times3) + (n\times4) + (n\times5)] / [t\times N]\};$$

Where n: number of plants in each rating, t: total number of plants, N: maximum rating.

Using DSI, Disease severity (%) in each treatment group was calculated using the formula:

$$\text{Disease severity (\%)} = DSI \times 100$$

b) Total length of the chilli plants

To check the effect of pathogen on growth, the plants of each treatment were uprooted at the end of the 15 days and washed carefully in running tap water, then with DW and the surface moisture was absorbed on a cushion of blotting

paper. The plant length (root length + shoot length) was measured with the help of a scale and expressed in centimeters.

c) Estimation of plant defense enzymes and total phenolic content (TPC)

Induced defense mechanism involves the production of reactive oxygen species, phytoalexins, phenolic compounds, or an enormous number of enzymes involved in plant defense, like polyphenol oxidase, β-1,3-glucanase, chitinase, phenylalanine ammonia lyase, peroxidase which get stimulated due to BCA application after the pathogenic infection (Kaur et al. 2022). To check the stimulation of these compounds, leaves from the lower nodes of plants were randomly collected from each treatment at day 0, 5, 10 and 15 after pathogen challenge for the analysis. The collected leaf samples were washed under running tap water, dried on blotting paper and used as the sample for enzyme / phenol extraction.

i. Phenylalanine ammonia-lyase (PAL) assay

PAL (EC 4.1.3.5) activity was assayed by a method devised by Brueske (1980). Leaf sample (0.1 g) from each treatment was homogenized uniformly at 4 ºC in 2 mL of 0.1 M borate buffer (pH 7.0) (Appendix II) containing 1.4 mM mercaptoethanol and 1.0 mM Polyvinylpyrrolidone, followed by centrifugation (Lab-i-fuge C series, Lab India Instruments, India) at 13,000 rpm at 4 ºC for 15 min. The supernatant obtained acted as an enzyme source. The reaction mixture consisted of 200 µL of enzyme extract, 500 µL of 0.2 M borate buffer (pH 8.7) (Appendix II) and 1.3 mL of water. To this 1 mL of 0.1 M L-phenylalanine (pH 8.7) was added to instigate the reaction followed by incubation at 32 ºC for 30 min. The reaction was terminated by the addition of 0.5 mL trichloroacetic acid (1 M). Enzyme activity was measured by the formation of transcinnamic acid at 290 nm and was expressed in terms of Activity U/min/g fresh weight (FW).

ii. Peroxidase (PO) assay

PO (EC 1.11.1.7) activity was assayed according to the method of Hammerschmidt et al. (1982) with minor alteration. The leaf sample (0.1 g) was homogenized separately from each treatment in 2 mL of 0.1 M phosphate buffer (pH 7.0) (Appendix II) at 4 ºC followed by centrifugation

at 13000 rpm at 4 ºC for 15 min. The supernatant obtained acted as an enzyme source. The reaction mixture comprised of 1.5 mL pyrogallol (0.05 M), 0.5 mL H_2O_2 (1 % v:v) and 0.05 mL enzyme extract. A reaction mixture without enzyme extract served as control. Change in the absorbance was measured at 420 nm for 5 min at every 30 sec intervals and enzyme activity was expressed as change in the Activity U/min/g FW.

iii. *Polyphenol oxidase (PPO) assay*

PPO (EC 1.14.18.1) activity was determined according to Gauillard et al. (1993). The leaf sample (0.1 g) was homogenized in 2 mL of ice cold 0.1 M phosphate buffer (pH 6.5) (Appendix II). The mixture obtained above was centrifuged at 13,000 rpm for 30 min at 4 ºC and the supernatant thus served as an enzyme source. The reaction mixture comprised 0.4 mL of enzyme extract and 0.4 mL catechol (1 mM) in 3 mL of 0.05 M phosphate buffer (pH 6.5) (Appendix II). The control consisted of a reaction mixture without any enzyme extract. Absorbance was recorded at 405 nm at 30 sec intervals for 5 min and PPO enzyme activity was expressed as a change in Activity U/min/g FW.

iv. *Total phenol content (TPC) assay*

Total of 0.1 g of leaf sample from each treatment was homogenized in 5 mL of 95 % ethanol followed by incubation for 48 h. This was then centrifuged at 13,000 rpm for 10 min and the supernatant was obtained. To 1 mL of supernatant, 1 mL of 95 % ethanol, 0.5 mL of 50 % Folin-Ciocalteau reagent (HiMedia RM10822) and 5 mL of sterile DW were added, followed by thorough mixing of the content. After incubation of 5 min, 1 mL of 5% sodium carbonate was added followed by incubation for 1 h and the colour developed in the reaction mixture was determined by recording the absorbance at 725 nm. A Standard curve for this assay was prepared using various concentrations of gallic acid dissolved in 95 % ethanol. Absorbance values were then converted and expressed as TPC U/min/g FW (Zheng & Shetty, 2000).

3.17.2 Experiment II: Efficacy of MPSK 23 in suppressing the wilt and boosting the growth of chilli plants against *F. solani*

This experiment was carried out for a period of 98 days. In this experiment assessment of wilt was done in all the treatment groups and the growth parameters of the plants were also studied (Etebarian et al., 2000; Poornima, 2011; Ahmed et al., 2022).

3.17.2.1 Experimental details

Crop plant: *Capsicum annuum* L.

Variety: Khola and Sitara

Treatments: Five

Replications: Nine

Design: Completely Randomized Design (CRD)

3.17.2.2 Treatment details and preparation

In addition to the four treatments mentioned in Table 4, one more set of plants not exposed to *F. solani* was also maintained. This set was denoted as T5 and was given no treatment. Rest all the treatments were prepared in a similar manner as described earlier in section 3.16.2.2.

Table 5: Description of the treatments used for pot trial (Experiment II)

Treatment Code	Treatment Detail
T1	Buffer Control (PBS)
T2	Biological Control (*Trichoderma*)
T3	Chemical Control (Carbendazim 12 % + Mancozeb 63 %)
T4	Test culture (MPSK 23)
T5	Unexposed

3.17.2.3 Pathogen exposure and treatment application

Inoculation of the pathogen *F. solani* and treatment application was done as previously described in section 3.16.2.3. However, here the treatments were re-applied at 45 and 90 days after planting. Each treatment had three seedlings in different pots and the treatments were replicated thrice (total 9 pots) in a Completely Randomized Design (CRD).

3.17.2.4 Observations recorded

Following observations were recorded from the Experiment II and correlation between disease incidence (DI), disease suppression, disease severity, number of leaves and the plant height was established using the statistical software.

a) Disease assessment in chilli plants

Wilting disease was assessed based on the incidence of disease and the suppression of disease in different treatment groups. In the groups which showed the incidence of wilting, the severity of the disease was assessed. Finally, the biocontrol ability also termed as the Protective value of the Biological control (T2), Chemical control (T3) and the Test culture MPSK 23 (T4) against *F. solani* was evaluated.

i. Disease incidence and disease suppression

Disease incidence and disease suppression was assessed at the end of a month after transplanting the seedlings, in the different treatment groups. The plants were observed for disease development after pathogen exposure and the number of plants that showed wilting symptoms was counted. From this data, disease incidence (DI) was calculated using the formula:

$$DI (\%) = (\text{Number of infected plants} / \text{Total number of plants}) \times 100$$

From the DI values, Disease suppression in each treatment group was also calculated by subtracting the DI values from 100.

ii. Disease severity and Protective Value

Severity of wilting was also monitored for three months after transplanting with an interval of 15 days and Disease severity (%) was calculated as described previously in section 3.16.2.4 a. Disease severity (%) values were used to derive the protective value three treatments groups (T2, T3 and T4) using the formula:

$$\text{Protective Value} = [(\text{Disease severity of control- Disease severity of test}) / \text{Disease severity of control}] \times 100$$

b) Analysis of growth parameters

Along with the disease assessment, analysis of the growth of the plants in all treatment groups was also carried out.

i. *Total plant height and number of leaves*

The total plant height and the number of leaves were studied continuously for a period of 98 days with an interval of 14 days after transplantation. The height of the plant was measured in cm from the base of the plant (ground level) to the tip of the plant's main axis after stretching the plant with the help of a meter scale and was expressed in cm. The total number of leaves (both symptomatic and non-symptomatic) were also counted and noted down.

ii. *Root and Shoot length and weight (wet and dry)*

For determining the length and weight (dry and wet) of the root and shoot, sampling was done randomly on day 7, day 35, day 63 and day 98 by sacrificing a plant. No data was recorded in a treatment group where there was no survival of any plant. One plant from each treatment was carefully uprooted, washed carefully in running tap water, then with DW and the surface moisture was absorbed on a cushion of blotting paper. The morphological growth parameter i.e. total plant length (root length + shoot length) was measured with the help of a scale and expressed in cm. The same plant was used for determining the physiological growth parameters (wet and dry weight). The fresh (wet) weight of the roots and shoot was measured by weighing them on an electronic balance and the value was expressed in grams. After measuring the fresh weight, plant samples were kept in a pre-heated hot air oven at 60 ℃ for 6 h and the dry weight was recorded in grams. Weight of roots and shoot were combined to obtain the total weight of the plant.

3.18 Assessing the shelf-life of MPSK 23

Bacterial isolate MPSK 23 was inoculated in ZMB and incubated at 37 ± 2 °C on a shaker incubator at 120 rpm. After 48 h, the pellet obtained by centrifuging the broth at 10,000 rpm for 10 min, was washed twice with sterile PBS and subjected to lyophilization (ScanVac Coolsafe Lyophilizer, Labogene, Denmark) to obtain the powdered form. The shelf life of this powder was studied at room temperature (28 ± 2 °C) and refrigerator temperature (4 ± 2 °C) after every 7 days for one month and then every 30 days for 12 months. The viable count (CFU/mL) of the lyophilized cells was

estimated by spread plating technique after re-suspended it in sterile tap water and 1 % gum acacia (Bartakke, 2018).

3.19 Statistical analysis of data

Statistical analysis was performed by using IBM© SPSS version 23.0 statistical software (IBM Corporation, USA, 2015). The results were expressed as mean with their corresponding standard deviation (SD). The data wherever applicable was log transformed to improve linearity prior to analysis. The results of screening for antifungal activity (n=3), germination tests (n=5), *in-vitro* inhibition of *F. solani* by MPSK 23 (n=3), pot trials (n=5, n=9) and shelf-life study (n=3) were subjected to one-way analysis of variance (ANOVA) followed by Levene‘s test to check homogeneity of variance. Subsequently, post-hoc with Duncan’s multiple range test (DMRT) was used to assess the significant difference between the treatment groups. The data were considered to be statistically significant at $p < 0.05$. Pearson's correlation analysis was used to study the correlation between the different parameters during the pot trials.

Chapter 4

Results

4.1 Location and physico-chemical parameters of the sampling site

The location of the three salt pans *viz*. Batim, Agarwado and Ribandar (two sampling sites from each) are tabulated below, giving the geographical coordinates along with the physico-chemical parameters of the water overlying the salt pan ponds from where the samples were collected for the isolation of the hypersaline bacteria (Table 6).

Table 6: Geographical co-ordinates and physico-chemical parameters of the sampling sites

Sr. No.	Saltpan	Site	Geographical coordinates	Temp (ºC)	TDS (ppt)	DO (mg/L)	pH	Salinity (psu)
1	Batim	S1	N15º27'29.6"E 073º52'50.4"	**37.1** ± 2.5	**90.84** ± 2.1	**9.17** ± 0.3	**7.68** ± 0.05	**60.5** ± 0.3
		S2	N15º27'29.9"E 073º52'49.0"	**37.8** ± 3.2	**149.3** ± 4.3	**7.85** ± 0.7	**8.30** ± 0.01	**98.5** ± 0.5
2	Ribandar	S1	N15º29'57.7"E 073º50'49.6"	**36.7** ± 1.8	**94.24** ± 1.4	**4.48** ± 0.9	**7.29** ± 0.02	**62.07** ± 0.8
		S2	N15º29'58.0"E 073º50'49.5"	**36.2** ± 1.3	**175.3** ± 2.2	**4.43** ± 0.6	**8.63** ± 0.04	**102.6** ± 1.0
3	Agarwado	S1	N15º38'36.6"E 073º45'56.4"	**30.1** ± 3.1	**64.04** ± 0.9	**5.93** ± 0.4	**7.66** ± 0.03	**52.25** ± 0.4
		S2	N15º38'30.4"E 073º45'56.2"	**33.1** ± 2.5	**139.6** ± 3.2	**4.61** ± 0.1	**8.90** ± 0.02	**86.52** ± 0.3

Each value is the mean ± Standard deviation (SD) of the triplicates (n=3)

Plate 1: Ribandar salt pan

4.2 Bacteria from salt pan water and sediment

A total of 89 bacterial colonies were isolated from three different salt pans. The visualized trend of the number of bacterial isolates obtained from each salt pans was Agarwado > Batim > Ribandar; where the highest number was from Agarwado salt pan (32) followed by Batim (29) and Ribandar (28). Figure 2 shows the abundance of the bacterial isolates in water and sediment samples collected from each salt pan.

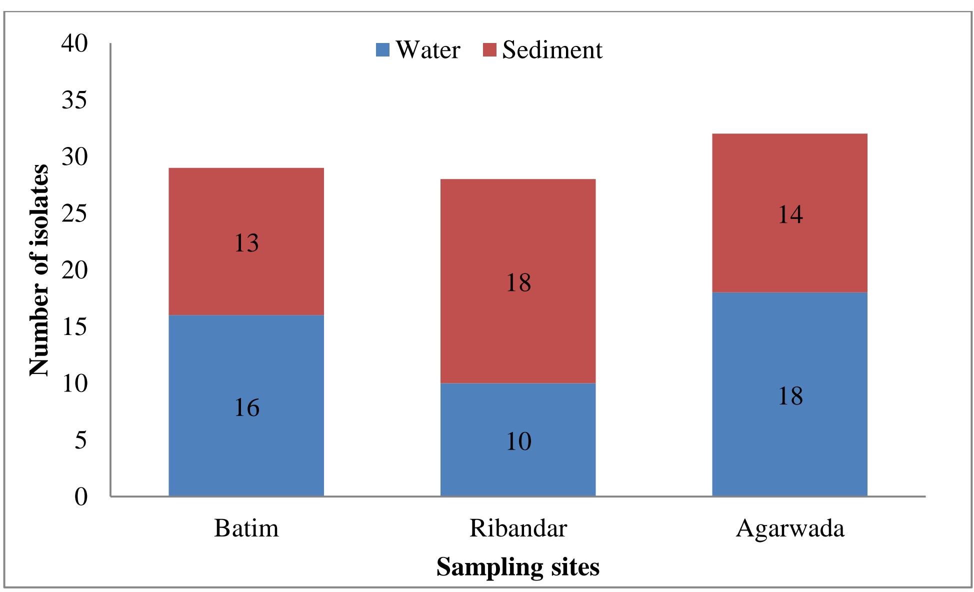


Figure 2: Abundance of bacteria in the salt pans

4.3 Critical examination of the diseased chilli plants for isolation of fungi

The infected chilli plants showed the symptoms like stunted planted growth, drooping of foliage, yellowing and loss of turgidity of the leaves and discoloration of the roots and lower portions of the stem. Thirty diseases plant samples revealing the above symptoms were uprooted from different fields of Goa and sectioned for the isolation of the fungus. Plate 2 (A and B) depicts the plant samples exhibiting the root rotting and wilting symptoms selected for the study.

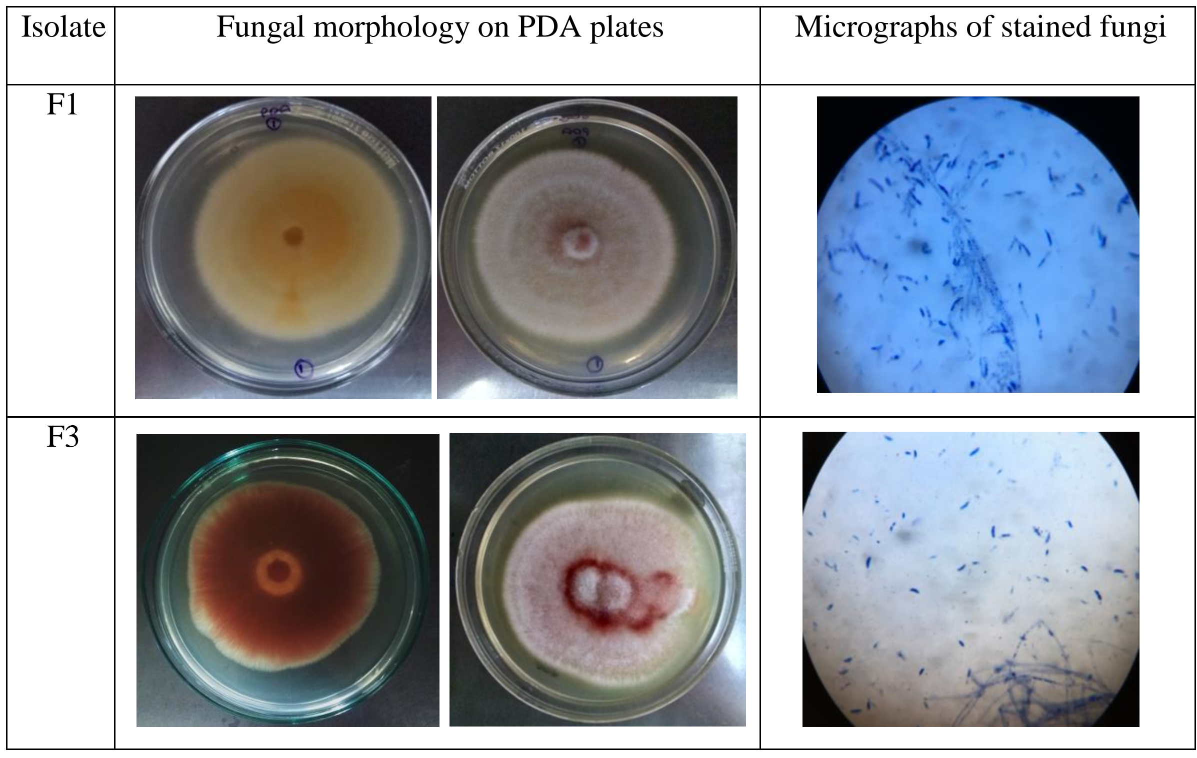
Plate 2: Plant samples uprooted from fields showing symptoms of root rotting (A) and wilting (B)

4.4 Fungal isolates obtained from the diseased chilli plant samples

After inoculation of the sections from the stem and root regions of the diseased chilli plants, fungal mycelia appeared on PDA plates from day 2 till day 7 as seen in plate 3. A total of 14 fungi were isolated from different fields of Goa (4 from Bicholim, 3 from Saligao, 2 from Valpoi, 3 from Margaon and 2 from Khola). Isolates were morphologically distinguished from each other based on the mycelial growth period (time taken to cover petriplates, i.e., slow or fast), colour of the mycelia after growth (front colour of mycelium and on the reverse of the petriplates) and spores formation (if formed, shape and size of the spores and if not, appearance of any specialized structures) under *in-vitro* conditions. Based on these characters, five morphologically

different isolates were selected for further studies (Plate 4). Fungal isolate F1 showed white mycelia on PDA plate and light orange coloration on the reverse of the petriplates with concave spores under the microscope. Similar spores were produced by F3 and F5 isolates; however the colour of the mycelia varied. F3 isolate formed light pink mycelia with purple coloration on the reverse side of the plate and F5 formed white mycelia with orange-brown coloration. In case of fungal isolates F6 and F14, no spores were observed. F6 isolate formed a hard compact mycelial structure with black color on both the sides, whereas F14 which initially formed white mycelia eventually got covered with black sclerotia like structures.

Plate 3: Isolation of fungus from the root and the stem sections



Isolate	Fungal morphology on PDA plates	Micrographs of stained fungi
F1		
F3		

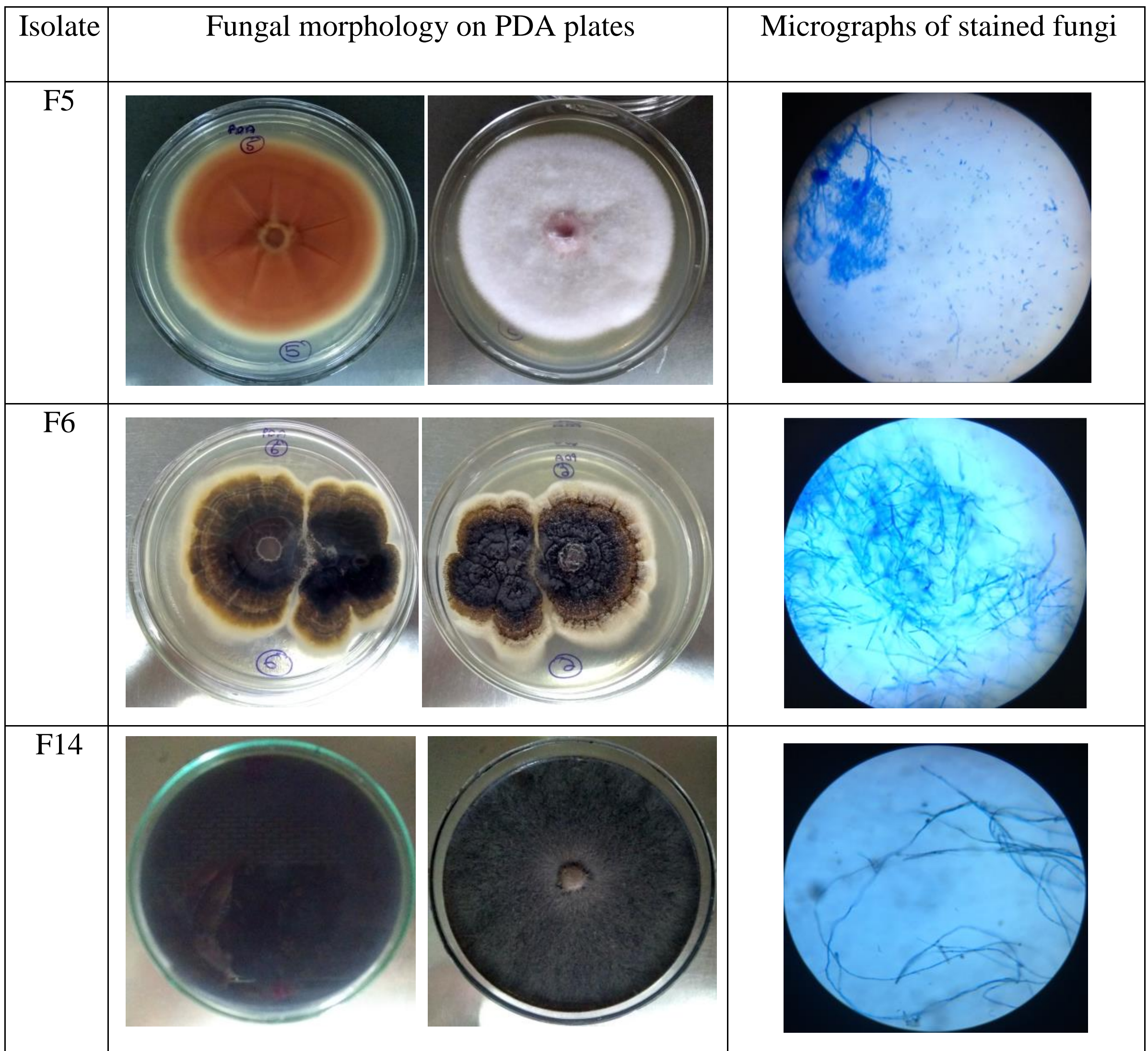


Plate 4: Morphology and microphotographs of stained fungi on PDA isolated from diseased chilli plants

4.5 Characterization of the fungal isolates obtained

Based on the growth period, morphological variations and the spores; the five fungi which were isolated from the diseased chilli plants were identified as *Fusarium solani, Fusarium oxysporum, Fusarium chlamydosporum, Boeremia exigua* and *Macrophomina phaseolina.* Further these cultures were deposited in Indian type culture collection (ITCC) and registration number (featuring in the IX Edition) was obtained as seen in Table 7. This morphological characterization of isolates *Fusarium solani* (F1)*, Fusarium oxysporum* (F3) and *Macrophomina phaseolina* (F4) which have been previously reported as chilli plant pathogens; was supported by the sequencing of the ITS region. The sequences obtained were then submitted to GenBank and accession number was obtained. The phylogenetic tree obtained by

sequence analysis of ITS region of the three isolates and the sequences of 11 other fungal species obtained from NCBI, GenBank is given below in Figure 3.

Table 7: Morphological identification of the fungal isolates with the deposition number

Isolate Code	Identification	ITCC number
F1	*Fusarium solani*	8299
F3	*Fusarium oxysporum*	8302
F5	*Fusarium chlamydosporum*	8300
F6	*Boeremia exigua*	8301
F14	*Macrophomina phaseolina*	8303

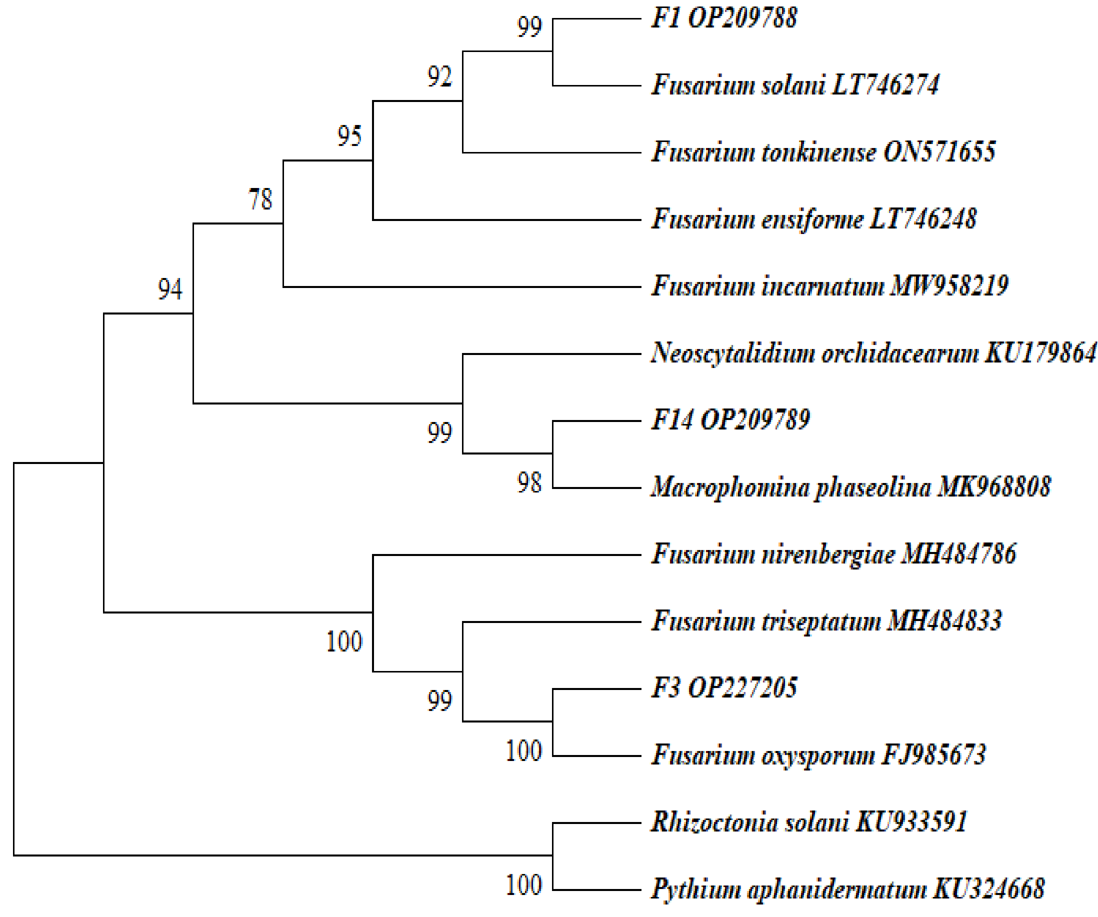


Figure 3: The phylogenetic tree obtained by sequence analysis of the ITS region of fungal isolates with 1000 bootstrap replicates

4.6 Antifungal activity of salt pan bacteria

A total of 76 out of 197 hypersaline bacteria (89 bacteria isolated in this study and 108 from culture collection) which were tested for their antifungal activity by dual culture method, were inhibitory towards at least one of the six tested fungal pathogens. Thirty of these hypersaline bacteria were isolated from water samples, 25 were from sediment samples and 21 from biofilm samples as depicted in Figure 4. Maximum number of hypersaline bacteria (60) obtained from all the three sources, showed inhibitory activity against *M. phaseolina,* followed by 40 hypersaline bacteria which were antagonistic to *F. solani, F. oxysporum, F. pallidoroseum* and *R. solani* and the least number of isolates (38) inhibited *P. aphanidermatum* (Figure 5). From these, 22 hypersaline bacteria inhibited all the six fungal pathogens tested. Percentage inhibition of the fungal mycelia exhibited by these hypersaline bacteria is depicted in Table 8. Isolates BGUM 14B, ABSK 9, MPSK 9, MPSK 14, MPSK 20, MPSK 22, MPSK 23, MPSK 109, MPSK 186 and SK 473 showed > 40 % mycelial inhibition of all the six fungal pathogens. The highest 60 ± 0.5 % inhibition of *F. pallidoroseum* was by SK 473; MPSK 109 inhibited *R. solani* by 65.9 ± 1.2 % and *F. oxysporum* by 60.5 ± 1.5 % and MPSK 23 showed the maximum inhibition of *P. aphanidermatum* (61 ± 1.1 %), *F. solani* (59.38 ± 1.5 %) and *M. phaseolina* (72.5 ± 0.7 %).

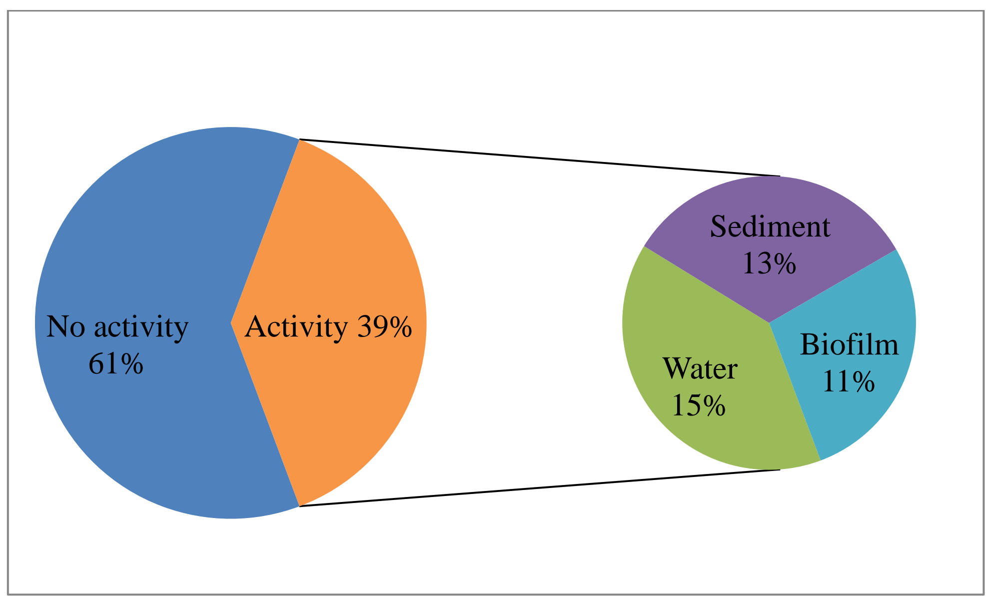


Figure 4: Hypersaline bacteria showing antifungal activity by Dual culture assay

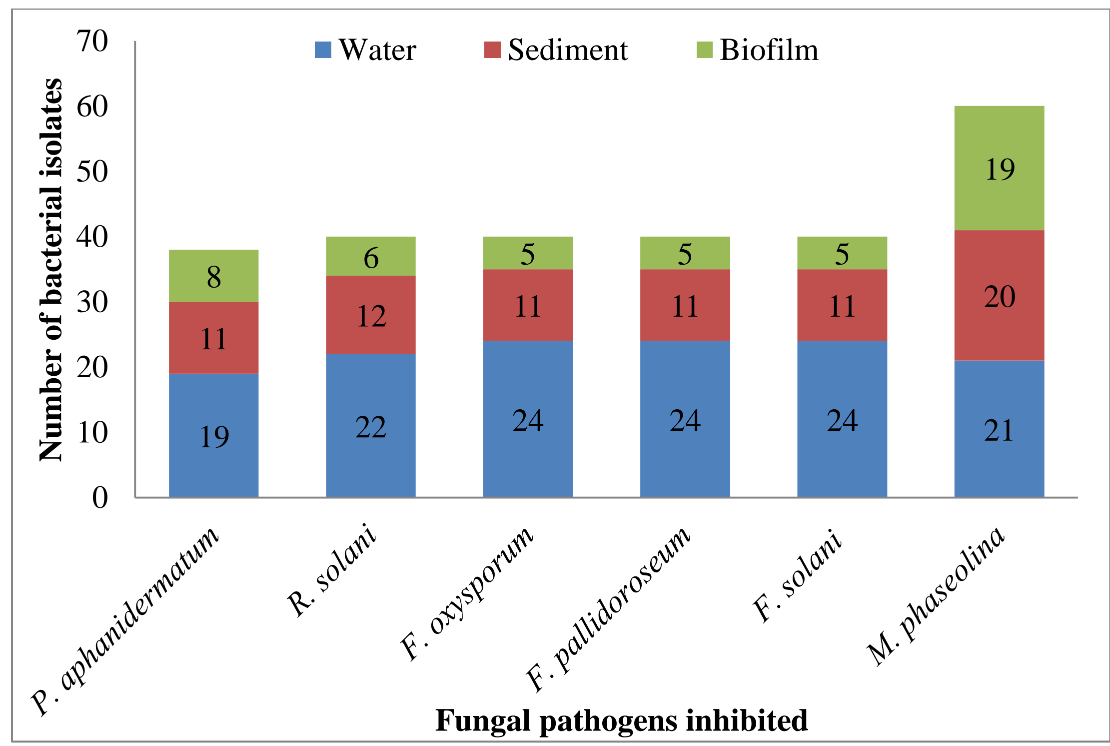


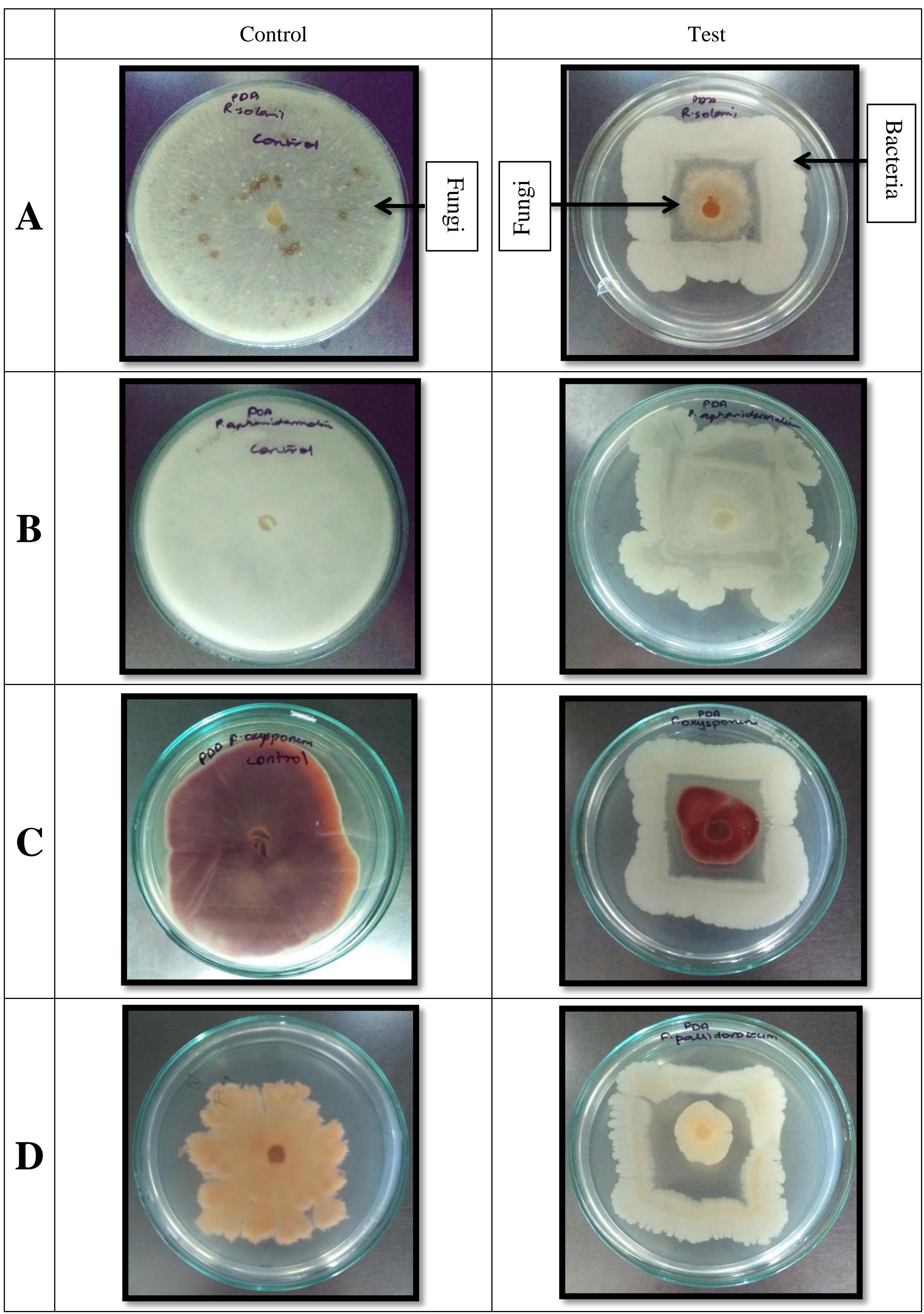
Figure 5: Hypersaline bacteria showing inhibition of the fungal pathogens

Table 8: Percentage inhibition of the fungal mycelia by the hypersaline bacteria

Isolates	**Inhibition (%)**					
	R. solani	***P. aphanidermatum***	***F. oxysporum***	***F. pallidoroseum***	***F. solani***	***M. phaseolina***
BGUM 14B	**54.3** ± 1.6^{gh}	**52.2** ± 1.3^{ghi}	**47.4** ± 1.8^{ef}	**49.0** ± 0.3^{d}	**56.25** ± 0.4^{g,h}	**67.5** ± 0.5^{j}
BGUM 93	**47.0** ± 1.3^{cd}	**44.0** ± 1.9^{b}	**42.1** ± 1.3^{d}	**40.0** ± 1.5^{c}	**56.25** ± 1.0^{g,h}	**30.0** ± 0.2^{b}
BGUM 136	**47.2** ± 2.2^{cd}	**46.9** ± 2.3^{bcd}	**42.1** ± 0.8^{d}	**32.0** ± 1.6^{a}	**40.25** ± 1.5^{c}	**12.5** ± 1.8^{a}
BGUM 256	**47.6** ± 1.3^{de}	**44.4** ± 2.5^{b}	**47.4** ± 2.0^{ef}	**32.0** ± 2.2^{a}	**40.25** ± 0.7^{c}	**50.15** ± 1.3^{g}
BGUM 359	**44.5** ± 0.5^{c}	**45.0** ± 0.5^{b}	**42.1** ± 1.3^{d}	**32.0** ± 1.3^{a}	**37.25** ± 1.7^{c}	**45.25** ± 0.6^{e,f}
BGUM 370	**36.0** ± 0.5^{b}	**45.5** ± 1.5^{bc}	**47.4** ± 0.9^{ef}	**40.0** ± 0.7^{c}	**45.15** ± 0.9^{d}	**33.35** ± 1.4^{b}
BGUM 440	**45.0** ± 2.2^{cd}	**44.4** ± 2.0^{b}	**34.2** ± 1.7^{b}	**36.0** ± 0.6^{b}	**35.21** ± 2.1^{b}	**48.25** ± 0.5^{f,g}
SK 473	**61.6** ± 2.3^{ij}	**54.0** ± 1.5^{ij}	**54.2** ± 1.8^{hi}	**60.0** ± 0.5^{h}	**53.13** ± 1.2^{f,g}	**70.0** ± 0.5^{j}
MPSK 6	**37.0** ± 1.1^{b}	**40.0** ± 0.5^{a}	**32.0** ± 1.0^{b}	**34.2** ± 0.9^{ab}	**30.6** ± 0.9^{a}	**50.0** ± 1.6^{g}

Isolates	**Inhibition (%)**					
	R. solani	***P. aphanid-ermatum***	***F. oxyspor-um***	***F. pallidor-oseum***	***F. solani***	***M. phaseol-ina***
MPSK 8	**29.5** ± 1.8^{a}	**40.0** ± 1.5^{a}	**29.0** ± 2.2^{a}	**35.4** ± 1.6^{b}	**37.5** ± 0.4^{b,c}	**12.51** ± 1.1^{a}
MPSK 9	**42.5** ± 0.8^{c}	**41.0** ± 1.5^{a}	**49.25** ± 1.2^{f}	**45.4** ± 1.8^{c,d}	**47.6** ± 2.0^{d,e}	**40.5** ± 3.1^{c,d}
MPSK 14	**55.5** ± 1.9^{h}	**53.0** ± 1.5^{hi}	**52.6** ± 1.6^{ghi}	**51.0** ± 2.1^{de}	**53.13** ± 2.0^{f,g}	**67.5** ± 1.8^{j}
MPSK 20	**51.0** ± 1.1^{f}	**50.0** ± 1.7^{efg}	**45.0** ± 1.5^{e}	**50.0** ± 1.3^{de}	**56.25** ± 1.9^{g,h}	**55.0** ± 2.0^{h}
MPSK 22	**51.4** ± 1.8^{ef}	**56.0** ± 1.2^{j}	**52.0** ± 2.1^{gh}	**54.2** ± 1.9^{fg}	**53.13** ± 0.7^{f,g}	**60.0** ± 2.3^{i}
MPSK 23	**63.2** ± 0.9^{j}	**61.0** ± 1.1^{k}	**54.2** ± 1.7^{hi}	**56.3** ± 1.9^{g}	**59.38** ± 1.5^{h}	**72.5** ± 0.7^{k}
MPSK 28	**52.4** ± 1.2^{fg}	**49.7** ± 1.8^{efg}	**50.0** ± 0.7^{fg}	**49.6** ± 2.2^{de}	**49.6** ± 1.3^{e,f}	**12.5** ± 2.7^{a}
MPSK 51	**52.5** ± 0.6^{fg}	**51.0** ± 0.5^{fgh}	**39.0** ± 0.5^{c}	**34.2** ± 1.1^{ab}	**37.5** ± 0.9^{b,c}	**50.0** ± 2.1^{g}
MPSK 109	**65.9** ± 1.2^{k}	**56.0** ± 1.5^{j}	**60.5** ± 1.5^{j}	**55.0** ± 1.6^{fg}	**53.13** ± 1.8^{f,g}	**62.5** ± 2.1^{i}
MPSK 186	**56.7** ± 1.3^{h}	**51.0** ± 1.8^{fgh}	**55.3** ± 2.1^{i}	**56.0** ± 0.6^{g}	**56.25** ± 1.1^{g,h}	**70.0** ± 2.3^{j,k}
ABSK 9	**50.0** ± 1.7^{ef}	**49.0** ± 0.7^{def}	**47.4** ± 1.8^{ef}	**40.0** ± 0.5^{c}	**47.5** ± 2.1^{d,e}	**42.5** ± 3.1^{d,e}
ABSK 11	**37.4** ± 1.8^{b}	**45.0** ± 1.3^{b}	**47.4** ± 1.2^{ef}	**40.0** ± 1.5^{c}	**37.5** ± 1.4^{b,c}	**37.5** ± 2.2^{c}
ABSK 171	**37.6** ± 0.5^{b}	**48.0** ± 1.4^{cde}	**47.4** ± 1.2^{ef}	**34.5** ± 0.5^{ab}	**37.5** ± 1.7^{b,c}	**50.0** ± 2.5^{g}

Values are mean ± SD of three independent experiments (n=3). Means followed by same letter within columns are not significantly different (ANOVA; $p<0.05$) according to Duncan's Multiple Range Test (DMRT) statistics.


Control
Test
A
B
C
D
Fungi
Fungi
Bacteria

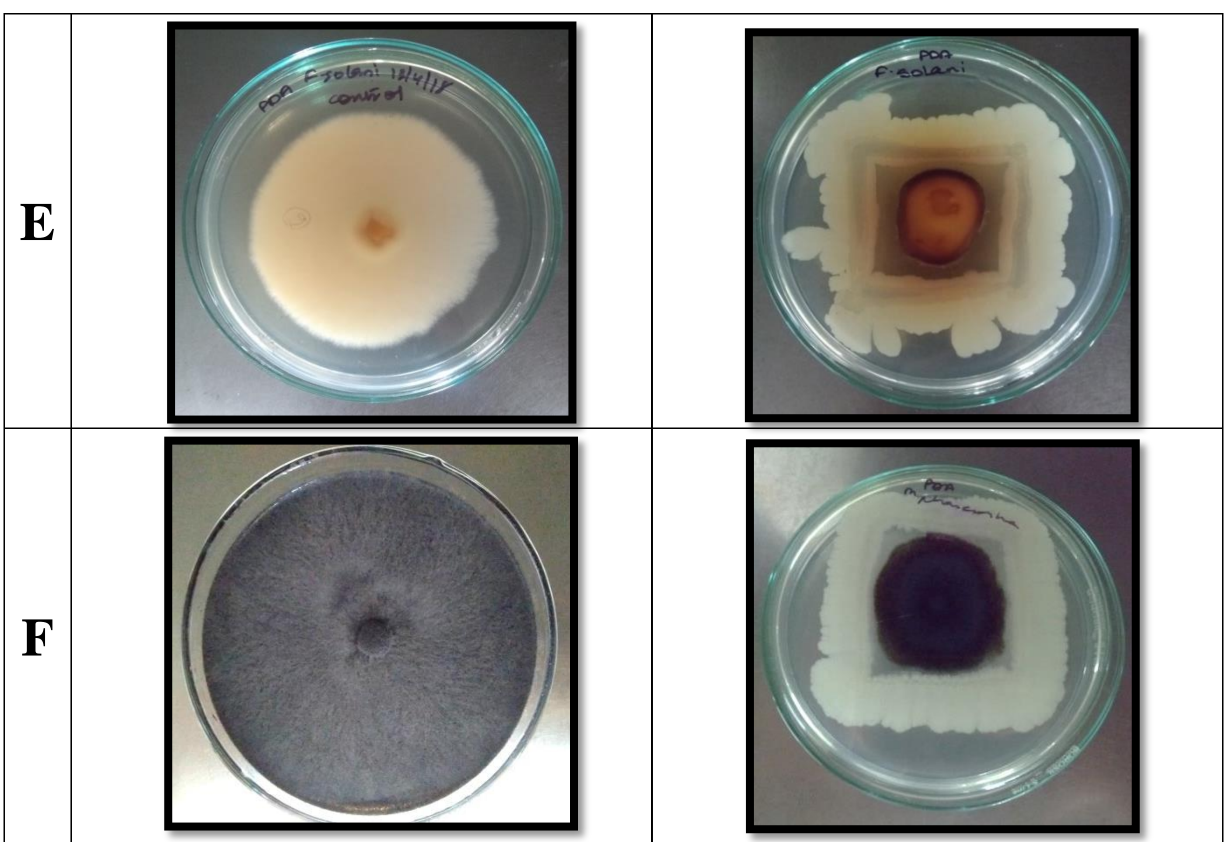


Plate 5: Bacteria MPSK 109 showing inhibition of fungus *R. solani* (A), *P. aphanidermatum* (B), *F. oxysporum* (C), *F. pallidoroseum* (D), *F. solani* (E) and *M. phaseolina* (F)

4.7 Growth assessment of salt pan bacteria in soil extract

The hypersaline bacteria normally grow in salt enriched soil or water. To evaluate their effectiveness and growth in the terrestrial environments, soil extract (SE) was used. Out of the 76 hypersaline bacteria which showed antifungal activity, all the isolates except MPSK 51 and ABSK 11 showed growth on SE agar plates after 5 days of incubation (as seen in Plate 6).

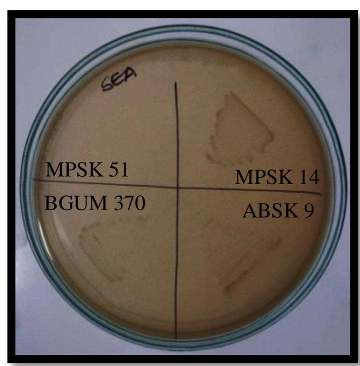


Plate 6: Growth of hypersaline bacteria on the SE agar plates

4.8 Antagonism type exhibited by selected salt pan bacteria

Selected hypersaline bacteria were studied for their mode of antagonism (Mixed path, indirect and direct).

4.8.1 Analysing salt pan bacteria qualitatively for mixed path antagonism and indirect antagonism tests

Ten hypersaline bacteria BGUM 14B, SK 473, MPSK 9, MPSK 14, MPSK 20, MPSK 22, MPSK 23, MPSK 109, MPSK 186 and ABSK 9 were tested for mixed path antagonism and indirect antagonism tests. Under this, screening for the release of the metabolites, secretion of the lytic enzymes, production of hydrogen cyanide (HCN), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophore and exopolysaccharide, solubilization of nutrients and fixation of atmospheric nitrogen was done by plate method. Culture broth was analyzed for the presence of ammonia and indole-3-acetic acid (IAA) produced by the hypersaline bacteria.

4.8.1.1 Assessing the volatile and diffusible metabolites antagonistic to fungi

Among the 10 hypersaline bacteria tested for the production of metabolites, three isolates *viz*. BGUM 14B, MPSK 9 and ABSK 9 inhibited *M. phaseolina*, *R. solani* and *F. solani* by inverted plated method (Plate 7). The remaining seven isolates MPSK 14, MPSK 22, MPSK 23 MPSK 109, MPSK 186, MPSK 20 and SK 473 inhibited the three fungal pathogens by well diffusion method (Plate 8). Table 9 depicts the inhibition (%) and Table 10 depicts the zone of inhibition (mm) of the fungal pathogens tested by the hypersaline bacteria.

Table 9: Percentage inhibition of the fungal mycelia by volatile metabolites produced by the hypersaline bacteria

	Percentage inhibition of fungal mycelia (%)		
Isolate	***M. phaseolina***	***R. solani***	***F. solani***
BGUM 14B	**44.44** ± 1.5	**22.22** ± 1.0	**45.44** ± 0.9
MPSK 9	**11.11** ± 1.7	**15.75** ± 1.1	**11.11** ± 1.5
ABSK 9	**11.11** ± 2.0	**11.11** ± 1.8	**20.25** ± 0.5

Each value is the mean ± SD of three replicates (n=3)

Table 10: Zone of inhibition of the fungal pathogens by the diffusible metabolites produced by the hypersaline bacteria

	Zone of inhibition (mm)		
Isolate	***M. phaseolina***	***R. solani***	***F. solani***
MPSK 14	**11** ± 2.0	**12** ± 1.0	**15** ± 3.0
MPSK 22	**20** ± 3.0	**18** ± 2.0	**22** ± 1.0
MPSK 23	**24** ± 1.0	**27** ± 1.0	**25** ± 3.0
MPSK 109	**19** ± 3.0	**16** ± 3.0	**20** ± 2.0
MPSK 186	**25** ± 2.0	**25** ± 1.0	**22** ± 3.0
SK 473	**17** ± 5.0	**15** ± 1.0	**19** ± 5.0
MPSK 20	**18** ± 1.0	**14** ± 2.0	**18** ± 5.0

Each value is the mean ± SD of three replicates (n=3)

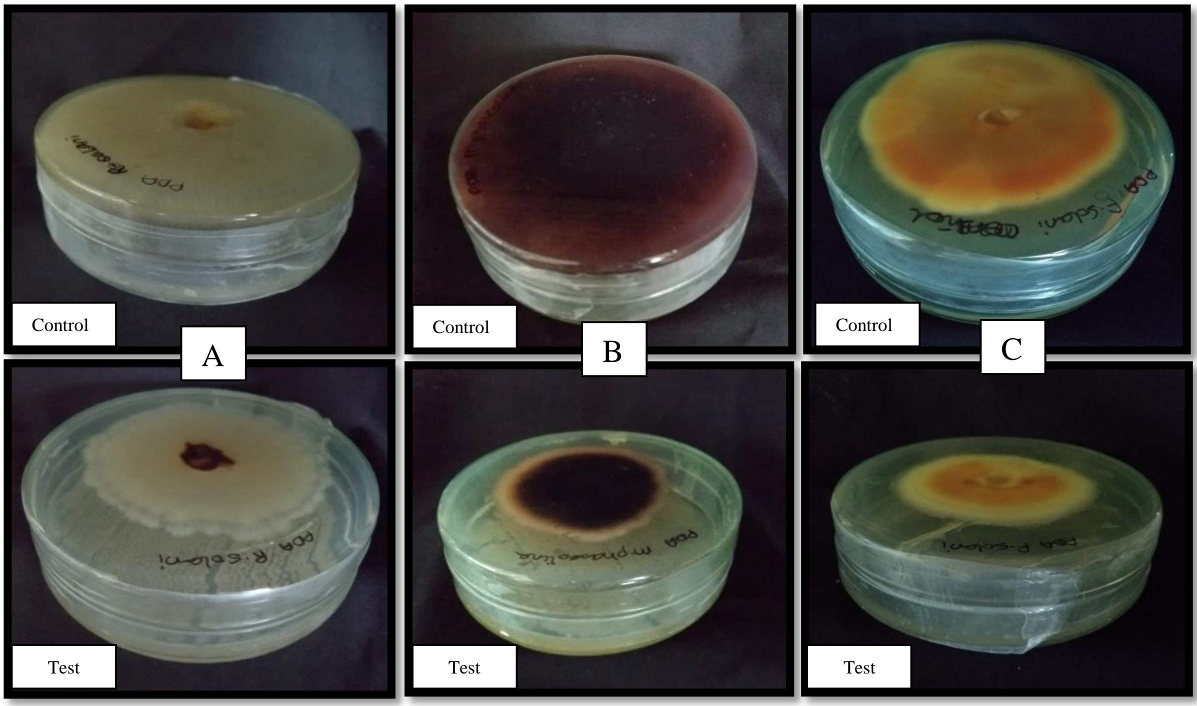


Plate 7: Antifungal activity exhibited by BGUM 14B by the volatile metabolite production against *R. solani* (A), *M. phaseolina* (B) and *F. solani* (C) by inverted plate method

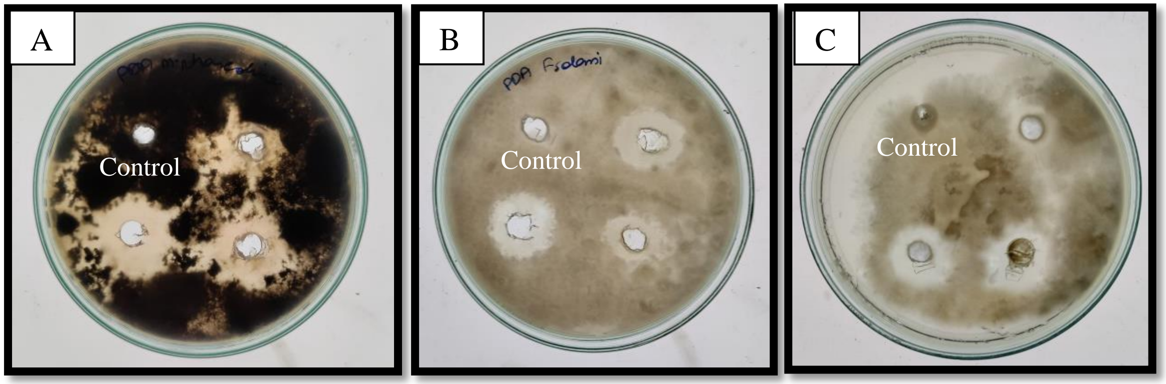


Plate 8: Antifungal activity exhibited by MPSK 23 against *M. phaseolina* (A), *F. solani* (B) and *R. solani* (C) by well diffusion method (carried out in triplicates)

4.8.1.2 Secretion of chitinase, glucanase, amylase, cellulase, protease and lipase enzymes

Out of 10 hypersaline bacteria, SK 473, MPSK 14, MPSK 20, MPSK 22, MPSK 23, MPSK 109 and MPSK 186 tested positive for amylase, protease, lipase and cellulase. In addition to this, MPSK 22, MPSK 23 and MPSK 186 also showed production of chitinase and β-glucanase enzymes (Plate 9). After measuring the zone of clearance, the solubilisation index which is the resultant of the assessed lytic activity, was calculated and tabulated (Table 11).

Table 11: Production of extracellular lytic enzymes by the selected hypersaline bacteria

	Solubilisation Index					
	Chitinase	**Glucanase**	**Amylase**	**Cellulase**	**Protease**	**Lipase**
BGUM 14B	-	-	-	-	-	-
ABSK 9	-	-	-	-	-	-
MPSK 9	-	-	-	-	-	-
MPSK 20	-	-	**1.0** ± 0.75	**1.6** ± 0.70	**2.1** ± 0.30	**1.8** ± 1.14
MPSK 22	**1.2** ± 0.50	**1.2** ± 0.75	**2.0** ± 0.50	**1.4** ± 0.40	**1.5** ± 0.40	**1.6** ± 0.77
MPSK 23	**1.5** ± 0.40	**1.2** ± 0.55	**1.7** ± 0.90	**1.6** ± 0.70	**1.4** ± 0.60	**1.8** ± 0.85
MPSK 186	**1.1** ± 0.30	**2.0** ± 0.35	**2.5** ± 0.70	**1.9** ± 0.50	**3.0** ± 0.50	**2.8** ± 1.30
MPSK 109	-	-	**3.0** ± 0.30	**1.9** ± 0.20	**3.1** ± 0.90	**2.4** ± 0.77
MPSK 14	-	-	**2.1** ± 0.30	**2.3** ± 0.30	**1.6** ± 0.75	**2.4** ± 0.48
SK 473	-	-	**2.2** ± 0.80	**1.9** ± 0.40	**1.7** ± 0.51	**2.6** ± 0.85

Each value is the mean ± SD of three replicates (n=3); -: Not observed

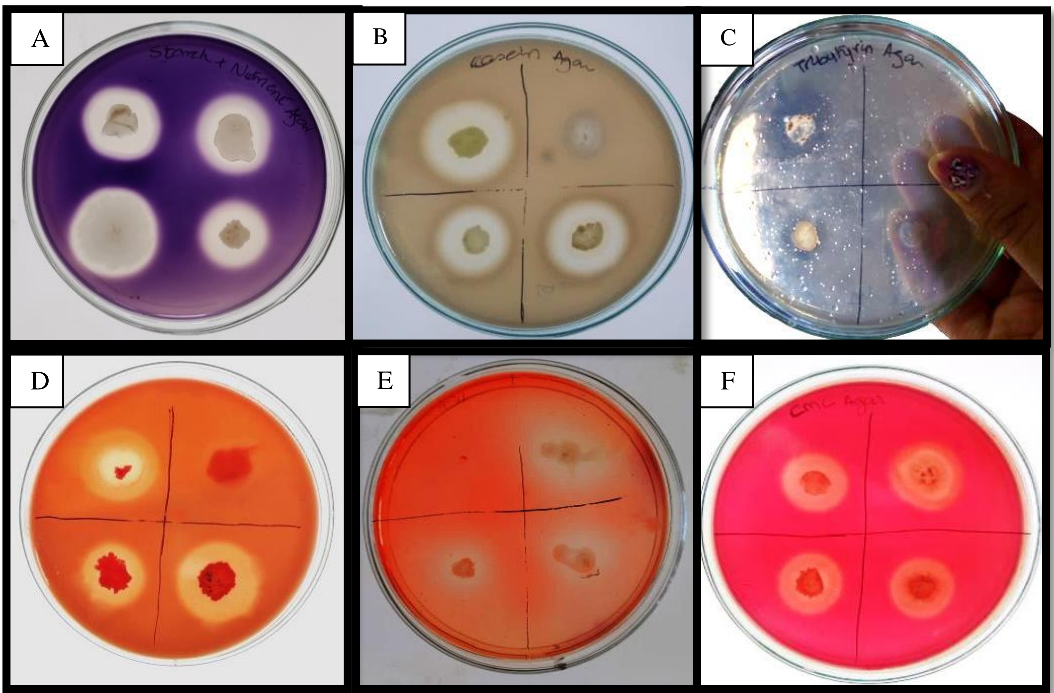


Plate 9: Production of extracellular enzymes amylase (A), protease (B), lipase (C), chitinase (D), glucanase (E) and cellulase (F) by hypersaline bacteria

4.8.1.3 Production of ammonia and hydrogen cyanide

All the hypersaline bacteria except MPSK 20 produced ammonia, indicated by the orange coloration of the peptone water broth in which they was grown individually (Plate 10). However, none of the hypersaline bacteria tested positive for production of hydrogen cyanide.

Plate 10: Ammonia production test of the hypersaline bacteria

4.8.1.4 *In-vitro* production of plant growth promoting attributes

All the hypersaline bacteria tested positive for IAA and ACC deaminase production. They were also able to grow on nitrogen free media indicating the ability to fix atmospheric nitrogen. Out of the 10 hypersaline bacteria, only ABSK 9 solubilized phosphate and MPSK 9 solubilized silicate and all the hypersaline bacteria except BGUM 14B and ABSK 9 solubilised the insoluble zinc from the medium. Further, none of the hypersaline bacteria tested positive for solubilisation of potassium. The solubilisation index was calculated from the zone of clearance (mm) indicating the solubilisation of zinc, silicate and phosphate, which is represented in Table 12.

Table 12: *In-vitro* plant growth promoting attributes of the hypersaline bacteria

	IAA production	**ACC deaminase production**	**N_2 fixation**	**Solubilisation Index**		
				Zinc	**Silicate**	**Phosphate**
BGUM 14B	+	+	+	-	-	-
ABSK 9	+	+	+	-	-	**1.6** ± 0.5
MPSK 9	+	+	+	**1.3** ± 1.0	**2.8** ± 0.5	-
MPSK 20	+	+	+	**1.4** ± 0.2	-	-
MPSK 22	+	+	+	**1.3** ± 0.1	-	-
MPSK 23	+	+	+	**1.6** ± 0.3	-	-
MPSK 186	+	+	+	**1.4** ± 0.1	-	-
MPSK 109	+	+	+	**2.4** ± 0.3	-	-
MPSK 14	+	+	+	**2.2** ± 0.1	-	-
SK 473	+	+	+	**1.1** ± 0.1	-	-

Each value is the mean ± SD of three replicates (n=3); +: Positive test; -: Not observed

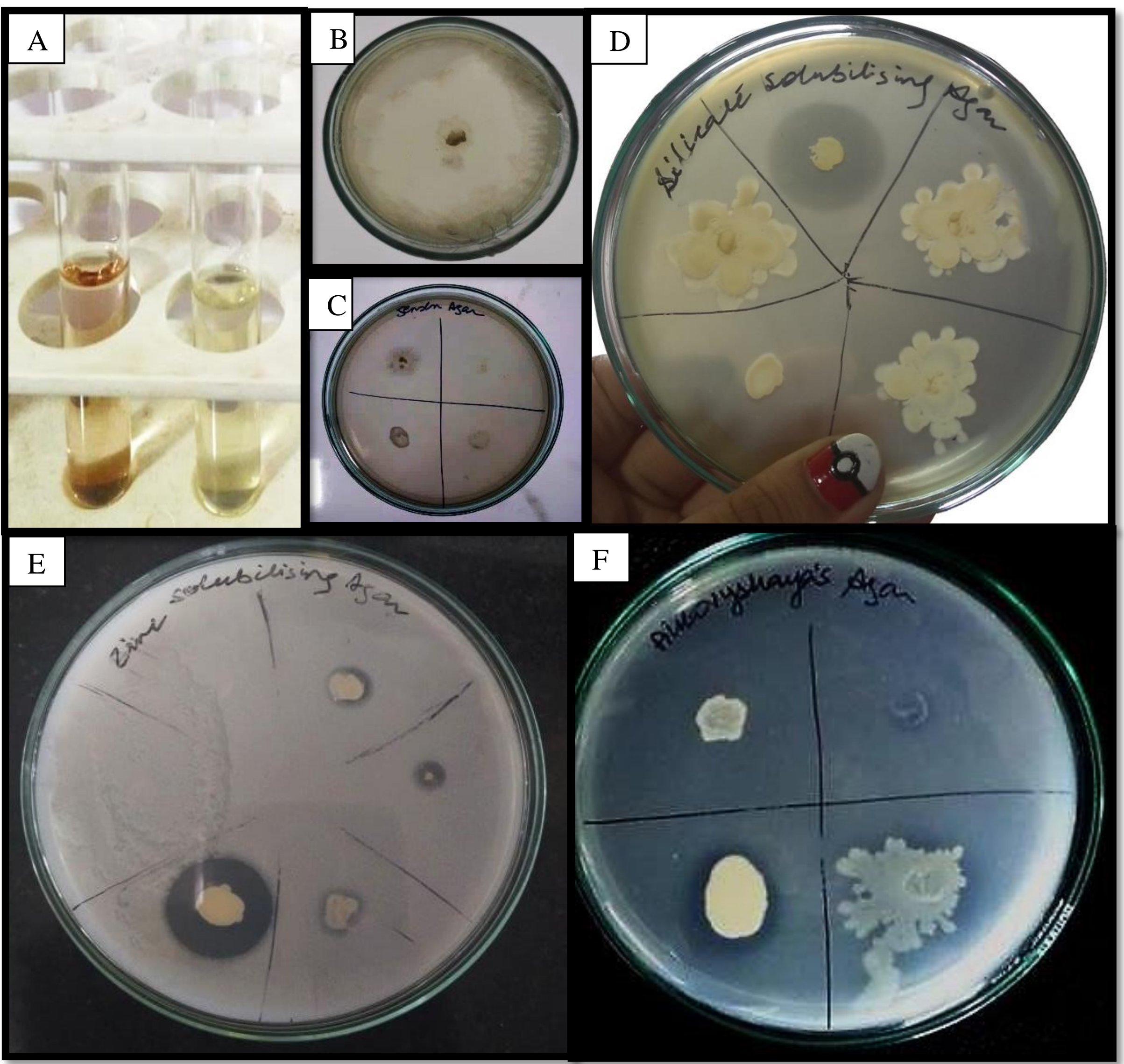


Plate 11: *In-vitro* plant growth promoting attributes IAA production (A), AAC deaminase production (B), N_2 fixation (C), Silicate solubilisation (D), Zinc solubilisation (E) and Phosphate solubilisation (F) by hypersaline bacteria

4.8.1.5 Siderophore production

ABSK 9, MPSK 109 and MPSK 186 were positive for siderophore production indicated by an orange halo around the colony (Plate 12 A).

4.8.1.6 Exopolysaccharide (EPS) production

Black coloration of all the bacterial colonies except that of BGUM 14B and MPSK 9 was observed on Congo red ZMA plates supplemented with sucrose. This marked a positive test for exopolysaccharide secretion by bacteria (Plate 12 B).

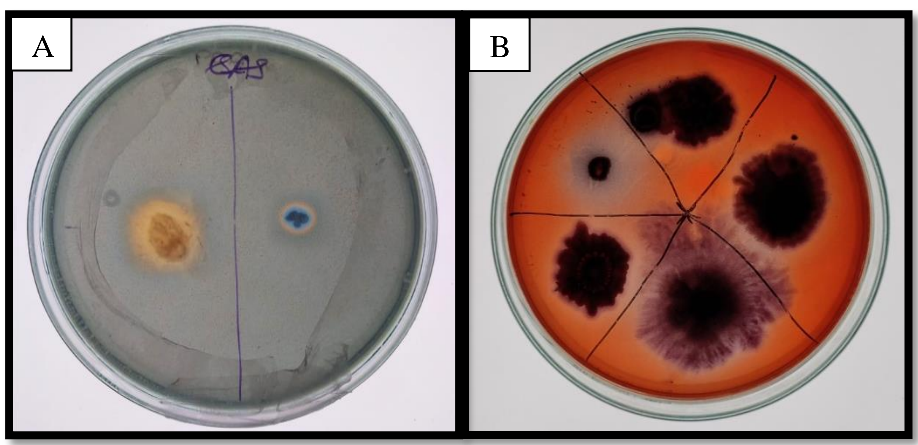


Plate 12: Production of siderophore (A) and exopolysaccharide (B) by hypersaline bacteria

4.8.2. Quantitative estimation of mixed path antagonism and indirect antagonism tests

All the hypersaline bacteria which tested positive for specific qualitative tests *viz.* lytic enzymes secretion, ammonia production, IAA production, solubilization of nutrients and siderophore production were quantitatively estimated.

4.8.2.1 Estimation of chitinase, glucanase, amylase, cellulase, protease and lipase enzymes activity

Quantitative estimation of the extracellular lytic enzymes secreted by the hypersaline bacteria MPSK 20, MPSK 22, MPSK 23, MPSK 186, MPSK 109, MPSK 14 and SK 473 was carried out and represented as enzyme activity (U/min) (Table 13). Highest amylase and protease activity was observed in MPSK 109 (3.00 ± 0.25 U/min and 3.08 ± 0.91 U/min respectively). MPSK 186 showed highest activity of lipase and glucanase (2.81 ± 1.30 U/min and 2.00 ± 0.35 U/min respectively). Highest chitinase activity was observed in MPSK 23 (1.50 ± 0.40 U/min) and highest cellulase activity was seen in MPSK 14 (2.25 ± 0.25 U/min).

Table 13: Quantitative estimation of the extracellular lytic enzymes secreted by the hypersaline bacteria

	Chitinase (U/min)	Glucanase (U/min)	Amylase (U/min)	Cellulase (U/min)	Protease (U/min)	Lipase (U/min)
MPSK 20	ND	ND	**1.00** ± 0.75	**1.57** ± 0.70	**2.14** ± 0.30	**1.81** ± 1.14
MPSK 22	**1.20** ± 0.50	**1.15** ± 0.75	**2.00** ± 0.50	**1.42** ± 0.40	**1.45** ± 0.40	**1.61** ± 0.77
MPSK 23	**1.50** ± 0.40	**1.15** ± 0.55	**1.67** ± 0.90	**1.57** ± 0.70	**1.40** ± 0.60	**1.83** ± 0.85
MPSK 186	**1.10** ± 0.25	**2.00** ± 0.35	**2.50** ± 0.65	**1.88** ± 0.50	**3.00** ± 0.50	**2.81** ± 1.30
MPSK 109	ND	ND	**3.00** ± 0.25	**1.86** ± 0.16	**3.08** ± 0.91	**2.44** ± 0.77
MPSK 14	ND	ND	**2.10** ± 0.30	**2.25** ± 0.25	**1.57** ± 0.75	**2.36** ± 0.48
SK 473	ND	ND	**2.17** ± 0.80	**1.85** ± 0.40	**1.67** ± 0.51	**2.55** ± 0.85

Each value is the mean ± SD of three replicates (n=3); ND: Not detected

4.8.2.2 Ammonia estimation

Ammonia production by hypersaline bacteria BGUM 14B, SK 473, MPSK 9, MPSK 14, MPSK 22, MPSK 23, MPSK 109, MPSK 186 and ABSK 9 was estimated by spectrophotometric method and represented as mg/mL (Table 14). Highest ammonia was estimated in the supernatant of MPSK 186 (526.01 ± 14.51 mg/mL), followed by MPSK 22 (414.39 ± 3.48 mg/mL) and MPSK 23 (392.18 ± 16.84 mg/mL). Least production was seen in ABSK 9 (36.07 ± 5.14 mg/mL).

4.8.2.3 Estimating solubilized nutrients and produced IAA

Among MPSK 9, MPSK 20, MPSK 22, MPSK 23, MPSK 186, MPSK 109, MPSK 14 and SK 473; highest amount of soluble zinc was detected in the supernatant of MPSK 14 (217.00 ± 4.24 ppm) which was almost the twice of what was detected in uninoculated media. In the supernatant of MPSK 9, 3.94 ± 0.44 mM/L of soluble silicon was traced and in the supernatant of ABSK 9, 6.47 ± 0.11 mM/L of soluble phosphate was detected; which were the only isolates exhibiting

solubilisation of silicate and phosphate respectively. All the hypersaline bacteria exhibited IAA production, highest being estimated in SK 473 (8.70 ± 0.21 µg/mL) and least production was estimated in MPSK 109 (0.46 ± 0.06 µg/mL) (Table 14).

Table 14: Quantitative estimation of ammonia, IAA, solubilised zinc, silicon and phosphate in the culture supernatant of selected hypersaline bacteria

	Ammonia (mg/ml)	**IAA (µg/ml)**	**Zinc (ppm)**	**Silicon (mM/L)**	**Phosphate (mM/L)**
BGUM 14B	**293.47** ± 2.3	**1.48** ± 0.08	ND	ND	ND
ABSK 9	**36.07** ± 5.1	**0.90** ± 0.02	ND	ND	**6.47** ± 0.11
MPSK 9	**117.50** ± 1.6	**2.26** ± 0.15	**185.0** ± 8.49	**3.94** ± 0.44	ND
MPSK 20	ND	**2.47** ± 0.13	**184.5** ± 6.36	ND	ND
MPSK 22	**414.39** ± 3.5	**1.93** ± 0.07	**190** ± 12.73	ND	ND
MPSK 23	**392.18** ± 1.7	**2.41** ± 0.11	**177.5** ± 3.54	ND	ND
MPSK 186	**526.01** ± 1.5	**2.42** ± 0.09	**189.5** ± 2.12	ND	ND
MPSK 109	**170.27** ± 3.2	**0.46** ± 0.06	**200** ± 14.14	ND	ND
MPSK 14	**40.05** ± 3.3	**7.69** ± 0.20	**217.0** ± 4.24	ND	ND
SK 473	**345.48** ± 4.8	**8.70** ± 0.21	**204.0** ± 5.66	ND	ND

Each value is the mean ± SD of three replicates (n=3); ND: Not detected

4.8.2.4 Quantifying the siderophores produced

Among the three hypersaline bacteria which showed orange halo formation on CAS agar plates, highest siderophore was estimated in ABSK 9 (96.70 % siderophore units), followed by MPSK 109 (76.02 % siderophore units) and then in MPSK 186 (72.03 % siderophore units) assessed by the CAS-shuttle assay.

4.8.3. PCR amplification of genes of lipopeptide synthesis

Out of 11 genes screened in the three hypersaline bacteria, both MPSK 22 and MPSK 23 showed the amplification of *Lic*A and *Lic*C genes of lichenysin family and *Fen*C gene of fengycin family. A crisp band was observed of 735 bp and 1195 bp for *Lic*A and *Lic*C respectively (Lane 7 and 9 of Plate 13 A and B) and 964 bp for *Fen*C (Lane 3 of Plate 13 A and B), which matched with the expected product size. Similarly, amplification product of MPSK 186 revealed a crisp band of 964 bp which matched

with the expected product size of *Fen*B gene of the fengycin family (Lane 4 of Plate 13 C).

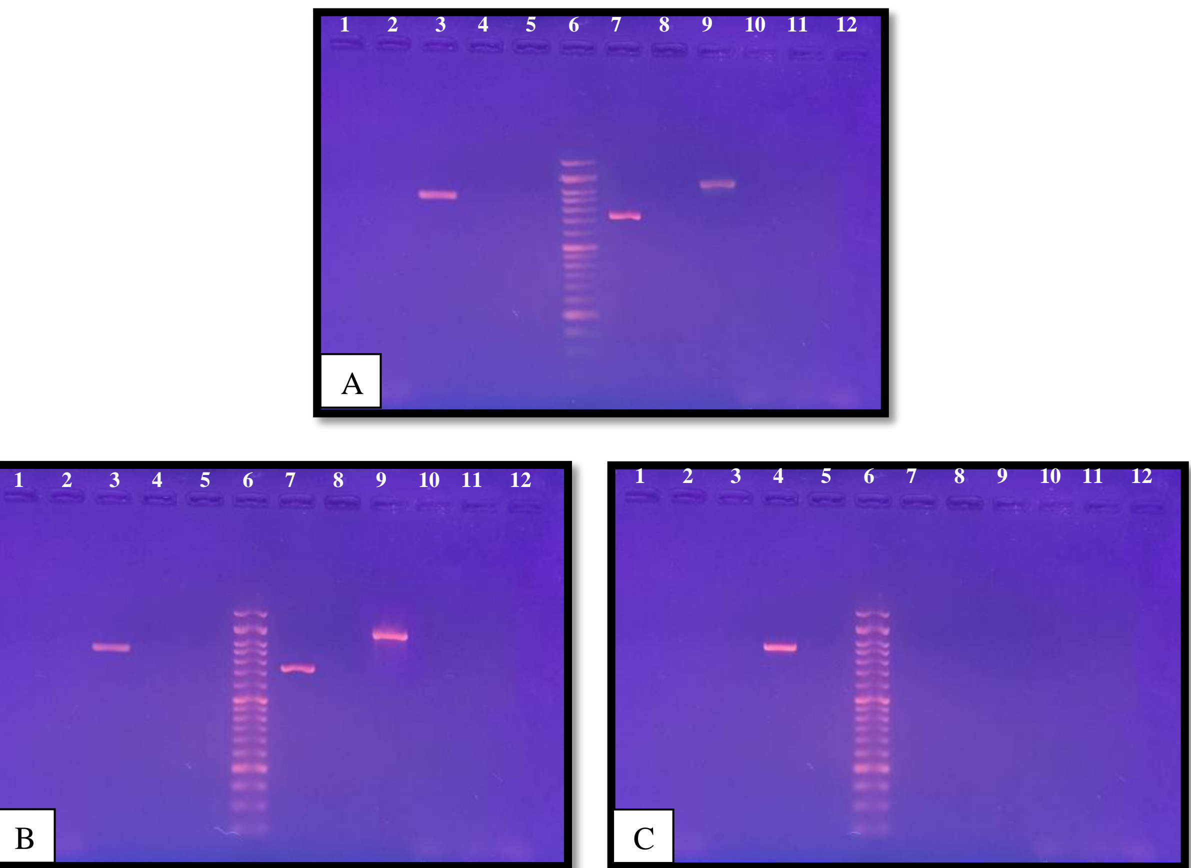


Plate 13: PCR amplification products of MPSK 22 (A), MPSK 23 (B) and MPSK 186 (C) Lane- 1:*Itu*A 2:*Fen*D 3:*Fen*C 4:*Fen*B 5:*Fen*A 6:50 kb ladder 7:*Lic*A 8:*Lic*B 9:*Lic*C 10:*Sfp* 11:*Itu*C 12:*Itu*D

4.8.4. Direct antagonism by interaction between fungal mycelia and salt pan bacteria

In Plate 14, the red boxes depict the fungal mycelia interacting with the hypersaline bacteria (Plate 14 A: *F. solani*, Plate 14 B: *R. solani* and Plate 14 C: *M. phaseolina*) and the green boxes depict the mycelia from the leading edge of the respective fungus (control mycelia). Light microscopy study of *F. solani* revealed leakage of cellular contents of the mycelia after interacting with the hypersaline bacteria (Plate 15 A). In case of *R. solani*, light microscopy revealed an abnormal swelling of the fungal hyphae (Plate 15 B) and *M. phaseolina* showed thinning and curling pattern in light microscopy (Plate 15 C). SEM microphotographs showed disintegrated and empty hyphae of *F. solani* which was not observed in the control (Plate 16 A). In case of *R. solani* and *M.*

phaseolina, SEM analysis displayed thinning and curling of the hyphae as compared to the control (Plate 16 B and C).

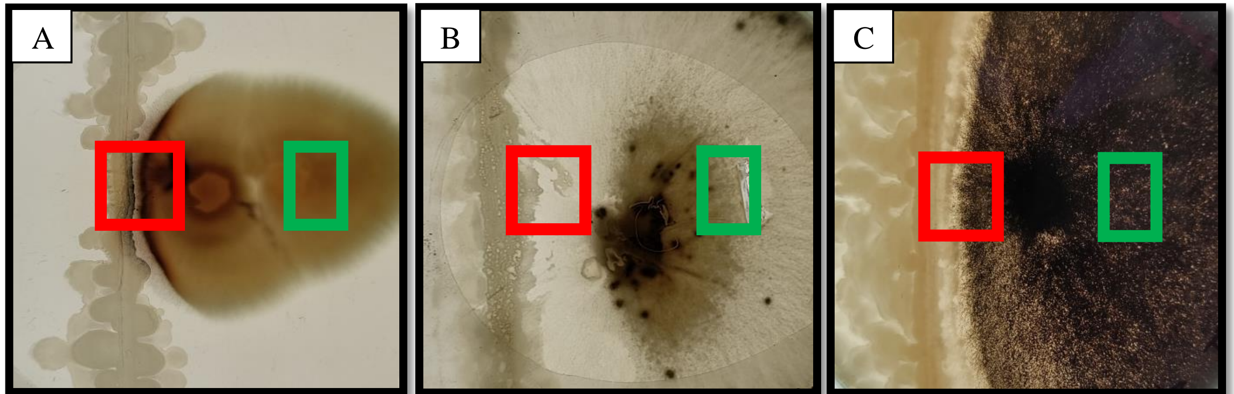


Plate 14: Fungal mycelia interacting with hypersaline bacteria (red boxes) and the control non-inhibited mycelia (green boxes) of *F. solani* (A), *R. solani* (B) and *M. phaseolina* (C)

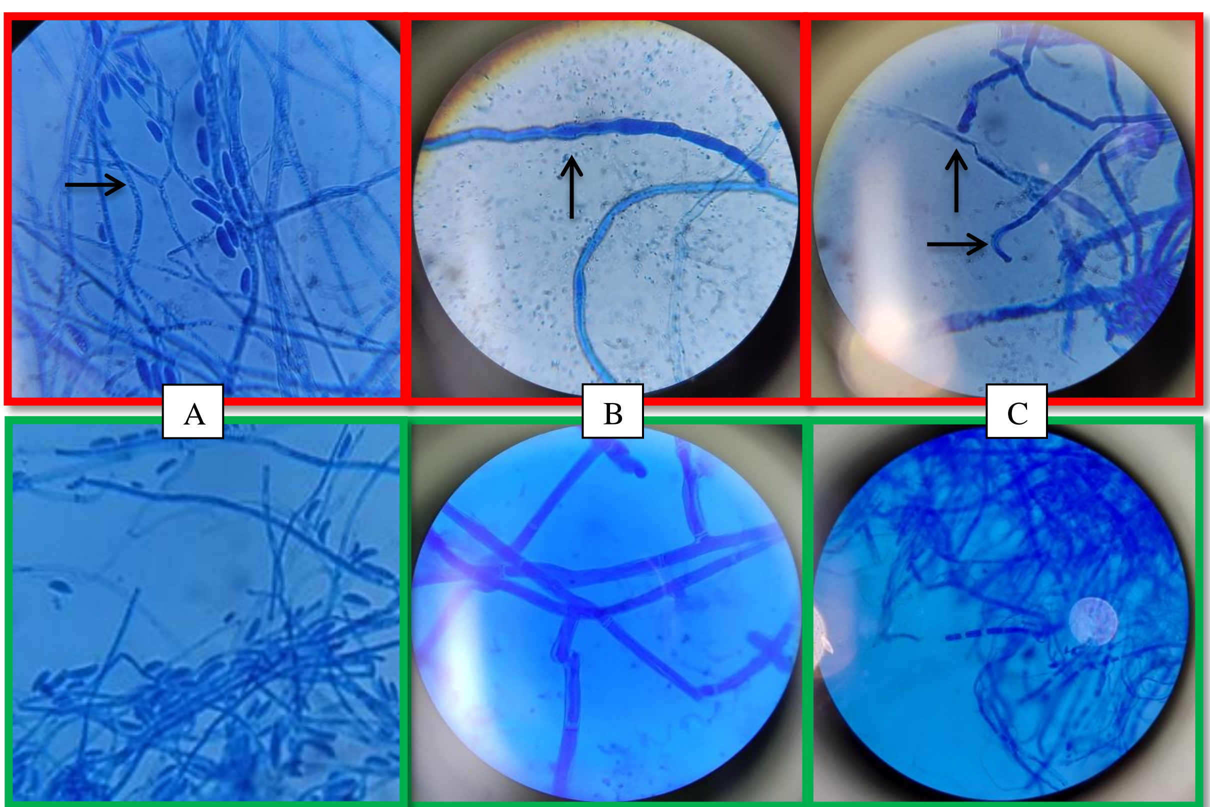
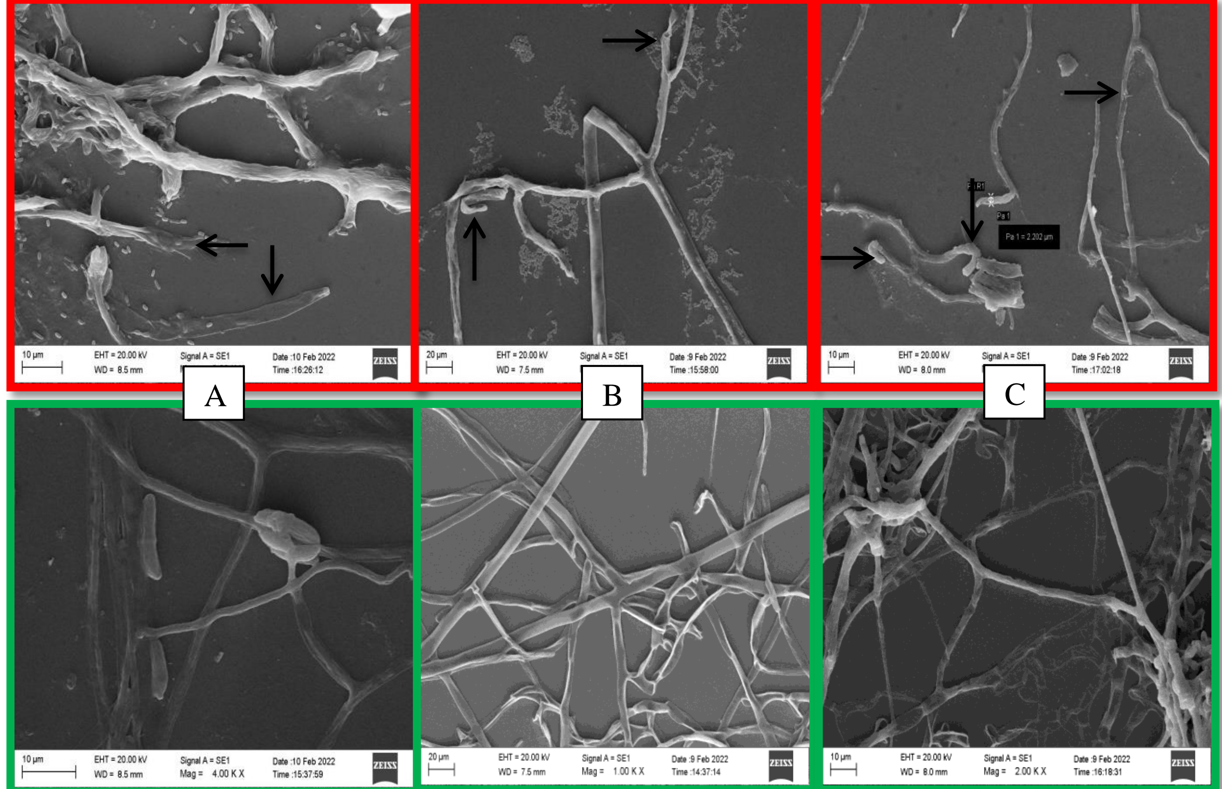


Plate 15: Light Microscopy images of test (highlighted in red boxes) and control mycelia (highlighted in green boxes) of *F. solani* (A), *R. solani* (B) and *M. phaseolina* (C)

Morphology of fungi in presence of hypersaline bacteria (Test)



Morphology of fungi without the hypersaline bacteria influence (Control)

Plate 16: SEM Microscopy microphotographs of test (highlighted in red boxes) and control mycelia (highlighted in green boxes) of *F. solani* (A), *R. solani* (B) and *M. phaseolina* (C)

4.9 Identification of salt pan bacteria MPSK 22, MPSK 23 and MPSK 186

Colony morphology and Gram character of MPSK 22, MPSK 23 and MPSK 186 is depicted in Plate 17. MPSK 22 and MPSK 23 grew as creamish-white colonies on ZMA which were mucoidy in texture. MPSK 186 formed creamish-white but flaky colonies on the same media. All the bacterial cells stained purple (Gram positive) and were rod shaped (Plate 17). SEM microphotographs confirmed that all the isolates were rods of varying dimensions (Plate 18). The length of the MPSK 22 cells was found to be 2.56 µm and width was 753.9 nm. The dimension of MPSK 23 was also similar (2.56 µm × 889.5 nm) to that of the former. MPSK 186 cells were comparatively smaller with 2.179 µm × 735.5 nm dimensions. Table 15 and Table 16 further summarize the results of the biochemical tests and carbohydrate utilization pattern of each individual hypersaline bacteria respectively. Further, the molecular

identification revealed a close match (100 % similarity) of MPSK 22 to *Bacillus licheniformis*, MPSK 23 to *Bacillus paralicheniformis* and MPSK 186 to *Bacillus subtilis* subsp. *inaquosorum*. These sequences have been submitted to the GenBank database under accession numbers MZ400680 (MPSK 22), MZ052210 (MPSK 23) and MZ400681 (MPSK 186). The taxonomic position of the three hypersaline bacteria in the phylogenetic tree along with their closest relatives is illustrated in Figure 6. Maximum Likelihood method was used to infer the evolutionary history of the respective bacteria with 1000 bootstrap replicates depicted next to the nodes. The figure validates the results of identification as the bootstrap values were found to be close to 100.

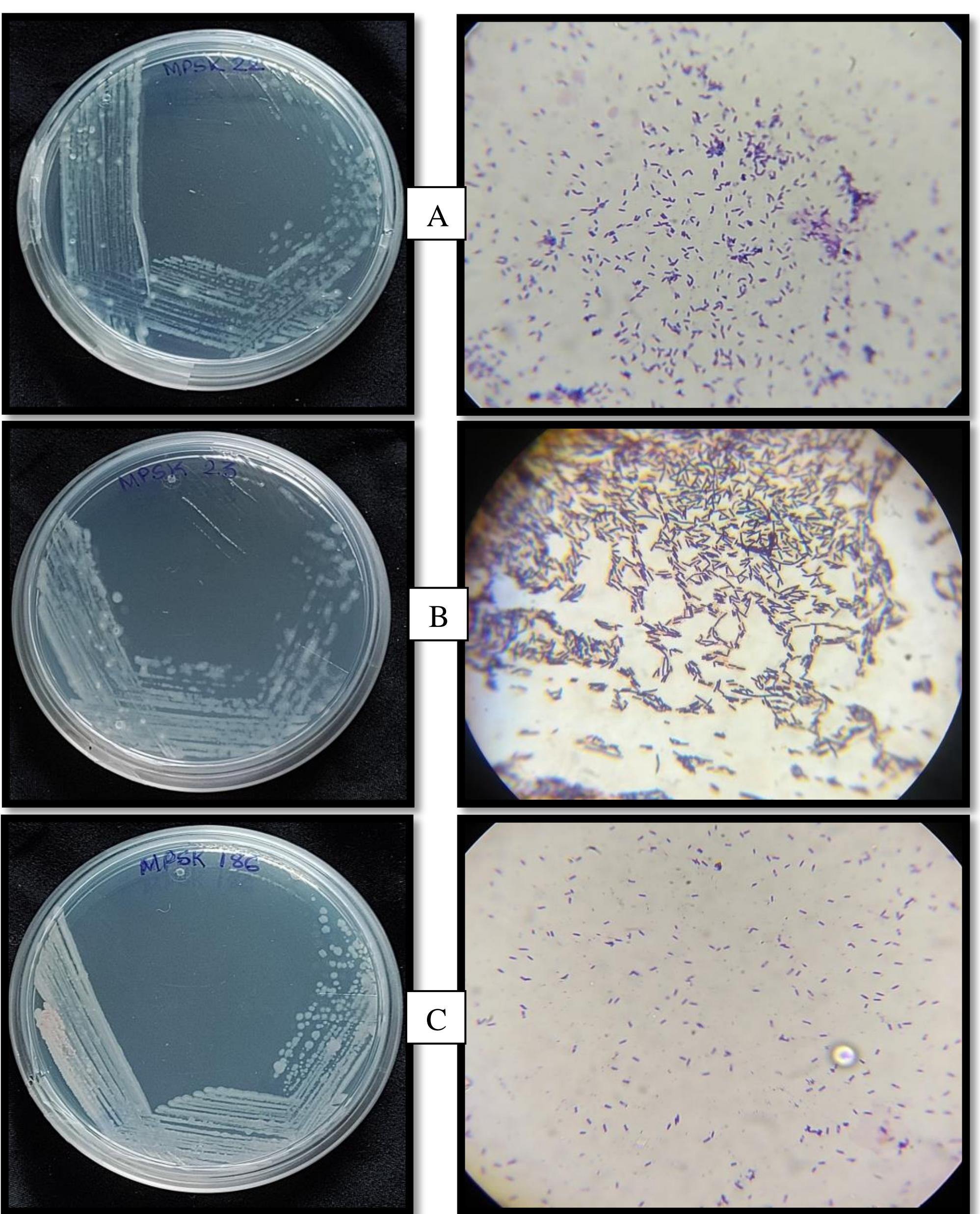


Plate 17: Colony morphology and Gram staining images of MPSK 22 (A), MPSK 23 (B) and MPSK 186 (C)

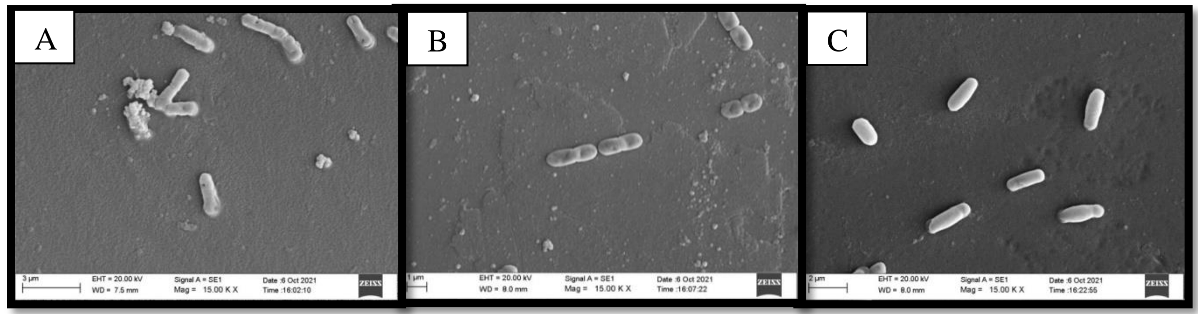


Plate 18: Scanning electron microphotographs of MPSK 22 (A), MPSK 23 (B) and MPSK 186 (C)

Table 15: Biochemical characteristics of the selected hypersaline bacteria

Biochemical Tests	MPSK 22	MPSK 23	MPSK 186
Oxidase Test	+	+	+
Catalase Test	+	+	+
ONPG Test	+	+	-
Urease Test	+	+	-
SIM Test			
Sulfur Reduction	-	-	-
Indole Production	-	-	-
Motility	+	+	+
Gelatinase Test	-	-	-
Citrate Utilization	+	+	+
MR-VP Test			
Methyl Red	-	-	+
Voges-Proskauer	+	+	+
Nitrate Test	+	+	+
Oxygen requirement	Facultative aerobes	Facultative aerobes	Obligate aerobes

+: Positive test; -: Negative test

Table 16: Carbohydrate utilization pattern of the selected hypersaline bacteria

Carbohydrate	**MPSK 22**	**MPSK 23**	**MPSK 186**	**Carbohydrate**	**MPSK 22**	**MPSK 23**	**MPSK 186**
Lactose	-	-	-	Sodium Gluconate	+	+	-
Xylose	-	+	+	Salicin	+	+	+
Maltose	+	+	+	Dulcitol	+	-	-
Fructose	-	+	+	Ionistol	+	-	-
Dextrose	+	+	+	Sorbitol	-	+	-
Galactose	+	+	+	Mannitol	-	+	+
Raffinose	+	+	+	Adonitol	-	+	-
Trehalose	+	+	+	Arabitol	-	-	-
Melibiose	-	-	+	Erythritol	-	-	-
α-Methyl D-Mannoside	-	+	-	α-Methyl D-Glucoside	-	-	-
L- Arabinose	+	-	+	Rhamnose	+	+	-
Malonate	-	-	-	Cellobiose	+	+	+
Mannose	+	-	+	Melezitose	+	+	+
Inulin	+	-	+	Sucrose	+	+	+
Glycerol	+	-	-	Xylitol	-	+	+
D- Arabinose	+	+	+	Sorbose	+	+	-

+: Positive test; -: Negative test

Table 17: Percent inhibition of the fungal pathogens, antagonism test results and the identification of the three shortlisted hypersaline bacteria

	MPSK 22	MPSK 23	MPSK 186
Inhibition (%)			
F. solani	**53.13** ± 0.7	**59.38** ± 1.5	**56.25** ± 1.1
F. oxysporum	**52.00** ± 2.1	**54.20** ± 1.7	**55.30** ± 2.1
F. pallidoroseum	**54.20** ± 1.9	**56.30** ± 1.9	**56.00** ± 0.6
R. solani	**51.40** ± 1.8	**63.20** ± 0.9	**56.70** ± 1.3
P. aphanidermatum	**56.00** ± 1.2	**61.00** ± 1.1	**51.00** ± 1.8
M. phaseolina	**60.00** ± 2.3	**72.50** ± 0.7	**70.00** ± 2.3
ACC deaminase production	+	+	+
Nitrogen fixation	+	+	+
Exopolysaccharide secretion	+	+	+
Lytic enzyme production (U/min)			
Amylase	**1.57** ± 0.08	**0.80** ± 0.04	**1.79** ± 0.08
Protease	**0.38** ± 0.03	**0.37** ± 0.02	**0.43** ± 0.14
Lipase	**2.58** ± 0.49	**3.91** ± 0.57	**4.83** ± 0.24
Chitinase	**0.57** ± 0.02	**0.70** ± 0.04	**0.47** ± 0.02
Glucanase	**0.15** ± 0.01	**0.15** ± 0.01	**0.29** ± 0.01
Cellulase	**0.72** ± 0.01	**0.74** ± 0.01	**0.91** ± 0.02
IAA (µg/ml)	**1.93** ± 0.07	**2.41** ± 0.11	**2.42** ± 0.09
Siderophore (% siderophore units)	ND	ND	**72.03** ± 1.40
Ammonia (mg/ml)	**414.39** ± 3.48	**392.18** ± 1.68	**526.01** ± 1.45
Zinc solubilization (ppm)	**190** ± 12.73	**177.5** ± 3.54	**189.5** ± 2.12
Antifungal compound genes	*Lic*A, *Lic*C, *Fen*C	*Lic*A, *Lic*C, *Fen*C	*Fen*B
Direct antagonism	+	+	+
Gram character and morphology	Positive rods	Positive rods	Positive rods
Sequence similarity to	*Bacillus licheniformis*	*Bacillus paralicheniformis*	*Bacillus subtilis* subsp. *inaquosorum*

Each value is the mean ± SD of three replicates (n=3); +: Positive test; ND: Not detected

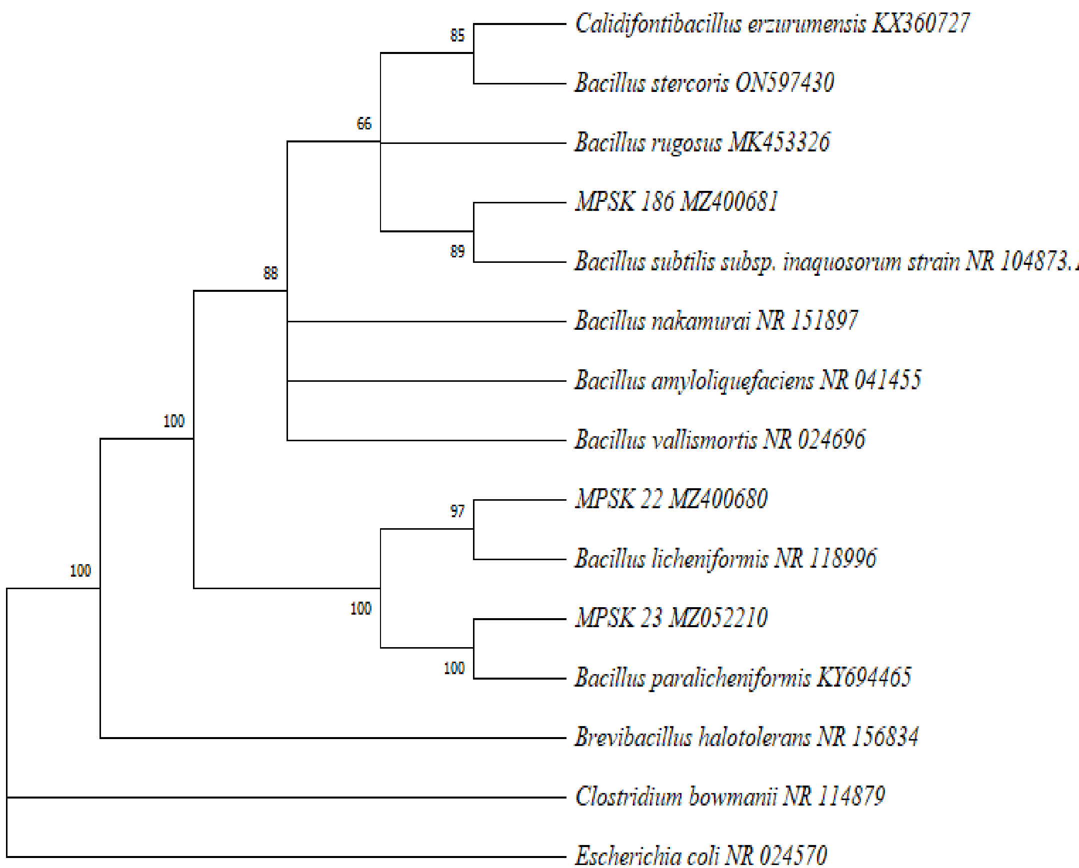


Figure 6: Phylogenetic tree depicting the sequence similarity of the three hypersaline bacteria with other bacteria based on 16S rRNA analysis

4.10 Blood hemolysis and antibiotic sensitivity tests of MPSK22, MPSK 23 and MPSK 186

All the three hypersaline bacteria MPSK 22, MPSK 23 and MPSK 186 exhibited gamma hemolysis on human blood agar plates (Plate 19 A). This was carried out as a primary safety evaluation criterion to assess the toxicity of the hypersaline bacteria to human blood cells. Table 18 represents the sensitivity of the three hypersaline bacteria to 24 antibiotics along with the interpretive criteria according to Clinical and laboratory standards institute. MPSK 22 and MPSK 23 were sensitive to 18 out of 24 antibiotics with an intermediate susceptibility to 5 antibiotics. MPSK 186 was sensitive to 14 and showed intermediate susceptibility to 7 antibiotics (Plate 19 B). MPSK 186 showed susceptibility to β-lactam, Chloramphenicol, Fluoroquinolones, Tetracycline, Quinolone and Macrolide class of antibiotics. MPSK 22 and MPSK 23 also showed susceptibility

to these groups of antibiotics and in addition were also susceptible to Aminoglycoside, Lincomycin, Sulfonamides and Nitrofuran.

Table 18: Antibiotic sensitivity of the hypersaline bacteria

Sr. No.	Antibiotics	Zone of Inhibition (mm)		
		MPSK 22	MPSK 23	MPSK 186
1.	Amikacin (30 µg)	**26 S**	**19 S**	**15 I**
2.	Amoxyclav(30 µg)	**14 I**	**15 I**	**18 S**
3.	Ampicillin: Sulbactam (20 µg)	**25 S**	**15 S**	**23 S**
4.	Cephalexin (30 µg)	**18 S**	**21 S**	**18 S**
5.	Cephalothin (30 µg)	**17 I**	**15 I**	**17 I**
6.	Cephotaxime (30 µg)	11 R	11 R	10 R
7.	Chloramphenicol (30 µg)	**18 S**	**18 S**	**27 S**
8.	Ciprofloxacin (5 µg)	**24 S**	**27 S**	**24 S**
9.	Clindamycin (2 µg)	**24 S**	**22 S**	**17 I**
10.	Co-Trimoxazole (25 µg)	**29 S**	**28 S**	**15 I**
11.	Doxycycline hydrochloride (30 µg)	**27 S**	**31 S**	**27 S**
12.	Erythromycin (15 µg)	**15 I**	**18 I**	**25 S**
13.	Gentamycin (10 µg)	**20 S**	**20 S**	**19 S**
14.	Kanamycin (30 µg)	**20 S**	**20 S**	**17 I**
15.	Levofloxacin (5 µg)	**30 S**	**27 S**	**26 S**
16.	Lincomycin (10 µg)	**18 S**	**20 S**	**12 I**
17.	Nalidixic Acid (30 µg)	**20 S**	**19 S**	**21 S**
18.	Neomycin (30 µg)	**14 I**	**14 I**	11 R
19.	Nitrofurantoin (300 µg)	**20 S**	**21 S**	12 R
20.	Ofloxacin (5 µg)	**28 S**	**26 S**	**22 S**
21.	Streptomycin (10 µg)	**15 S**	**15 S**	**12 I**
22.	Tetracycline (30 µg)	**28 S**	**33 S**	**24 S**
23.	Tobramycin (10 µg)	**22 S**	**16 S**	**15 S**
24.	Vancomycin (30 µg)	**16 I**	**15 I**	**15 I**

Superscript: 'S' represents susceptible, 'I' represents intermediate and 'R' represents resistant (according to the Clinical and Laboratory Standards institute, 2013)

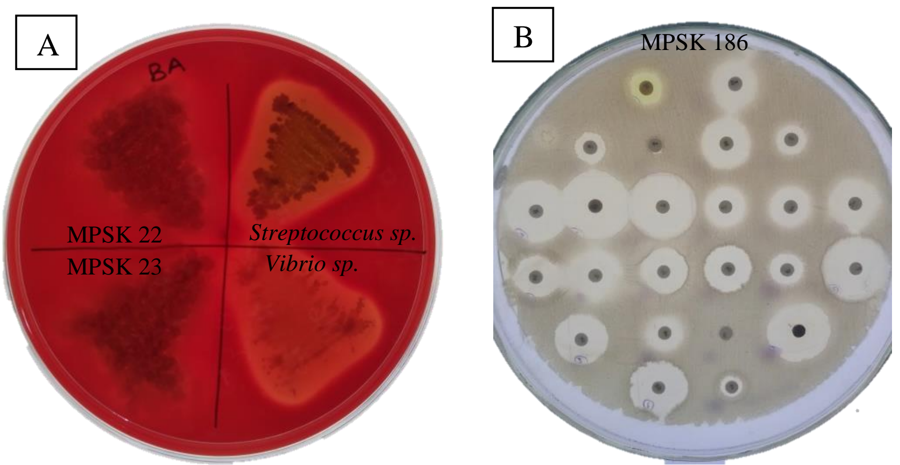


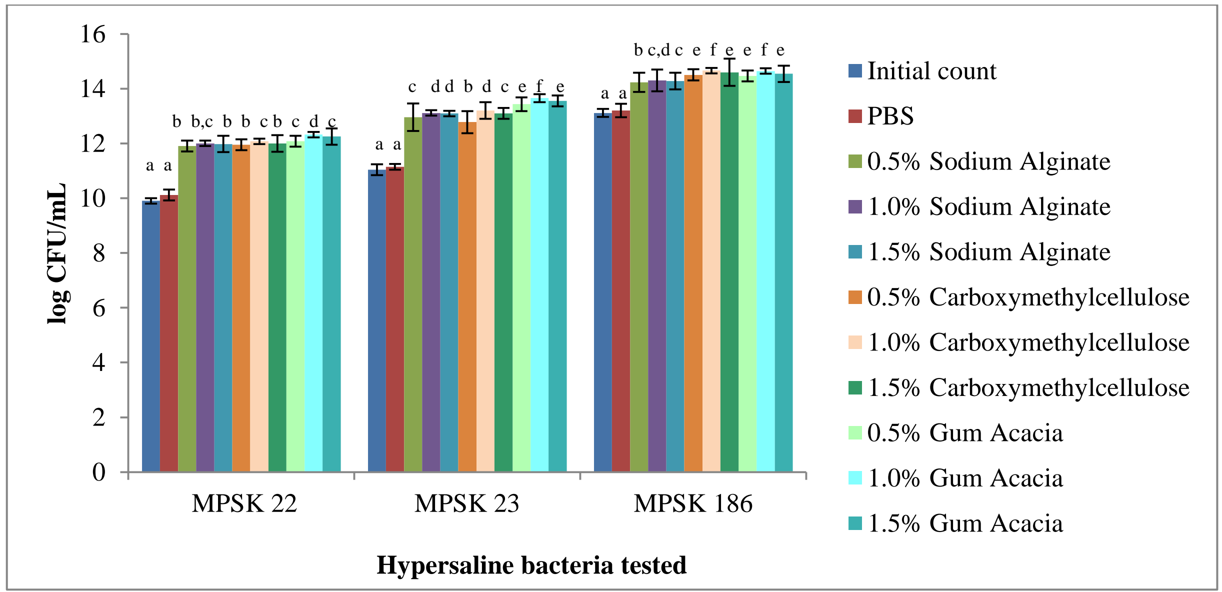
Plate 19: Primary safety evaluation by Blood agar hemolysis (A) and Antibiotic sensitivity test (B) of MPSK 22, MPSK 23 and MPSK 186

4.11 Effect of bioagents MPSK 22, MPSK 23 and MPSK 186 on the *in-vitro* germination of chilli seeds

For coating the cells of the salt pan bacteria MPSK 22, MPSK23 and MPSK 186 onto the seeds of the Khola and Sitara variety of chilli, three biopolymers sodium alginate, carboxymethylcellulose and gum acacia were selected as adjuvants.

4.11.1 Bioagent - Biopolymer compatibility assessment

Sodium alginate (SA), carboxymethylcellulose (CMC) and gum acacia (GA) showed compatibility to the cells of bioagents MPSK 22, MPSK 23 and MPSK 186 and increased the cell viability from the initial count, after 24 h of incubation as compared to the control (Figure 7). Among the three biopolymers, GA (1% w: v) reported significant increment ($p < 0.05$) in viable bacterial count of MPSK 22 (2.1×10^{12} CFU/mL) from an initial count of 0.8×10^{10} CFU/mL. Similarly it also increased the viable count of MPSK 23 from $1. \times 10^{11}$ CFU/mL to 4.5×10^{13} CFU/mL. Whereas in case of MPSK 186, maximum viable count was observed with 1 % CMC (4.6×10^{14} CFU/mL) as compared to initial count of 1.3×10^{13} CFU/mL. However, the increase in the viable count of MPSK 186 with 1 % CMC was significantly similar ($p > 0.05$) to that of 1 % GA (4.4×10^{14} CFU/mL). Thus 1 % GA was evaluated as the best biopolymer, since the proliferation of the hypersaline bacteria was maximal in comparison to the other biopolymers evaluated.



Different letters represent statistical significance for respective isolates (ANOVA $p < 0.05$), followed by Duncan's Multiple Range Test (DMRT) post hoc test (n=3)

Figure 7: Initial and final viable count of MPSK 22, MPSK 23 and MPSK 186 after incubating with different biopolymers as compared to control

4.11.2 Coating efficacy of 1 % gum acacia in seed biopriming

The coating efficacy of 1 % GA was calculated by coating the cell pellet of MPSK 22, MPSK 23 and MPSK 186 onto the Khola and Sitara seeds of chilli and tabulated (Table 19). It was observed that 1 % GA successfully coated > 95 % of the total cells of MPSK 22, MPSK 23 and MPSK 186 cells onto both Khola and Sitara seeds (Plate 20).

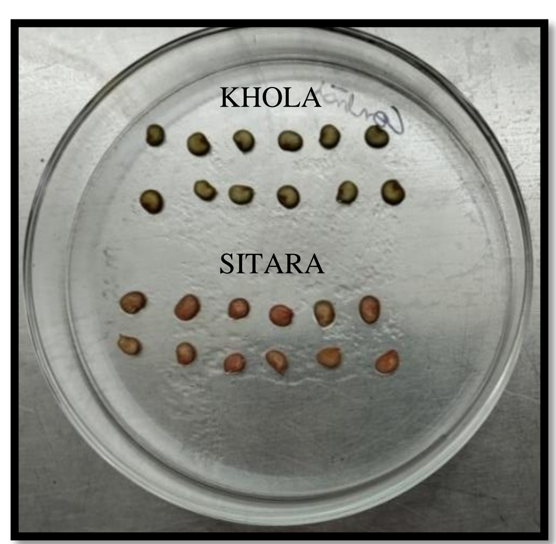


Plate 20: Khola and Sitara seeds coated with the cells of MPSK 23 using 1 % Gum acacia

Table 19: Coating efficacy (E %) of 1 % Gum acacia to coat chilli seeds with bacterial cells

Bioagent-Biopolymer	E (%) Khola seeds	E (%) Sitara seeds
MPSK 22 + 1% GA	**97.85** ± 1.03	**95.91** ± 1.63
MPSK 23 + 1% GA	**98.22** ± 0.07	**96.52** ± 1.08
MPSK 186 + 1% GA	**98.09** ± 0.05	**97.74** ± 0.09

Each value is the mean ± SD of three replicates (n=3)

4.11.3 Germination and growth of chilli seeds exposed to *F. solani* spores after coating with bioagent-biopolymer

Plate 21 represents the coated seeds of chilli germinated after fungal spore exposure in growth chamber with the wet paper towel. MPSK 23 showed the maximum Germination Percentage (GP, 100 %) in case of both Khola and Sitara variety, wherein all the 15 seeds successfully germinated at the end of 30 days. It was followed by MPSK 22 with 80 % GP in case of Khola seeds and 86.7 % GP in Sitara. MPSK 186 showed the least GP among the bacterial treatment (60 % in Khola and 66.7 % in Sitara). Only 4 seeds out of 15 of Khola variety germinated in the control with 26.7 % GP and 1 in Sitara with 20 % GP (Figure 8). Thus all the three bacterial treatments exhibited significantly higher GP ($p < 0.05$) as compared to control.

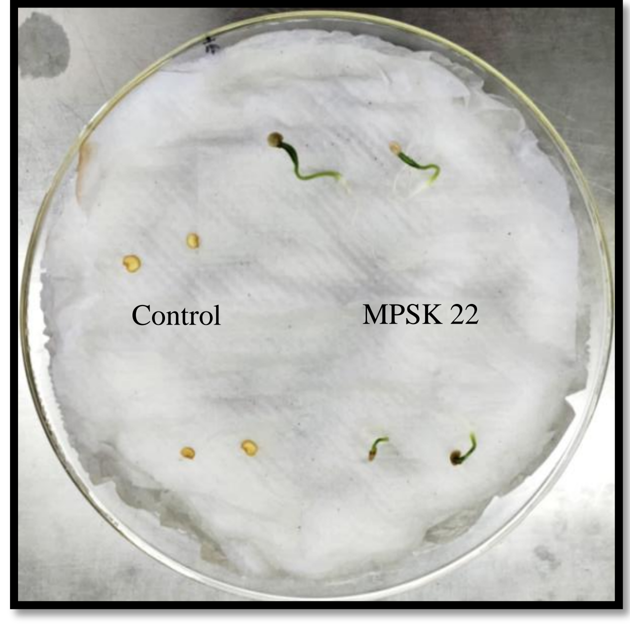
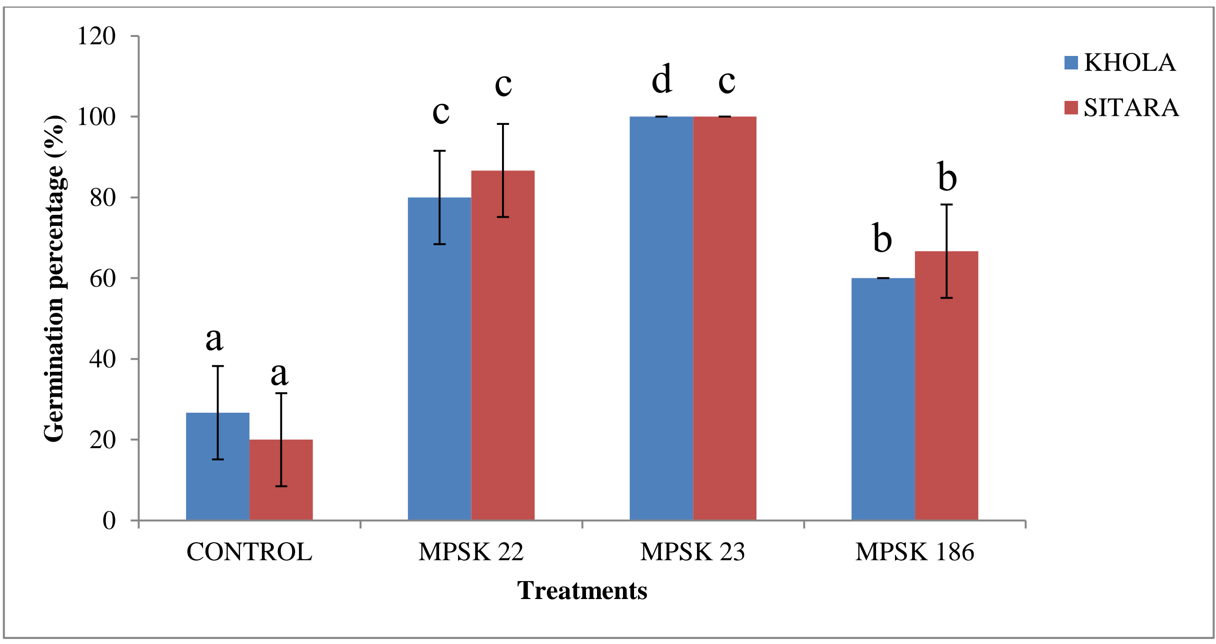


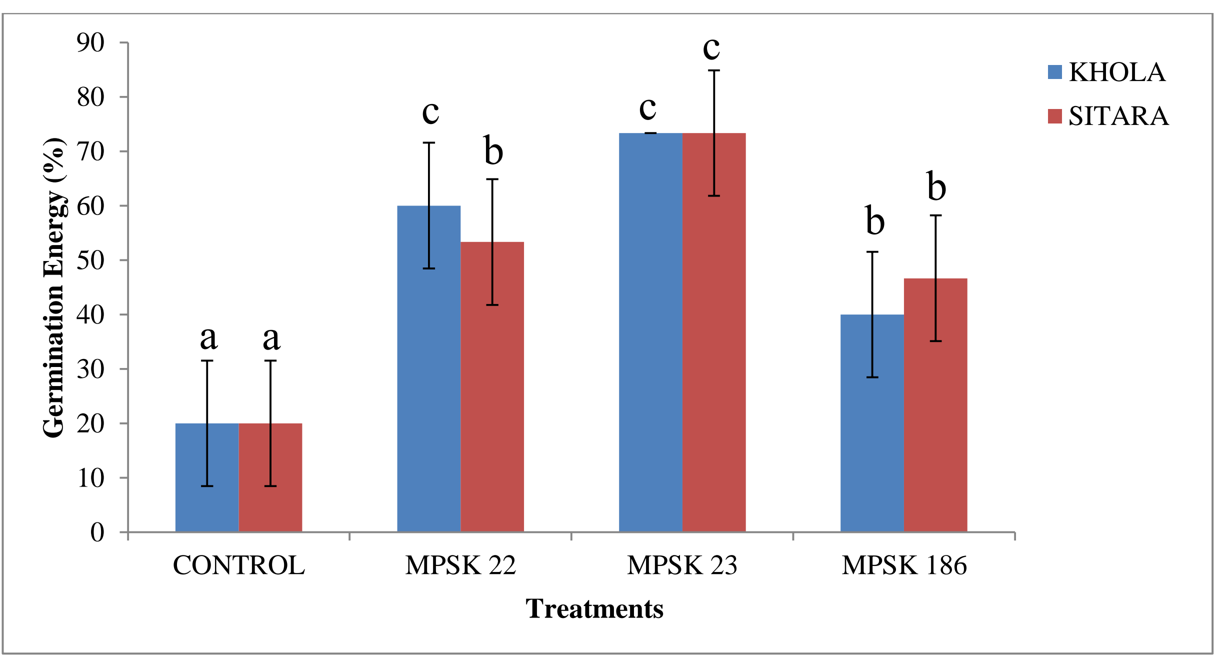
Plate 21: Growth chamber containing the wet paper towel for germination of seeds



Different letters represent statistical significance for respective treatments (ANOVA, $p < 0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT).

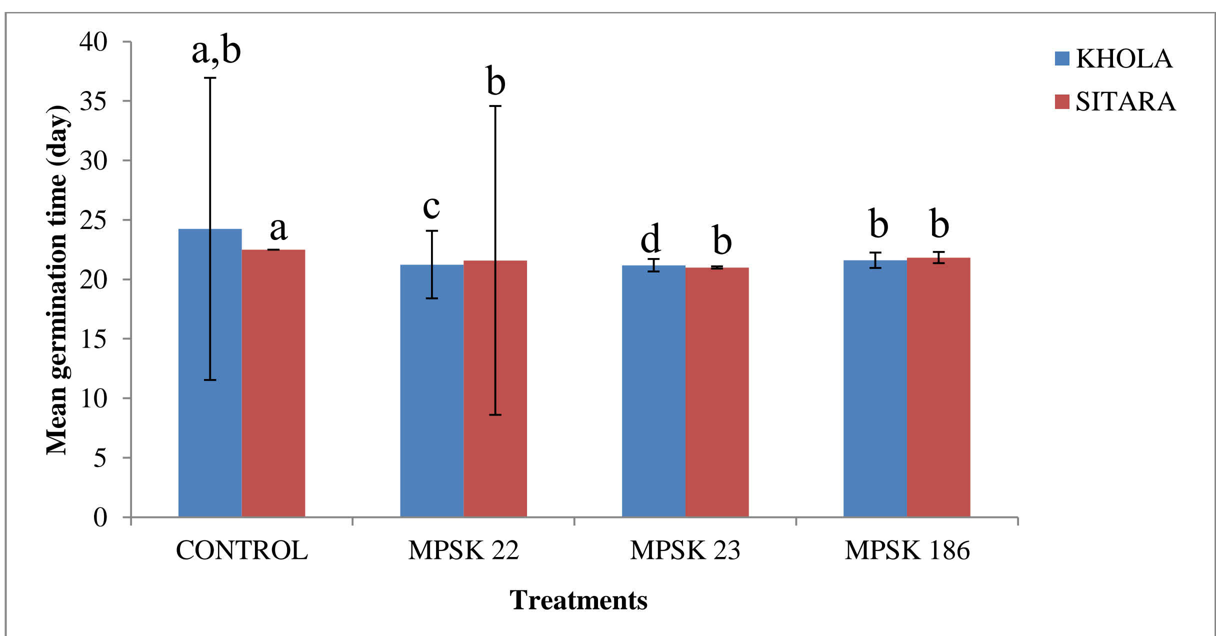
Figure 8: Graph of Germination Percentage (GP) of Khola and Sitara seeds

Next, Germination Energy (GE) was analyzed which accounts the number of seeds germinated on the day where at least 1 seed of every set has germinated. For Khola seeds the GE was calculated on the 15th day when one seed from control showed germination. On the same day 11, 9 and 6 coated seeds of MPSK 23, MPSK 22 and MPSK 186 respectively showed germination. Similarly for Sitara GE on 14th day was calculated with 11, 8 and 7 seeds coated with MPSK 23, MPSK 22 and MPSK 186 germinating respectively (Figure 9). Thus all the three bacterial treatments exhibited significantly higher GE ($p < 0.05$) as compared to control. The Mean Germination Time (MGT) was statistically lesser ($p < 0.05$) in case of bacterial coatings as compared to the control seeds. Khola seeds primed with MPSK 23 germinated on the 9th day and seeds primed with MPSK 22 and MPSK 186 germinated on the 10th day. A similar trend was seen in the Sitara variety, with MPSK 23 coated seeds germinating on 8th day, MPSK 22 seeds germinating on 10th day and MPSK 186 treated seeds germinating on the 11th day (Figure 10).



Different letters represent statistical significance for respective treatments (ANOVA, $p < 0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT).

Figure 9: Graph of Germination Energy of Khola and Sitara seeds



Different letters represent statistical significance for respective treatments (ANOVA, $p< 0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT).

Figure 10: Graph of Mean Germination Time of Khola and Sitara seeds

Both the hypocotyl and the root length of the seedling emerged from the seeds primed with hypersaline bacteria MPSK 23 were statistically higher than that of other hypersaline bacteria and the control ($p < 0.05$, Plate 21). The total length of the seedlings emerged from the seeds coated with MPSK 23 (7.59 ± 0.63 cm of Khola and 7.81± 0.27 cm of Sitara) was higher than the other bacterial isolates (MPSK 22 primed: 4.36 ± 0.90 cm of Khola and 3.09 ± 0.31 cm of Sitara seedling and MPSK 186 primed: 3.93 ± 0.56 cm of Khola and 4.06 ± 0.32 cm of Sitara seedling) and control (1.13 ± 0.55 cm of Khola and 1.5 ± 0.55 cm of Sitara) (Figure 11). Finally the seed vigour index of Khola and Sitara variety was calculated from the seedling length and GP. The vigour index of the seeds primed with MPSK 23 was statistically ($p < 0.05$) much higher (760 in the case of Khola seeds and 780.7 in the case of Sitara seeds) than that of MPSK 22 (348.7 in the case of Khola seeds and 343.3 in the case of Sitara seeds), MPSK 186 (236.7 in the case of Khola seeds and 270.7 in the case of Sitara seeds) and that of the control (30 for both Khola and Sitara seeds) (Figure 12). Thus MPSK 23 was selected for *in-vivo* trials against *F. solani* infection in Khola and Sitara varieties of chilli plant.

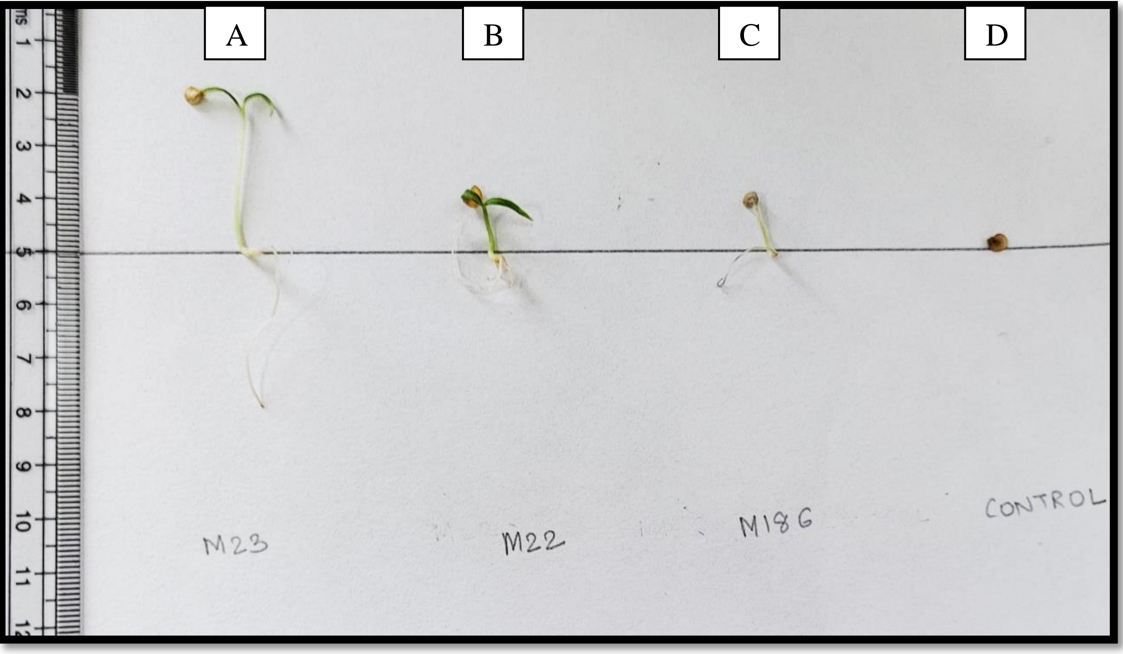
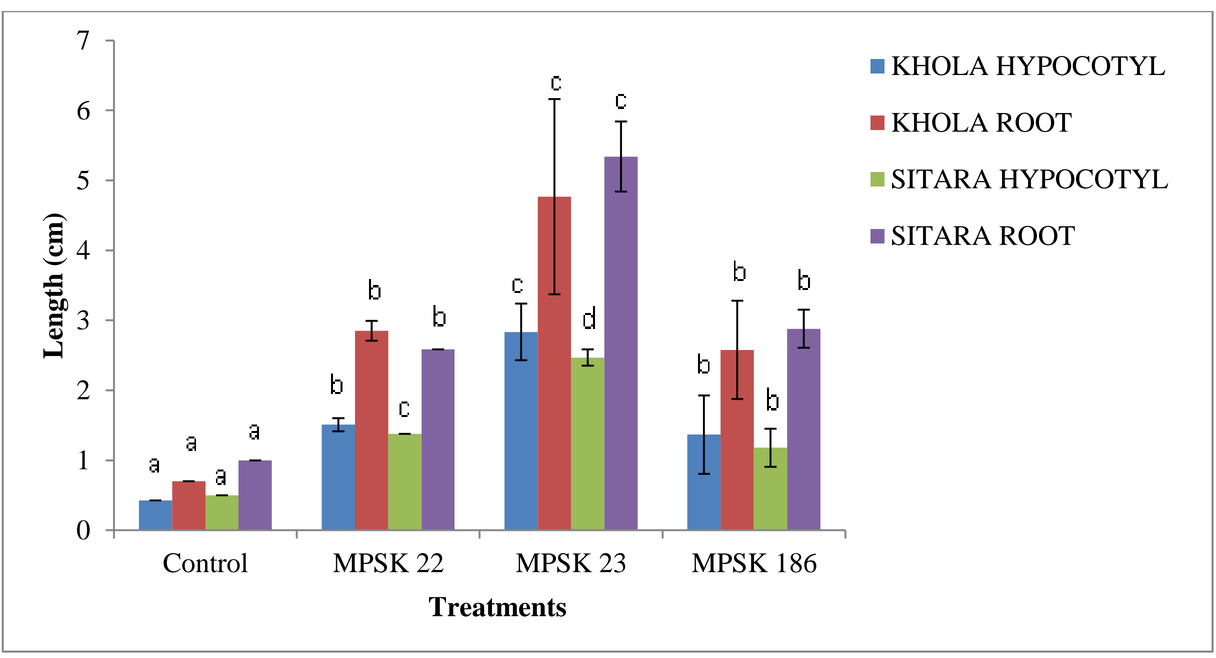
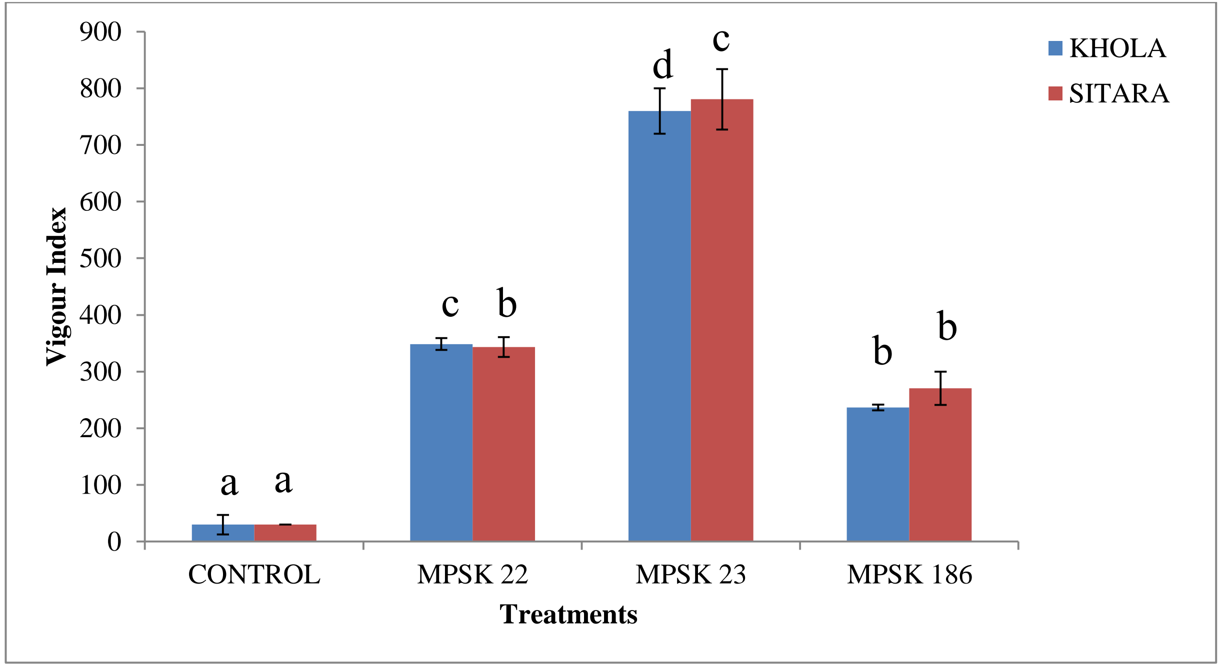


Plate 21: Growth chamber containing the wet paper towel for germination of seeds coated with cells of MPSK 23 (A), MPSK 22 (B), MPSK 186 (C) and control PBS coated seeds (D)



Different letters represent statistical significance for respective treatments (ANOVA, p<0.05, n=5), followed by Duncan's Multiple Range Test (DMRT).

Figure 11: Graph of Hypocotyl and Root length of germinated Khola and Sitara seedlings

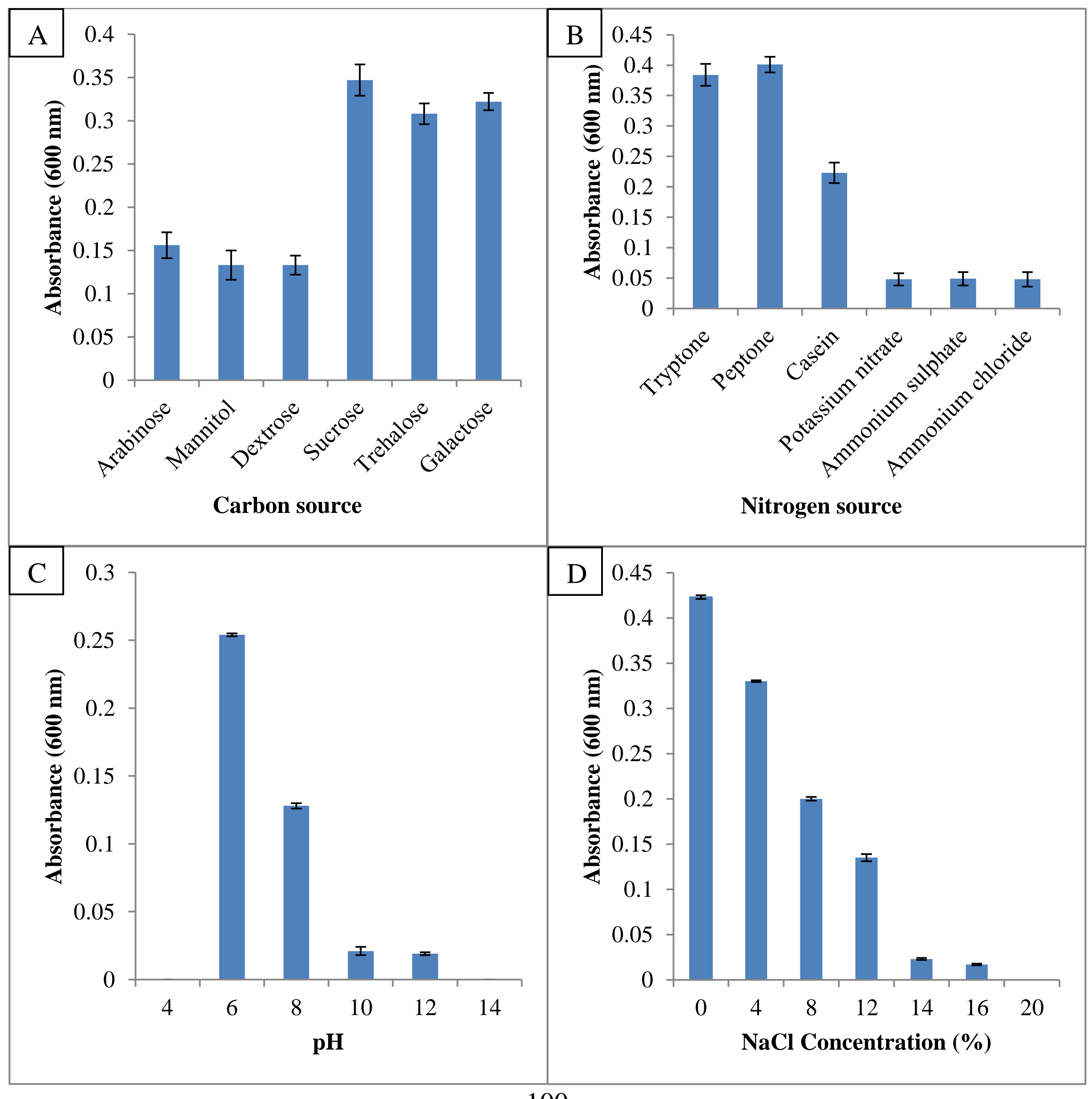


Different letters represent statistical significance for respective treatments (ANOVA, p<0.05, n=5), followed by Duncan's Multiple Range Test (DMRT).

Figure 12: Graph of Vigour Index of Khola and Sitara seeds

4.12 Growth of MPSK 23 in different carbon and nitrogen sources at varying salinity, pH and temperature

The hypersaline bacterium MPSK 23 was further studied for its growth characteristics. Among the various C sources tested, sucrose supported the maximum growth of MPSK 23 (Figure 13 A) and among the various N sources tested; peptone was the preferred source (Figure 13 B). MPSK 23 could tolerate a pH range of 6 to 12, showing an optimal growth at pH 6 (Figure 13 C). When assessed for salinity tolerance, the isolate exhibited growth from 0 - 16 % NaCl with optimal growth at 0 % NaCl indicating MPSK 23 was a halotolerant bacterium (Figure 13 D). Further the maximum temperature showing growth of MPSK 23 was 50 °C, however it could also grow at 10 °C which was the minimal growth temperature tolerated. The optimal growth of MPSK 23 was measured at 40 °C (Figure 13 E).



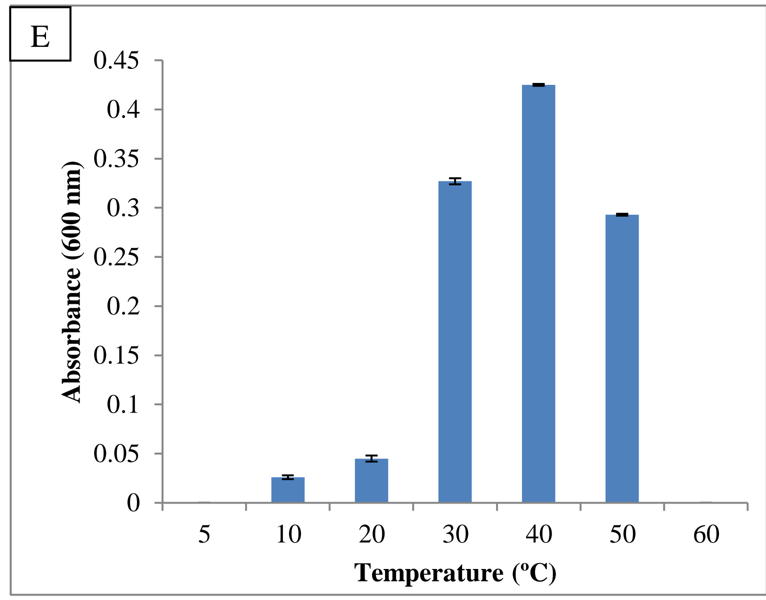


Figure 13: Growth of the hypersaline bacterium MPSK 23 at varying carbon sources (A), nitrogen sources (B), pH (C), NaCl concentrations (D) and temperatures (E)

4.13 Growth profile of MPSK 23 and the antifungal activity

MPSK 23 was assessed for its growth and production of the antifungal metabolite by the growth curve (Figure 14). A sigmoidal growth curve was observed with a lag phase which lasted for about 6 h, after which the log phase initiated and culminated within 24 h into the stationary phase. The growth was assessed till 48 h till which consistent optical density was observed. Maximum antifungal activity (20 mm zone of inhibition against *F. solani*) was observed between 24 - 32 h which related to the stationary phase of the growth curve of MPSK 23.

4.14 The stability and viability of MPSK 23 in soil

When assessed for its growth in the actual soil, an increase in the viable count from the initial 10^{10} CFU/mL of MPSK 23 was observed for a period of 96 h (12.1×10^{12} CFU/mL), after which a steady minimal decline until 40 days was observed (66.31×10^{9} CFU/mL). Further a substantial decrease in viability was observed; however the culture remained viable even after 60 days in low numbers (2.5×10^{7} CFU/mL) in the same soil (Figure 15).

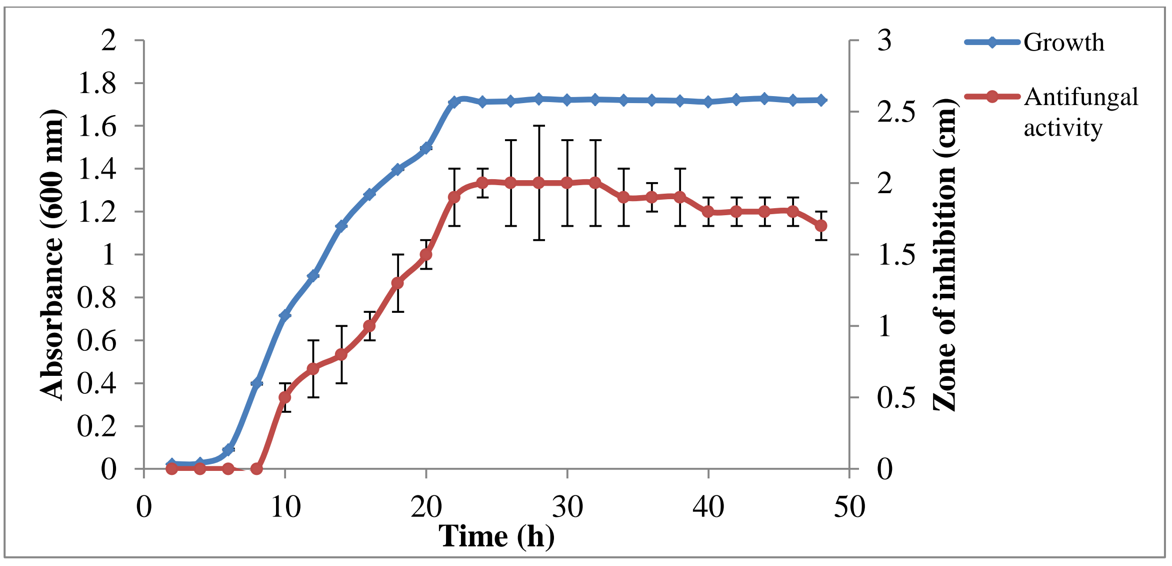


Figure 14: Growth profile and antifungal activity exhibited by the bacterium MPSK 23

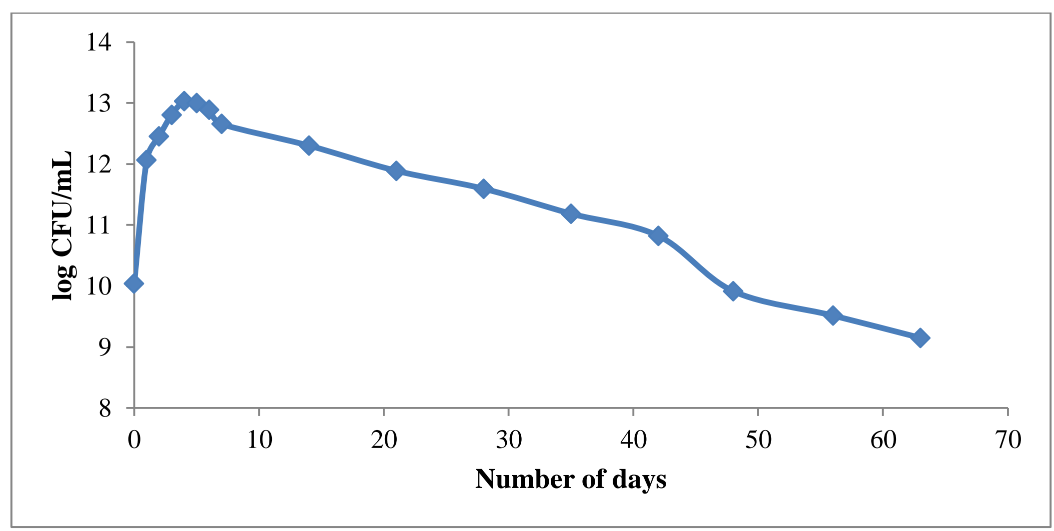
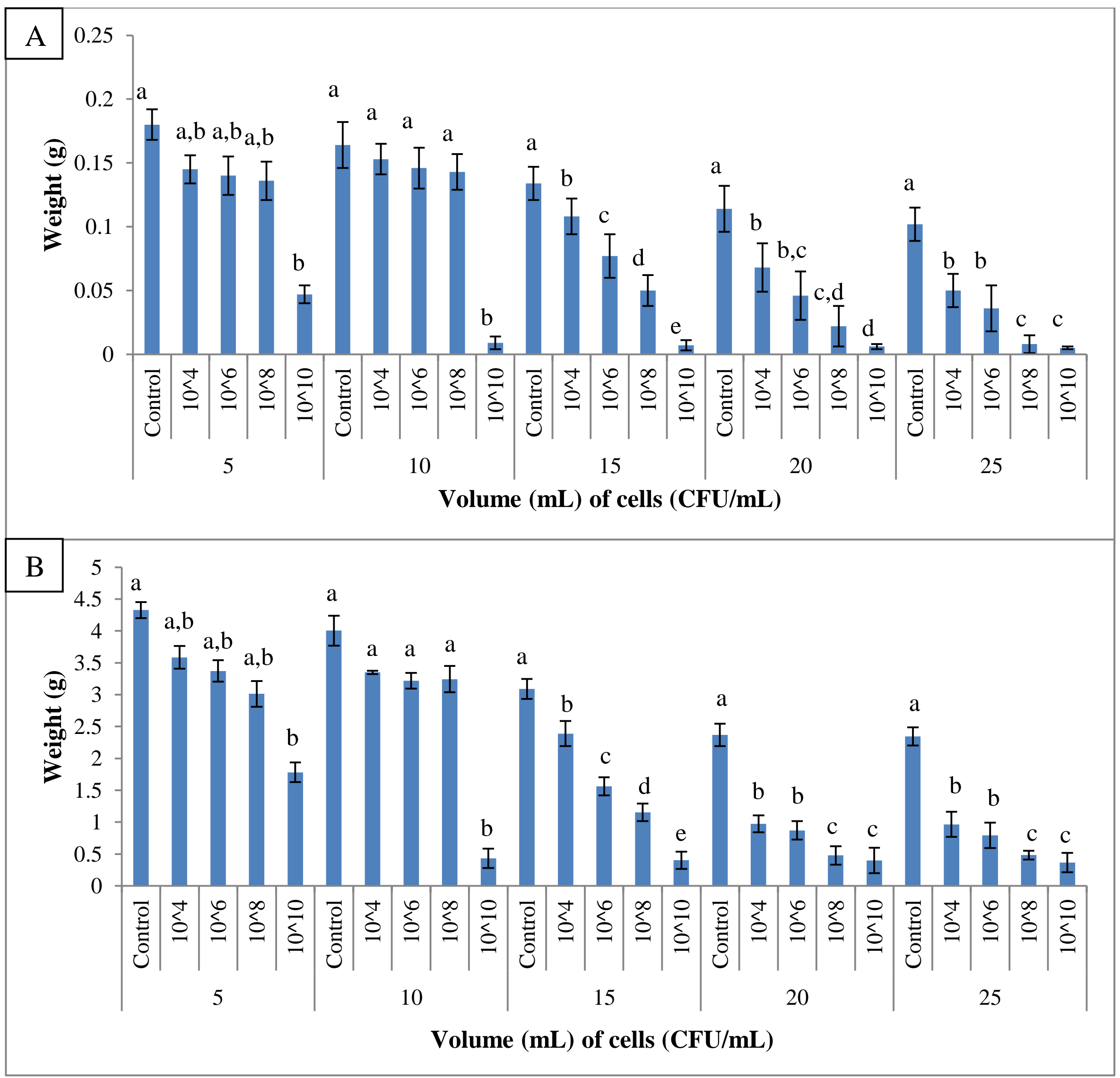


Figure 15: Viable count of the hypersaline bacterium MPSK 23 in the soil

4.15 Inhibition of *F. solani* by MPSK 23 i*n-vitro*

The co-inoculation of the fungi and the bacterium in liquid broth resulted in decrease of both the dry and wet weight of the *F. solani* mycelia. This decrease was inversely proportional to the volume of the viable cells of MPSK 23 added (Figure 16). As seen in the graph, when 5 or 10 mL of the MPSK 23 cells with the viable count of 10^4, 10^6 and 10^8 CFU/mL were added separately, the fungal weight (wet and dry) did not decrease significantly ($p < 0.05$) as compared to the control. However, 5 and 10 mL of 10^{10} CFU/mL viable cells did show a significant decrease. Remaining volumes (15, 20

and 25 mL) of viable count 10^4, 10^6 and 10^8 CFU/mL exhibited decrease in the fungal mycelial weight but the highest inhibition of *F. solani* (% inhibition) was observed in 25 mL of 10^{10} CFU/mL of MPSK 23. This volume of 10^{10} CFU/mL resulted in 94.71% decrease in the wet weight and 94.12 % decrease in the dry weight of the fungus as compared to the control. However, 10 mL of 10^{10} CFU/mL viable cells also showed a statistically ($p > 0.05$) similar decrease (93.45 % decrease in wet weight and 90.56 % decrease in dry weight), therefore was selected as the best concentration of MPSK 23 for *in-vivo* trials.



Different letters represent statistical significance for respective volume (ANOVA, $p<0.05$, n=3), followed by Duncan's Multiple Range Test (DMRT).

Figure 16: Weight of the *F. solani* measured as dry weight (A) and wet weight (B) in presence of MPSK 23 in liquid broth

4.16 Pathogenicity of *F. solani* in Khola and Sitara chilli cultivars

Agricultural soil on inoculation with fungal spores of *F. solani* was converted into sick soil after 5 days of incubation. When transplanted into this soil, within 15 days chilli seedlings of both the varieties showed manifestation of the disease as leaves of the plants started wilting. All the 15 seedlings of Sitara variety wilted gradually, dying at the end of 15 days, whereas one of Khola seedling survived the infection. The pathogen was then successfully re-isolated from the collar region of the diseased plants on PDA; where the growth of the white mycelia was observed after 5 days of incubation. Further, staining of this fungal mycelia exhibited the characteristic curved spores of *Fusarium* (macroconidia), confirming the pathogenicity of *F. solani* in both the chilli varieties (Plate 22).

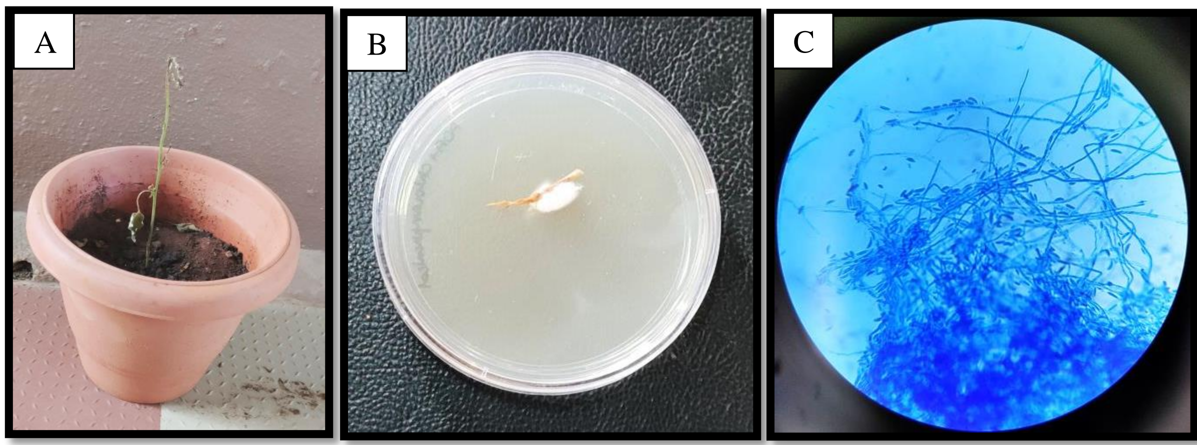


Plate 22: Pathogenesis of *F. solani* exhibiting wilting symptoms in chilli plant (A), re-isolation of the fungus (B) and lactophenol cotton blue staining of the fungus (C)

4.17 Establishing the potential of MPSK 23 as a biocontrol agent by *in-vivo* pot trials

The pot trials were carried out at the polyhouse of Don Bosco College of Agriculture-Sulcorna, Goa; wherein the three pots of each treatment were arranged in completely randomized design (Plate 23). Table 20 summarizes the characteristics of the soil used for the experiments before turning it into the sick soil.

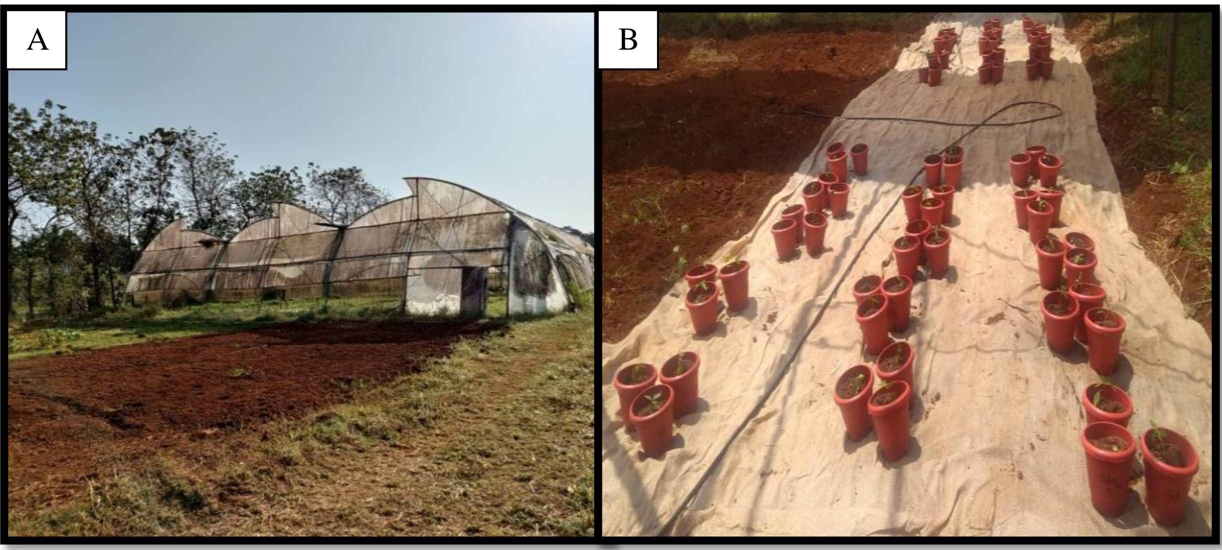


Plate 23: The polyhouse used for the pot trials (A) and the arrangement of the pots in polyhouse (B)

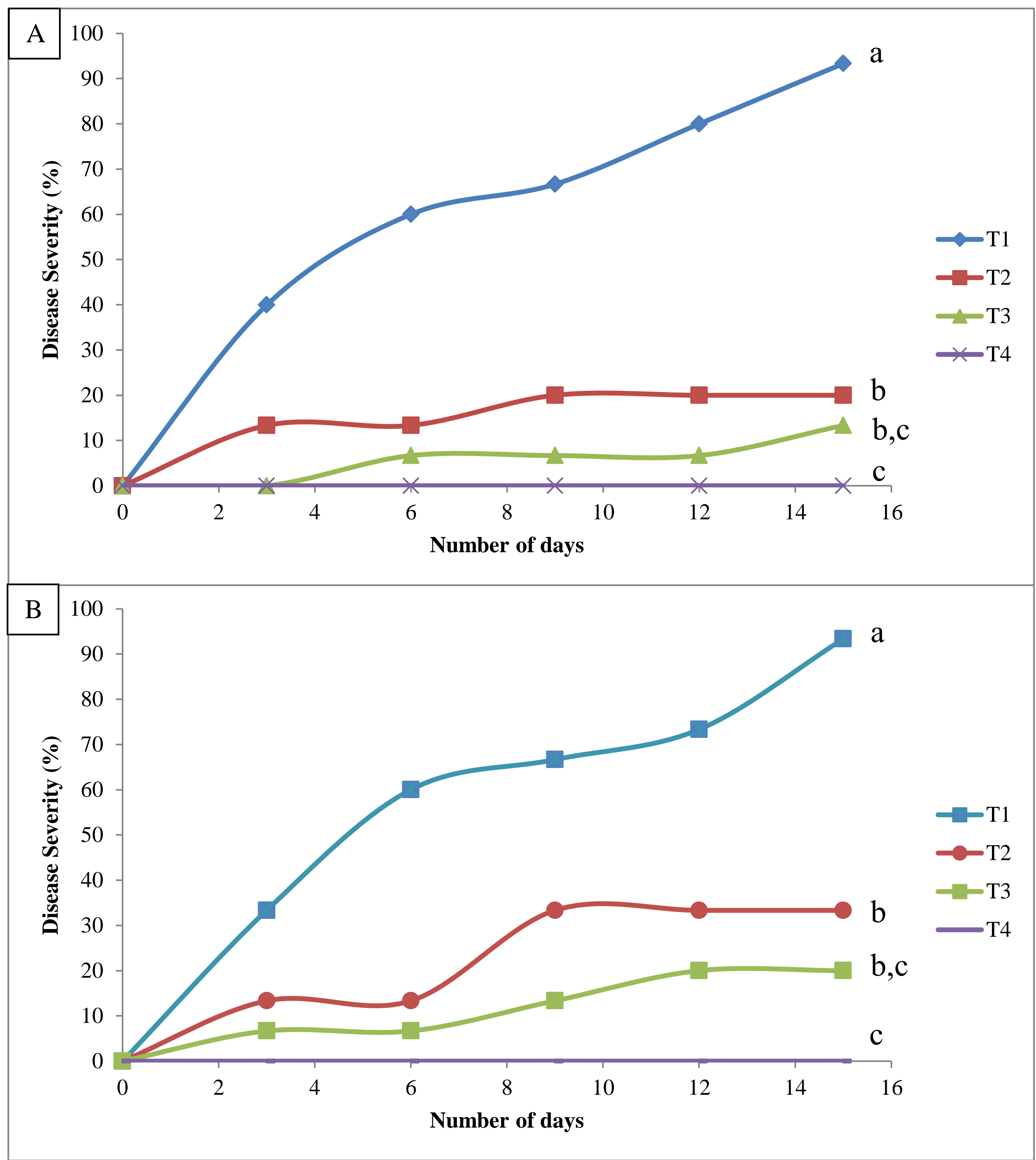
Table 20: Characteristics of the agricultural soil used in the pot trials

pH	EC dS/m	Organic Carbon %	Available N Kg/ha	Available P Kg/ha	Available K Kg/ha
5.41	0.059	2.57	150.5	12.65	163

4.17.1 Experiment I: Regulation of defense enzymes and phenols by MPSK 23 in chilli plants after *F. solani* exposure

a) Severity of wilt symptoms in chilli plants

Disease severity seen as wilting of leaves in chilli plants of both the varieties, increased soon after the pathogen exposure in the plants of the control group T1 (buffer). At the end of 15 days, 93.33 % disease severity was observed in T1 of Khola variety and 95.56 % disease severity was observed in T1 of Sitara variety. Disease severity in biological control group (T2) and chemical control group (T3) of both the varieties also increased after the pathogen exposure but the increase was significantly lesser ($p < 0.05$) as compared to T1. In the last treatment group (T4), where hypersaline bacterium MPSK 23 was applied before transplantation of the chilli plant, did not show any wilting symptom during the study period (15 days). Thus the severity of disease was the least (almost negligible) in both the varieties of chillies in this group (Figure 17).

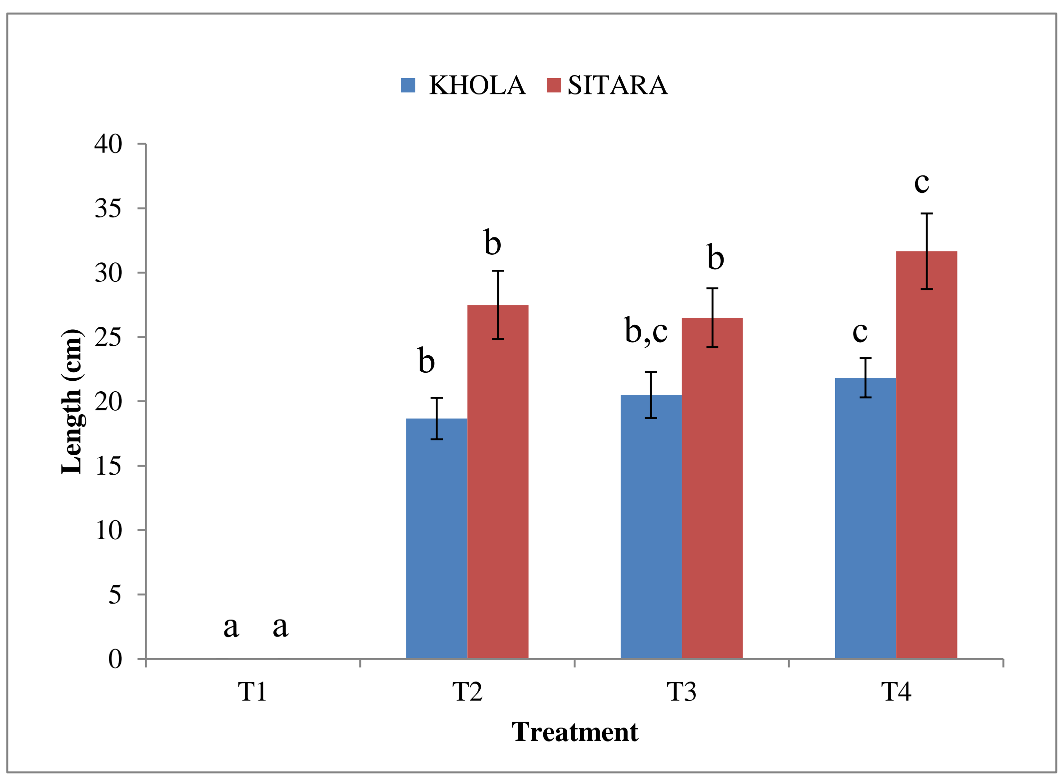


Different letters represent statistical significance for respective days between each treatment (ANOVA, $p<0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 17: Progression of disease severity (wilting) in Khola (A) and Sitara (B) chilli plant varieties over a period of 15 days

b) Length of the plant at the end of 15 days

At the end of the study period, none of the chilli plants of T1 group survived due to the wilting. Whereas plants from other T2 and T3 treatment group, exhibited the wilting of leaves but survived till the end. T4 treatment group not only evaded the infection but also showed the highest total length which was statistically significant compared to other treatments ($p < 0.05$). The total length (root length + shoot length) of T4 plants of Khola variety was 21.8 cm and of Sitara variety was 31.7 cm. After T4; T2 group showed the higher total length as compared to T3 (Khola: T2 18.7 cm and T3 20.5 cm, Sitara: T2 27.5 cm and T3 26.5 cm) (Figure 18).



Different letters represent statistical significance for respective treatments (ANOVA, $p<0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 18: Length of chilli plants after 15 days of pathogen exposure to *F. solani*

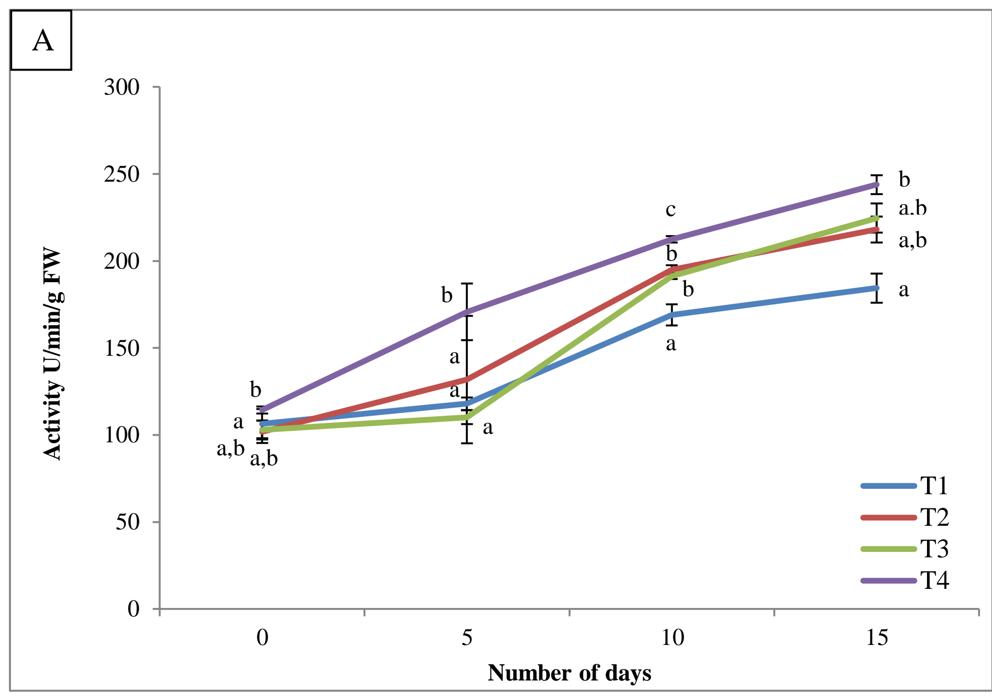
c) **Defense enzymes and phenols produced in plants of different treatments**

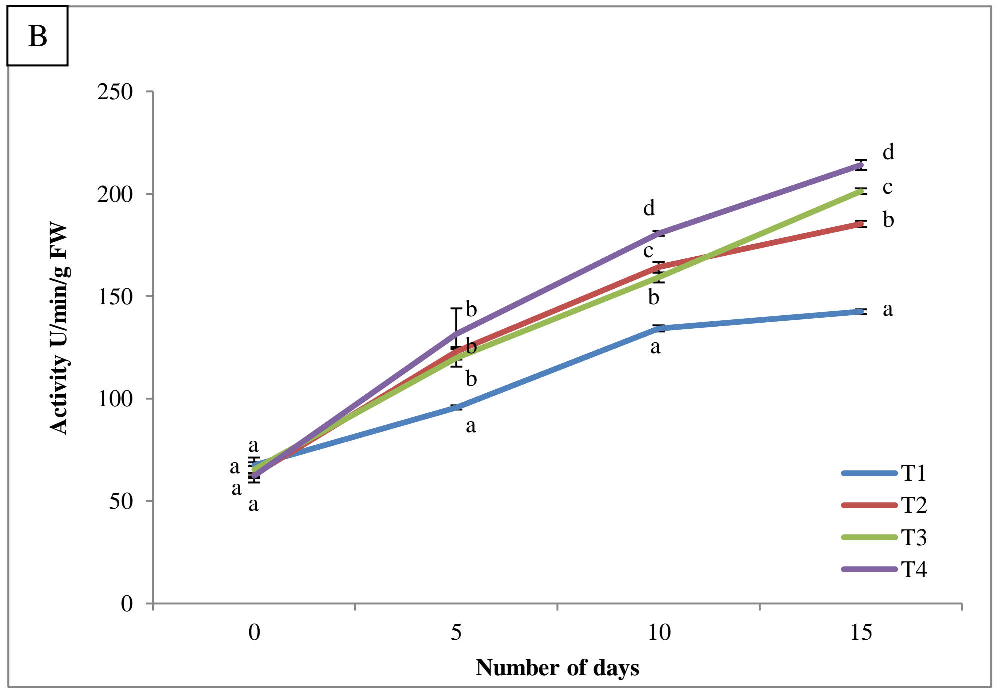
i. Phenylalanine ammonia-lyase (PAL)

An increase in PAL activity in the plant leaves of all the treatment groups was observed subsequent to the pathogen exposure and the trend of enzyme activity was similar in both the varieties. In case of T1 treated plants, an initial increase in PAL activity was observed but, at day 15 compared to other treatments, the enzyme activity was significantly lower ($p<0.05$) (Khola: 184.45 U/min/g FW and Sitara: 142.45 U/min/g FW). Plants of this treatment showed complete wilting at the end of the study period. Whereas accumulation of PAL at Day 15, in the plants with T4 treatment was significantly higher ($p<0.05$) which was 243.91 U/min/g FW in Khola and 214.04 U/min/g FW in Sitara variety, as compared to T3 and T2 treatments respectively (Khola T3: 224.68 U/min/g FW, Khola T2: 218.12 U/min/g FW, Sitara T3: 201.20 U/min/g FW and Sitara T2: 185.35 U/min/g FW) (Figure 19).

ii. Peroxidase (PO)

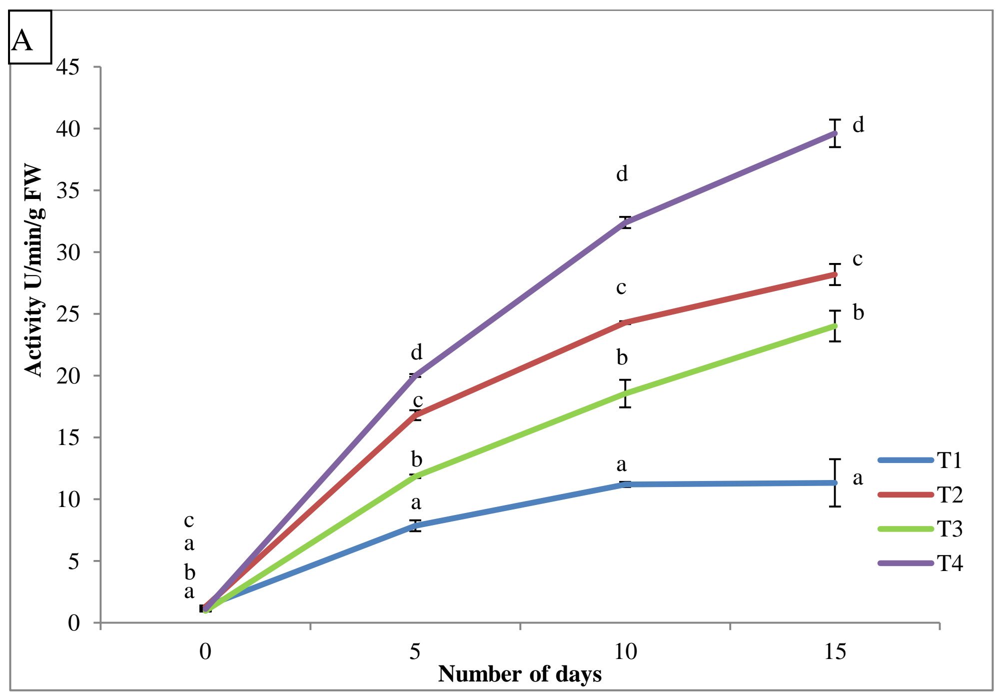
Treatment T4 showed spiked PO activity after the exposure to the pathogen from day 0 in both the varieties. This activity remained significantly higher ($p<0.05$) in T4 treatment as compared to other treatments, up till 15 days (Khola: 39.62 U/min/g FW and Sitara: 26.42 U/min/g FW). After an initial rapid increase in PO activity, T3 and T2 treatments showed a slower increase after 5 days. At day 15, enzyme activity of T2 treated plants was estimated to be 28.20 U/min/g FW in the case of Khola and 20.80 U/min/g FW in the case of Sitara variety and that of T3 treated plants was 24.02 U/min/g FW in the case of Khola and 18.05 U/min/g FW in the case of Sitara variety. In T1, an initial increase in PO activity was observed which thereon remained constant till day 15, where complete wilting was observed (Khola: 11.33 U/min/g FW and Sitara: 10.17 U/min/g FW). At the end of the study period as compared to the T1 control, T4 treatment showed 3.5 fold increase in PO activity in case of Khola variety and 2.5 fold increase in Sitara variety (Figure 20).

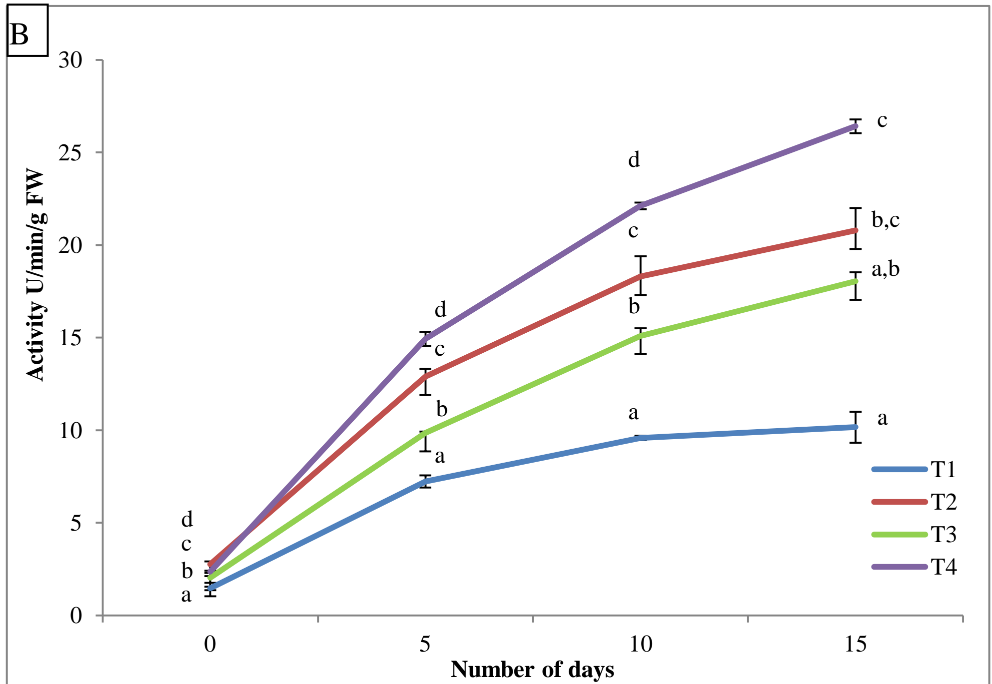




Different letters represent statistical significance for respective days between each treatments (ANOVA, $p<0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 19: Phenylalanine ammonia lyase activity in Khola (A) and Sitara (B) plants over a period of 15 days after pathogen exposure





Different letters represent statistical significance for respective days between each treatments (ANOVA, $p<0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

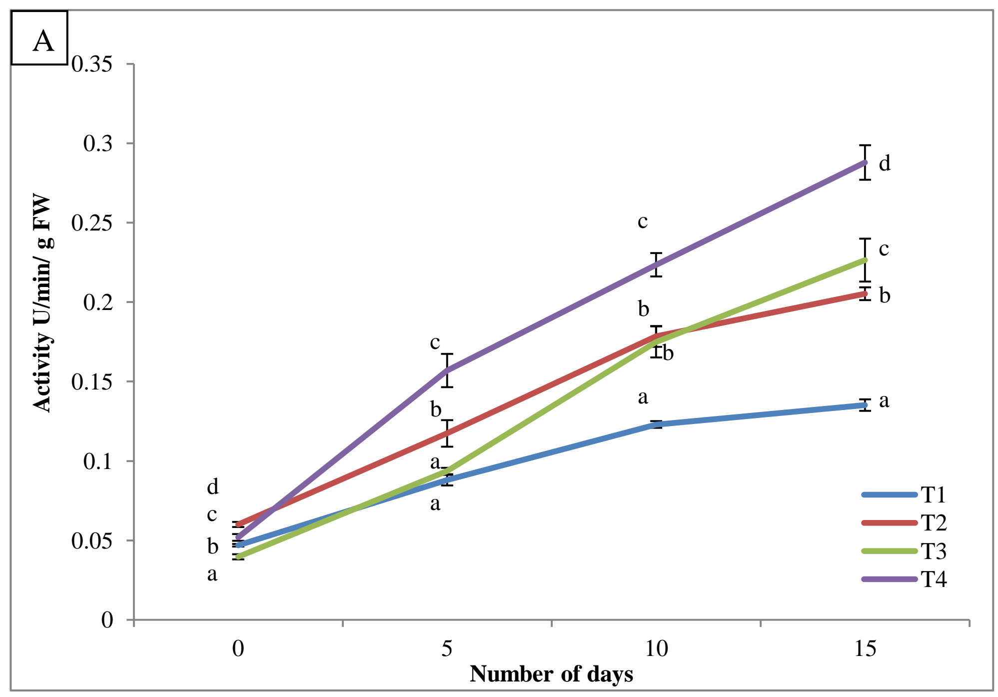
Figure 20: Peroxidase activity in Khola (A) and Sitara (B) plants over a period of 15 days after pathogen exposure

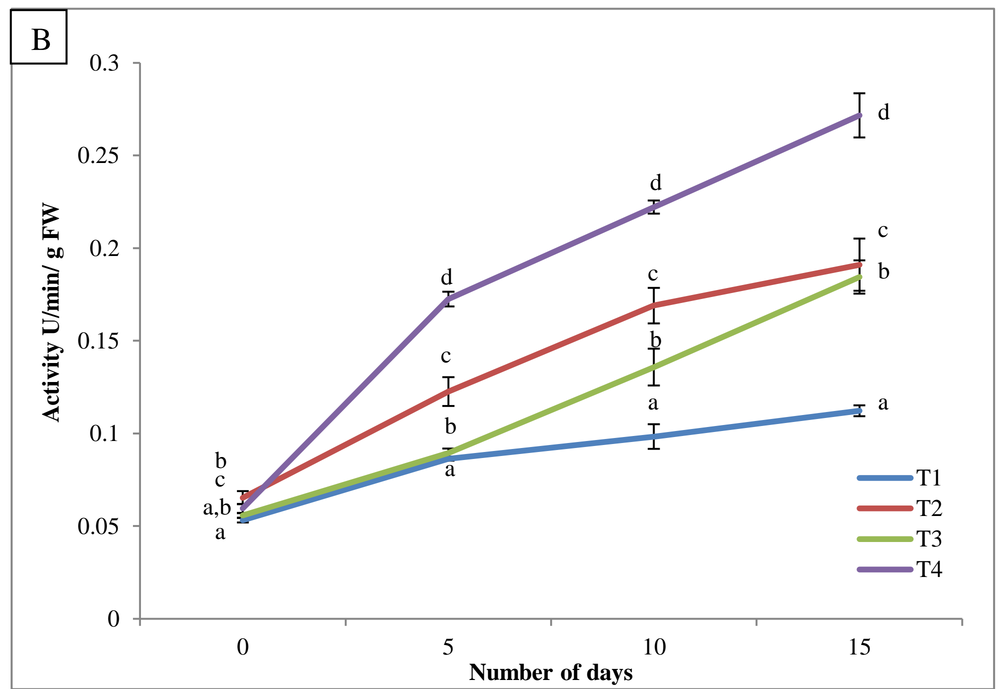
iii. Polyphenol oxidase (PPO)

As observed in the case of first two defense enzymes, the PPO activity was also significantly higher ($p<0.05$) in the plants of T4 treatment group as compared to control T1, T2 and T3 treatments in both the varieties. At the end of 15 days, the enzyme activity in the plants of T4 treatment was calculated to be 0.29 U/min/g FW in the Khola and 0.27 U/min/g FW in the Sitara variety. This was followed by T3 treated plants (0.17 U/min/g FW) in the Khola variety and T2 treated plants (0.19 U/min/g FW) in the Sitara variety. Least activity was observed in the plants with T1 treatment (Khola: 0.14 U/min/g FW and Sitara: 0.11 U/min/g FW), where wilting and death was observed 15 days post pathogen exposure (Figure 21).

iv. Total phenol content (TPC)

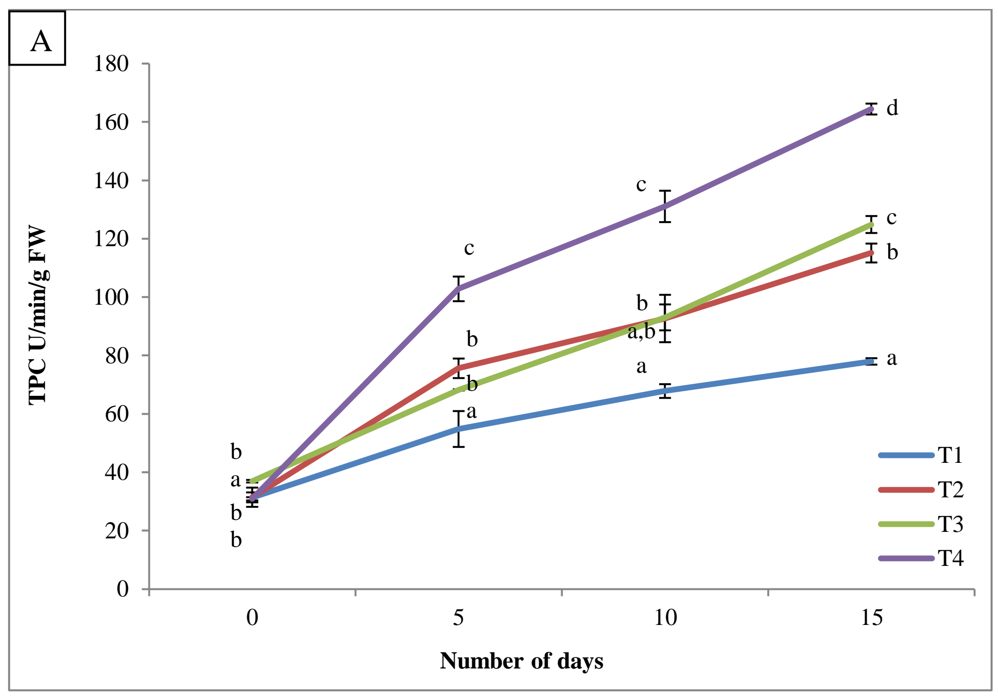
There was an increase in the total phenolic content in the plants of all the treatments after the pathogen exposure; however T1, T2 and T3 treated plants showed steady increase as compared T4 treated plants. Total phenolic content in the plants treated with MPSK 23 (T4), was two times higher in both the varieties as compared to that of PBS control (T1) at the end of 15 days. These values of T4 treated plants (Khola: 164.41 U/min/g FW and Sitara: 167.68 U/min/g FW) were also significantly higher ($p<0.05$) than the plants of T2 (Khola: 115.11 U/min/g FW and Sitara: 130.38 U/min/g FW) and T3 (Khola: 124.86 U/min/g FW and Sitara: 114.88 U/min/g FW) treatment group. T1 group showed steady and least increase in total phenols (Khola: 77.96 U/min/g FW and Sitara: 79.72 U/min/g FW) at day 15 (Figure 22).

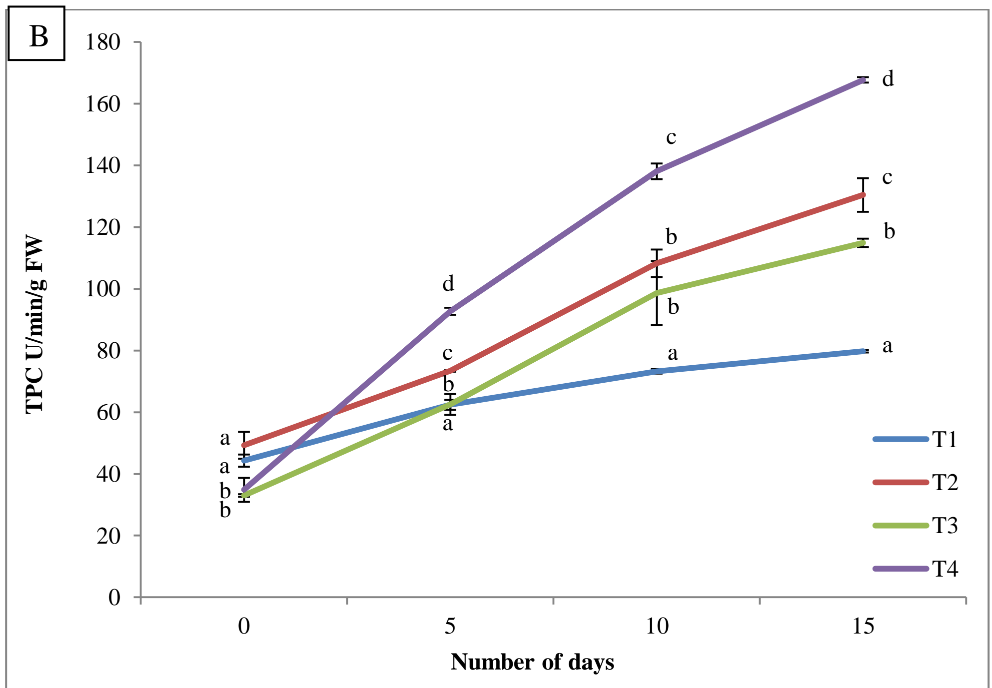




Different letters represent statistical significance for respective days between each treatments (ANOVA, $p<0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 21: Polyphenol oxidase activity in Khola (A) and Sitara (B) plants over a period of 15 days after pathogen exposure





Different letters represent statistical significance for respective days between each treatments (ANOVA, $p<0.05$, n=5), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 22: Total phenolic content in Khola (A) and Sitara (B) plants over a period of 15 days after pathogen exposure

The correlation between the defense enzymes, total phenolic content, disease severity and the total length of the plants analyzed by Pearson's correlation coefficient is presented in Table 21. A positive correlation was seen between defense enzymes and TPC and total length of the plants ($p<0.01$). The disease severity showed negative correlation with all the parameters tested.

Table 21: Correlation matrix of defense enzymes, total phenolic content, disease severity and the plant total length of Khola (A) and Sitara (B) chilli plants in pot trials

(A) Pearson's Correlation						
	PO	PPO	PAL	TPC	Disease severity	Total length
PO	1					
PPO	0.960**	1				
PAL	0.958**	0.991**	1			
TPC	0.965**	0.998**	0.982**	1		
Disease severity	-0.902**	-0.924**	-0.967**	-0.902**	1	
Total length	0.857**	0.882**	0.934**	0.855**	-0.988**	1

**. Correlation is significant at the 0.01 level, n=5

(B) Pearson's Correlation						
	PO	PPO	PAL	TPC	Disease severity	Total length
PO	1					
PPO	0.983**	1				
PAL	0.913**	0.916**	1			
TPC	0.994**	0.991**	0.883**	1		
Disease severity	-0.921**	-0.888**	-0.980**	-0.876**	1	
Total length	0.926**	0.877**	0.957**	0.879**	-0.995**	1

**. Correlation is significant at the 0.01 level, n=5

4.17.2 Experiment II: Suppression of the disease incidence in chilli plants infected with *F. solani* and growth enhancement by MPSK 23

a) Disease assessment

i. Disease incidence and disease suppression

In-vivo bacterial inoculation of chilli seedlings with biocontrol agent MPSK 23 (T4) caused a complete reduction in the disease incidence (100 %) in both the varieties. The efficacy of disease suppression was also prompted following MPSK 23 application in comparison to Trophy (T3) and *Trichoderma* (T2) treated plants. In control PBS treated plants (T1) of Khola variety there was 100% disease incidence, whereas in Sitara variety there was 88.89% disease incidence. There was very low disease suppression in this treatment for both the varieties (Figure 23).

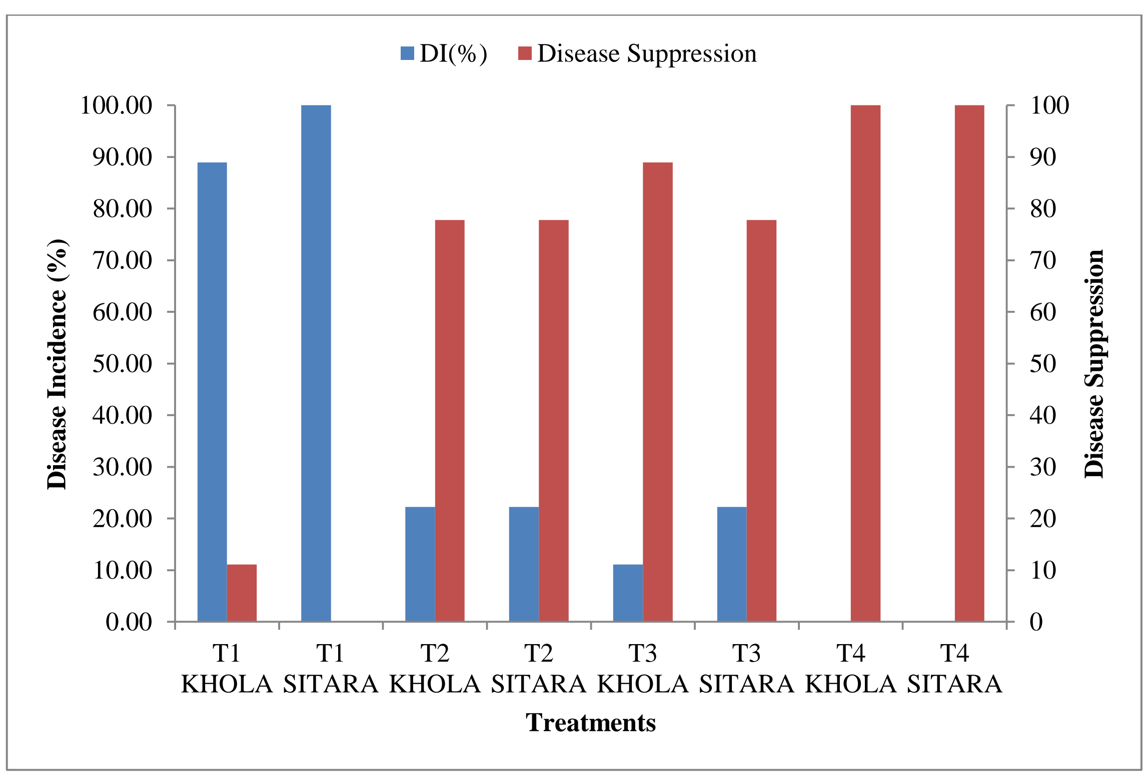
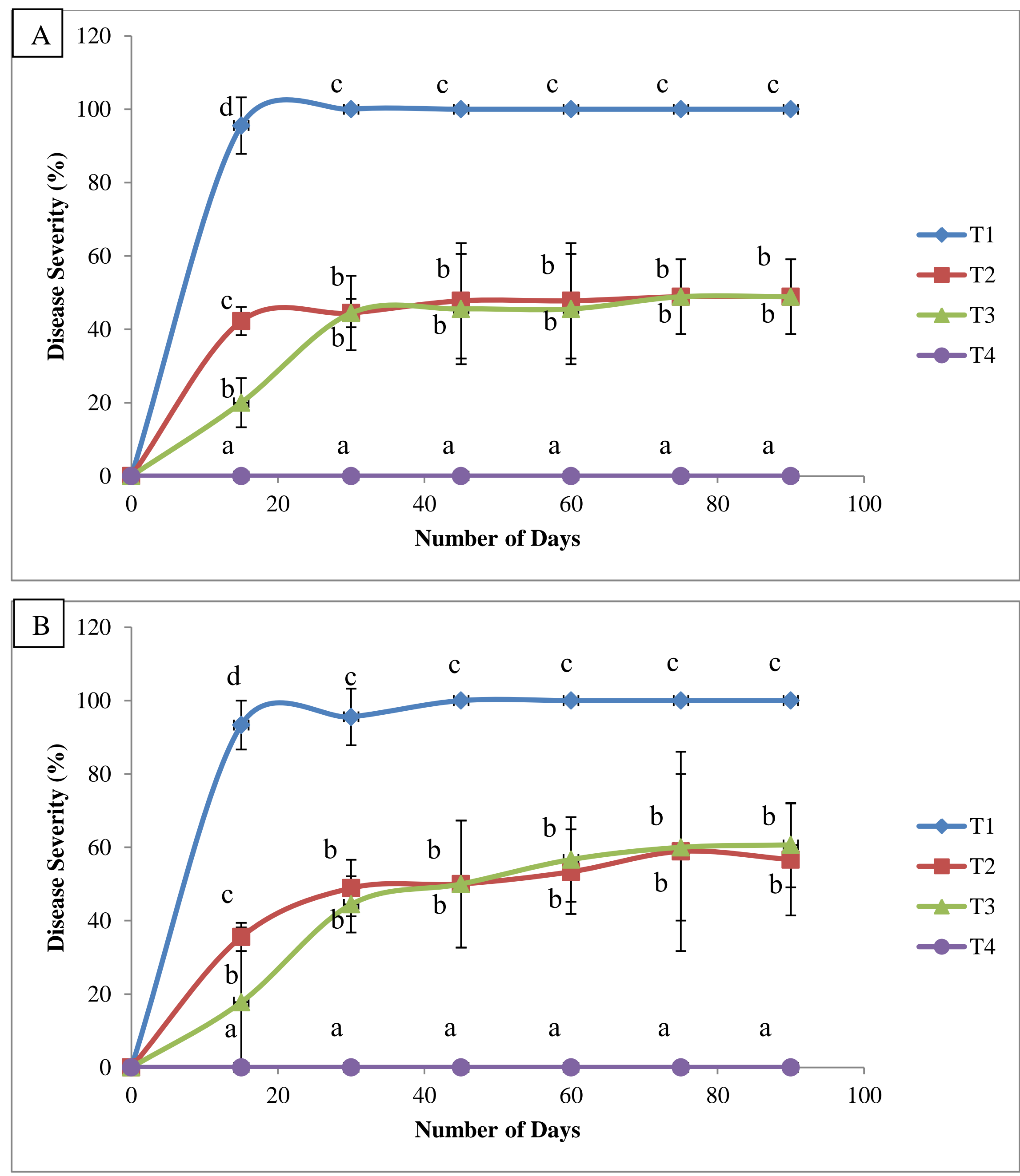


Figure 23: Graph of Disease Incidence and Disease Suppression among different treatments in Khola and Sitara chilli plants

ii. Disease severity and Protective Value

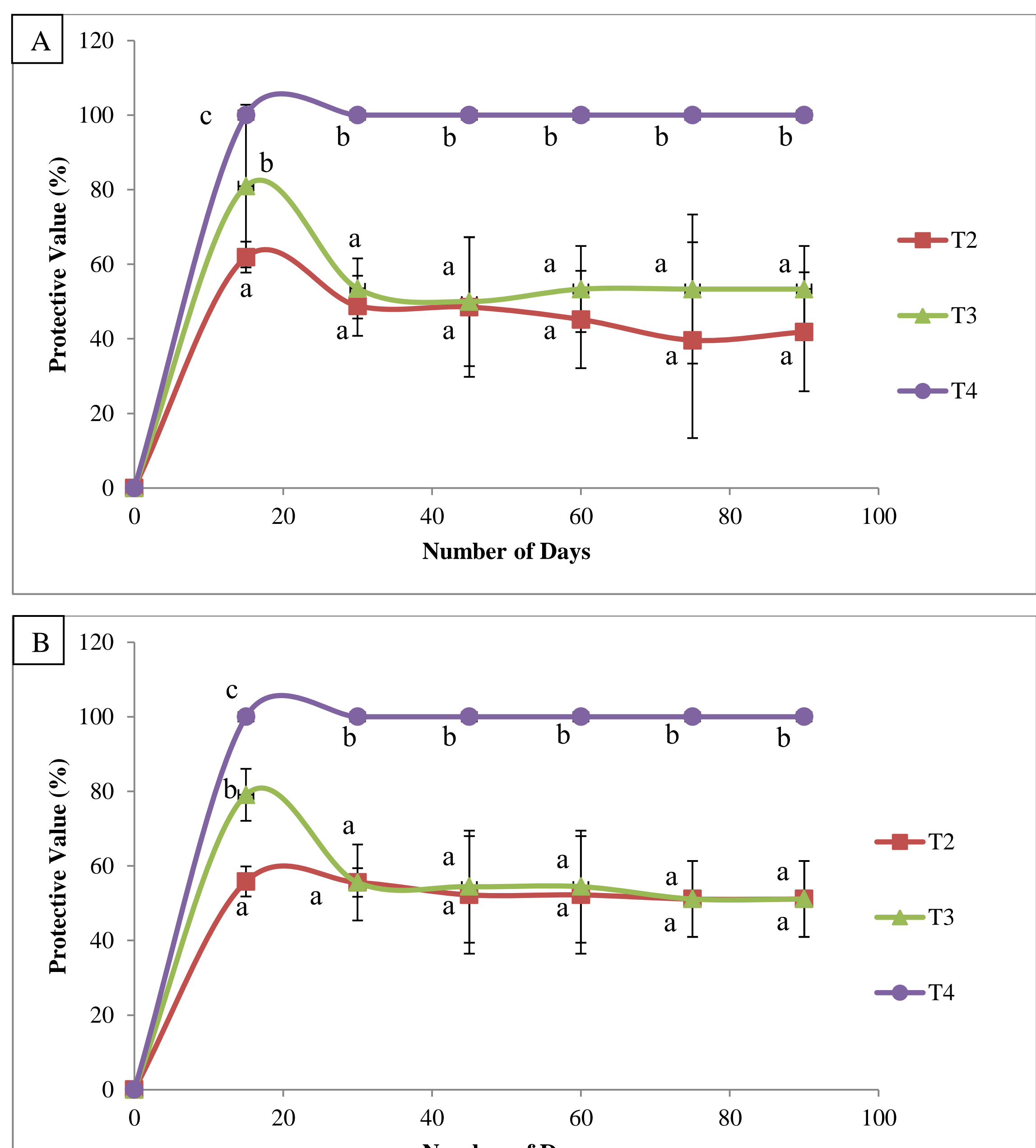
Disease severity increased, soon after the pathogen exposure in the plants of buffer control (T1) group. At the end of 90 days, highest disease severity (100 %) was observed in T1 treatment of both Khola and Sitara variety. Disease severity in biological control T2 treatment (Khola: 48.9 % and Sitara: 56.7 %) and chemical T3 treatment (Khola: 48.9 % and Sitara: 46.6%) of both the varieties also increased after the pathogen exposure but the increase was significantly lesser ($p < 0.05$) as compared to T1. In the last group (T4) where hypersaline bacterium MPSK 23 was used as treatment, plants did not show any wilting symptoms over the period of study. Thus the disease severity was negligible in both the varieties (Figure 24).

Also from the graph of protective value v/s number of days (Figure 25) it was observed that out of the three treatments, antagonistic bacteria MPSK 23 (T4) showed significantly higher ($p < 0.05$) protective value (100 %) in both the varieties followed by T3 treatment (Khola: 51.11 % and Sitara 53.33 %) and T2 treatment (Khola: 51.11 % and Sitara 41.85 %). As the control T1 treatment showed no additional protection to plants, the protective value of this group was assumed to be negligible. Hence it was seen that the MPSK 23 treatment showed the highest biocontrol efficacy against wilting caused by *Fusarium solani* in both the Khola and Sitara cultivars of the chilli plants which was statistically significant compared to other treatments ($p < 0.05$).



Different letters represent statistical significance for respective days of each treatment (ANOVA, $p<0.05$, n=9), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 24: Graph of Disease Severity in Sitara (A) and Khola (B) plants caused by *F. solani* over a period of 98 days



Different letters represent statistical significance for respective days of each treatment (ANOVA, $p<0.05$, n=9), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

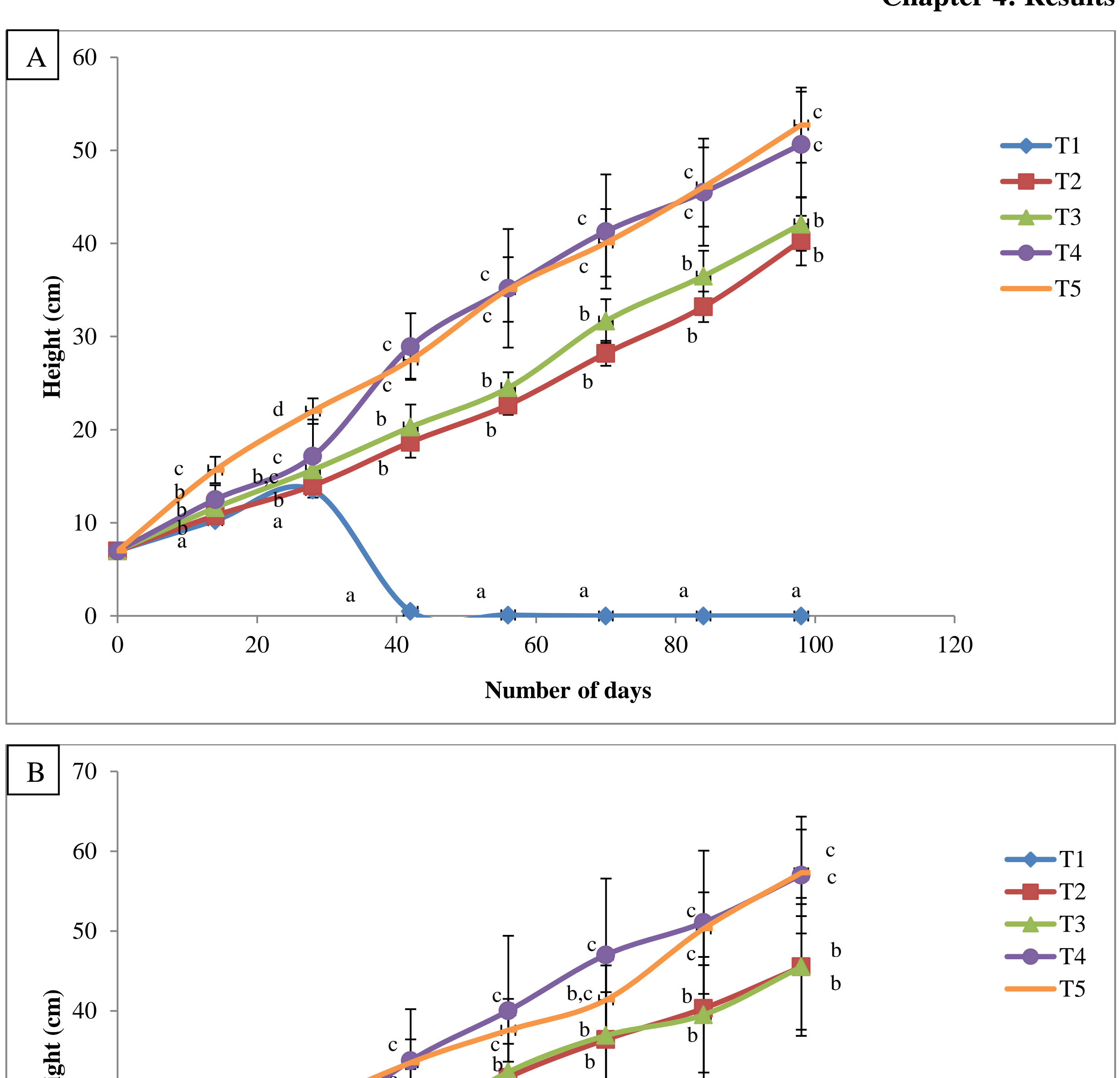
Figure 25: Graph of Protective Value of different treatments against the *F. solani* infection in Sitara (A) and Khola (B) chilli plants over a period of 98 days

b) Analysis of growth parameters

i. Total plant height and number of leaves

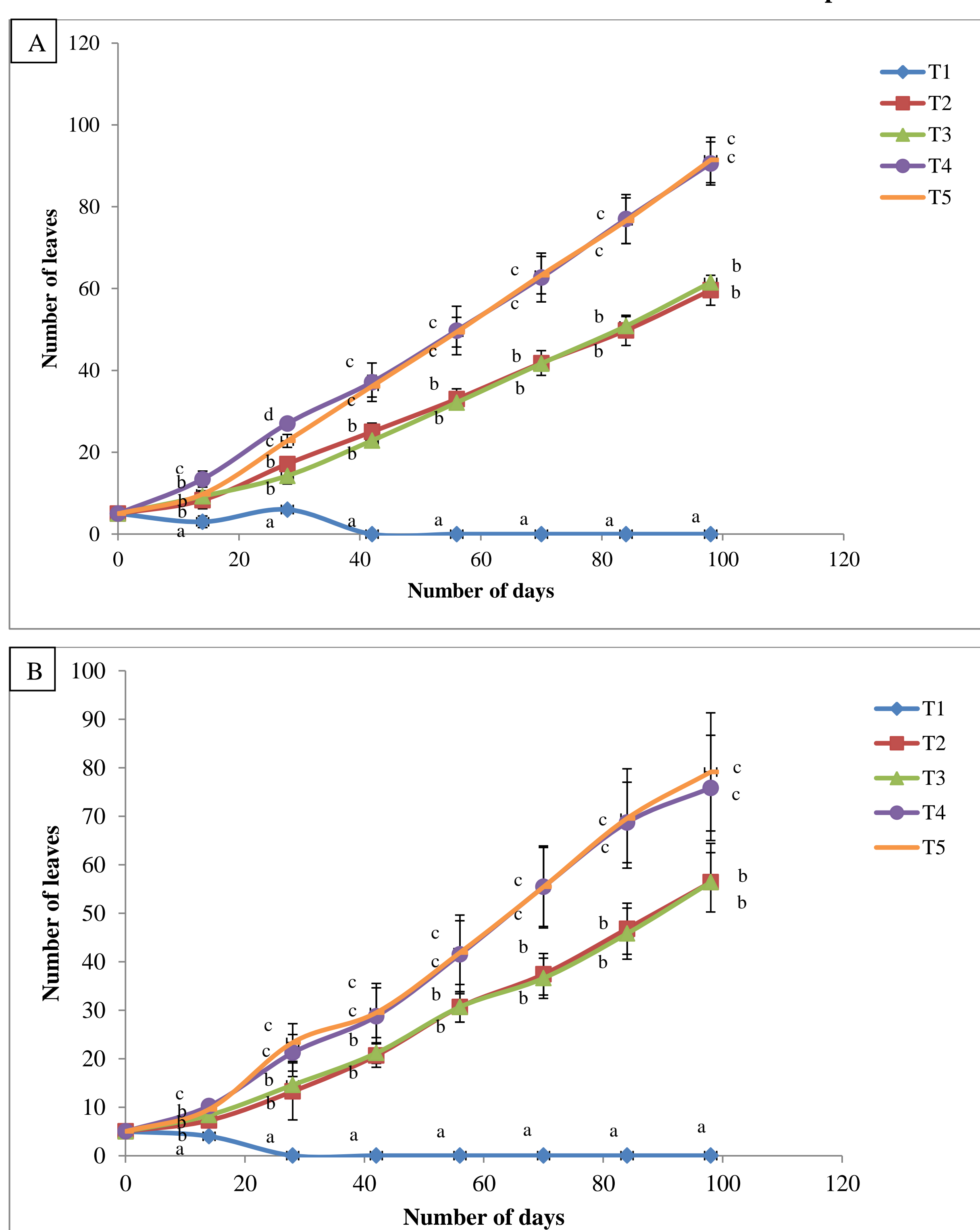
The antagonistic bacteria significantly enhanced ($p < 0.05$) the foliage and the total height of chilli plants as compared to that of PBS treated control plants (T1) (Plate 24, 25). Within 15 days, 7 out of 9 of Khola and 8 out of 9 of Sitara variety plants exhibited wilting in the T1 treatment. Wilting was also observed in two plants of each variety in biological control treatment (T2) and in one of Khola and two of Sitara in chemical control treatment group (T3). However none of the plants inoculated with the hypersaline bacterium MPSK 23 (T4) showed any major disease symptoms. At the end of 98 days, plants treated with hypersaline bacterium MPSK 23 (T4) had the maximum height (Khola: 50.64 cm and Sitara: 57.29 cm), followed by the T3 (Khola: 42.08 cm and Sitara: 45.5 cm) and T2 (Khola: 40.30 cm and Sitara: 45.4 cm) treated plants respectively (Figure 26). Also the height of MPSK 23 treated plant was comparable to that of plants which were unexposed to the fungal pathogen designated as T5 treatment (Khola: 52.71 cm and Sitara: 57.29 cm).

The average number of leaves of plants from T4 treatment (Khola: 90.57 and Sitara: 75.86) was also not significantly different ($p > 0.05$) from those in T5 treatment (Khola: 91.43 and Sitara: 74.14). Plants from T2 treatment showed 59.6 leaves in Khola and 56.4 leaves in Sitara variety and from T3 treatment showed 61.5 leaves in Khola and 56.4 leaves in Sitara variety. Whereas at the end of 98 days all the plants from T1 treatment had wilted completely. Figure 27 depicts the number of leaves of both Sitara and Khola variety plants over a period of 98 days.



Different letters represent statistical significance for respective days of each treatment (ANOVA, $p<0.05$, n=9), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 26: Graph of change in Khola (A) and Sitara (B) chilli plant height over a period of 98 days



Different letters represent statistical significance for respective days of each treatment (ANOVA, $p<0.05$, n=9), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 27: Graph of change in number of leaves of Khola (A) and Sitara (B) chilli plants over a period of 98 days

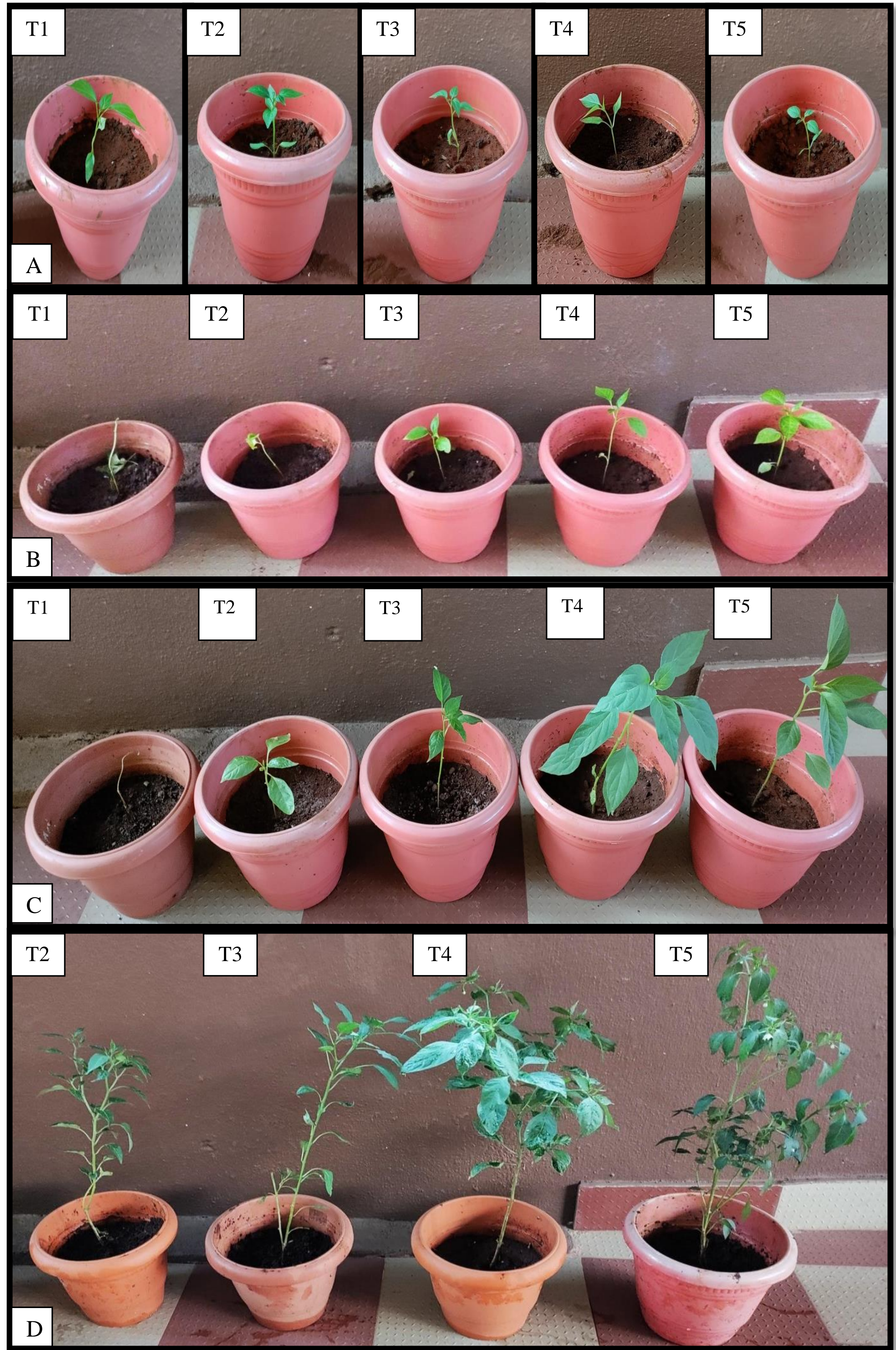


Plate 24: Growth of plants at day 0 (A), 7 (B), 15 (C) and 63 (D) days after transplantation

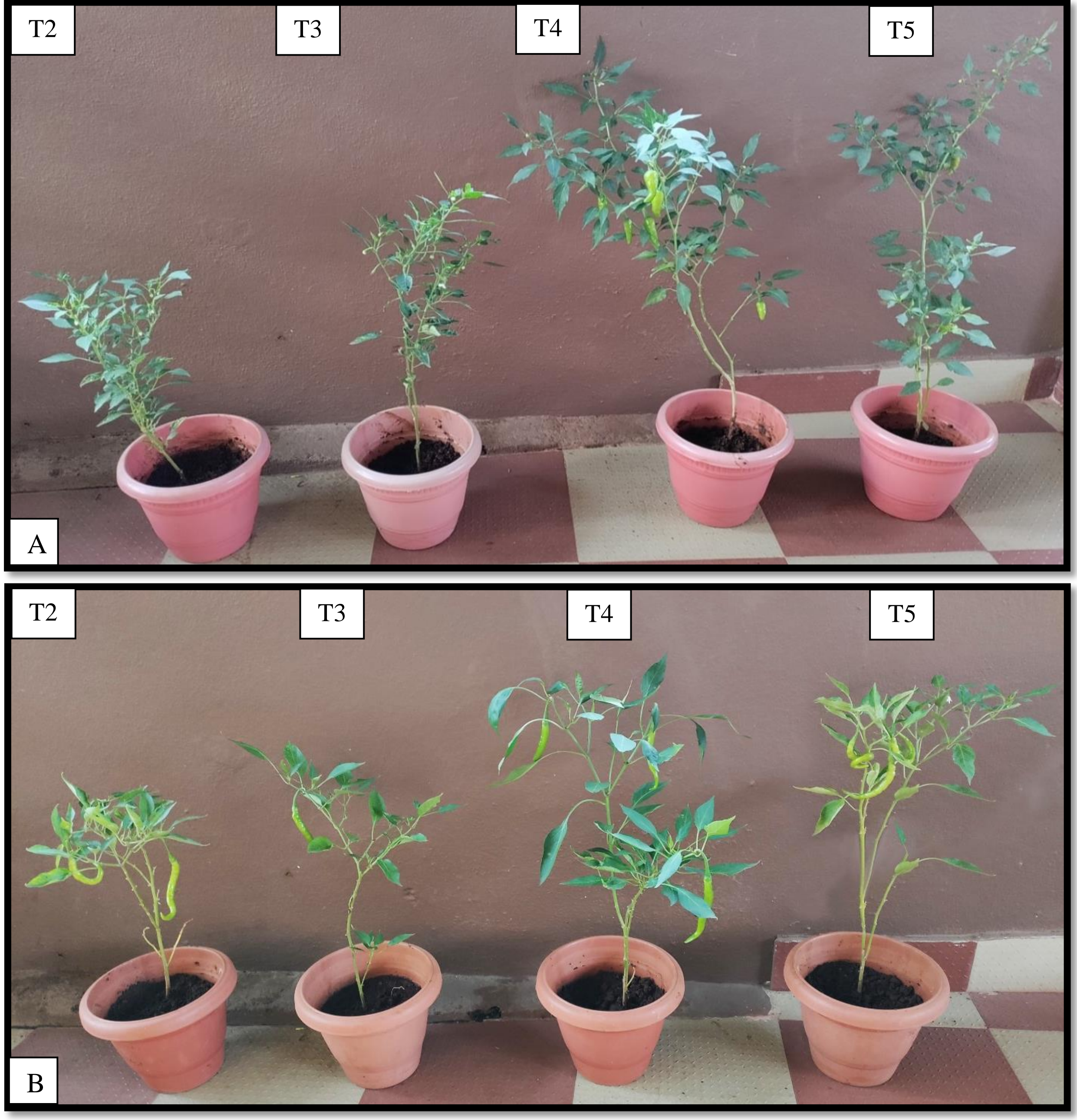


Plate 25: Plants of Khola (A) and Sitara (B) variety at day 84 after transplantation

The correlation between the disease incidence (DI), disease suppression, disease severity, number of leaves and the plant height determined by Pearson's correlation coefficient is presented in Table 22. A strong negative correlation (-1.00) was observed between disease suppression and DI in both the varieties and between DI and plant height in case of Sitara variety. Disease suppression also showed negative correlation with disease severity and positive correlation with number of leaves and plant height. A positive correlation was also observed between DI and disease severity and between plant height and number of leaves, whereas negative correlation was observed between DI and number of leaves and between disease severity and plant height and number of leaves (p<0.01).

Table 22: Correlation matrix of disease incidence (DI), disease suppression, disease severity, number of leaves and the plant height of Khola (A) and Sitara (B) chilli plants in pot trials

(A) Pearson's Correlation					
	DI	Disease suppression	Disease severity	Plant height	Number of leaves
DI	1				
Disease suppression	-1.000**	1			
Disease severity	0.926**	-0.926**	1		
Plant height	-0.997**	0.997**	-0.934**	1	
Number of leaves	-0.976**	0.976**	-0.982**	0.983**	1

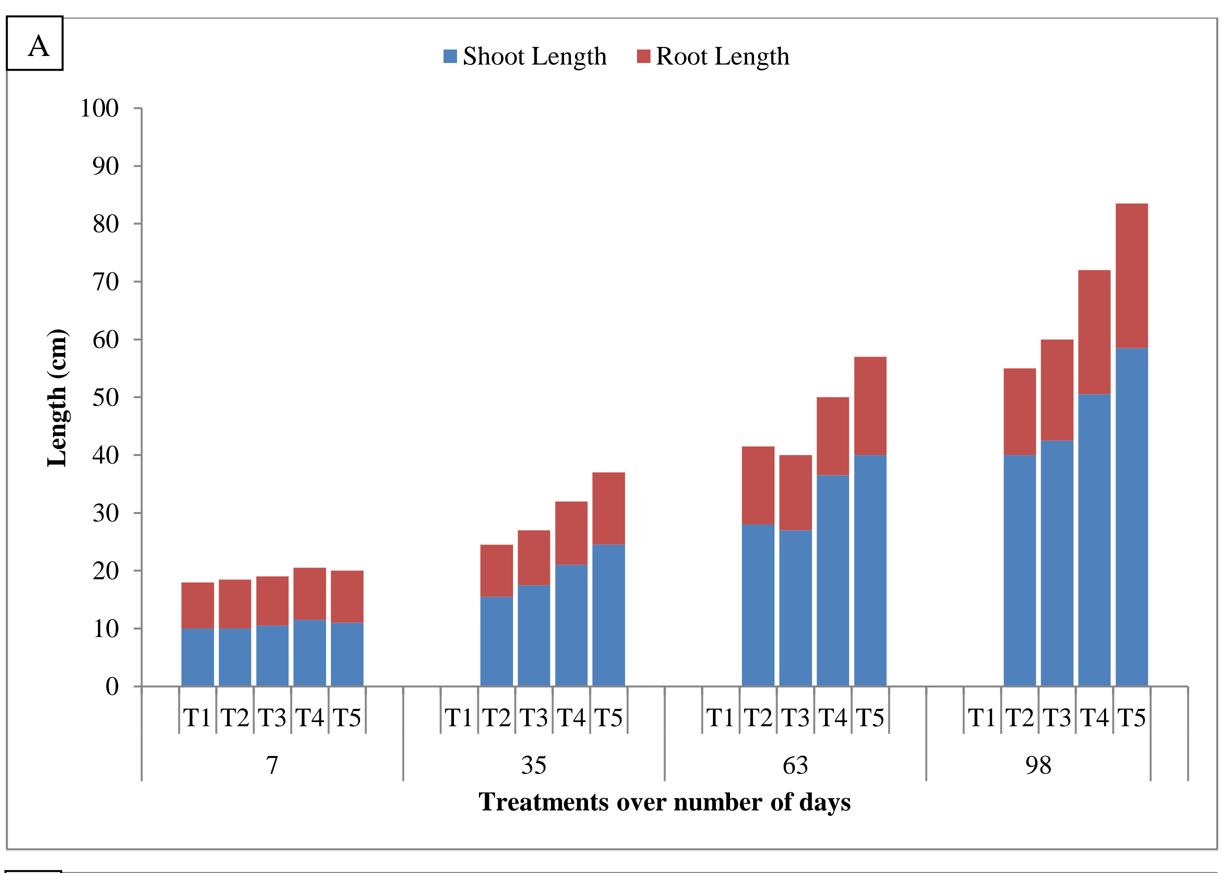
**. Correlation is significant at the 0.01 level, n=9

(B) Pearson's Correlation					
	DI	Disease suppression	Disease severity	Plant height	Number of leaves
DI	1				
Disease suppression	-1.000**	1			
Disease severity	0.949**	-0.949**	1		
Plant height	-1.000**	1.000**	-0.940**	1	
Number of leaves	-0.994**	0.994**	-0.977**	0.991**	1

**. Correlation is significant at the 0.01 level, n=9

ii. Root and Shoot length and weight (wet and dry)

Both root length and shoot length increased linearly from day 7 to day 98 in all treatments except in T1 which died due to wilting (Khola and Sitara). Maximum root length was recorded in T5 treatment of Khola plants (25 cm) followed by plants primed with MPSK 23 in T4 treatment (21.5 cm), but the above two treatments did not vary significantly ($p > 0.05$). While the *Trichoderma* treated plants recorded the least root length (T2: 15 cm) at 98 days after transplantation. A similar trend was observed in Sitara variety (T5: 25.5 cm; T4: 24 cm; T3: 20 cm and T2: 18.5 cm). Shoot length was maximum in T5 treatment of Khola and Sitara plants (T5: 58.5 cm in both varieties), followed by plants primed with MPSK 23 (T4: 50.5 cm of Khola and 55 cm of Sitara variety), but the difference was not significant ($p > 0.05$). While control PBS treated plants wilted after 7 days (T1) (Figure 28). Both wet and dry weight of root and shoot of Khola and Sitara variety increased linearly from day 7 to day 98 in all treatments, except in T1 which exhibited wilting. Maximum wet weight was recorded in unexposed Khola plants (T5: 34.6 g) followed by plants primed with MPSK 23 (T4: 31.8 g), but the above two treatment did not vary significantly ($p > 0.05$). While the minimum wet weight was recorded in *Trichoderma* treated plants (T2: 23.8 g) which were almost similar to the wet weight of plants treated with Trophy (T3: 24.3 g) at 98 days after transplantation. Least dry weight was observed in plants of T2 treatment (2.9 g), followed by T3, T4 and T5 treatments (3.2 g, 5.0 g and 5.6 g respectively). Similarly in case of Sitara variety both wet and dry weight was highest in T5 treatment (34.9 g and 5.8 g respectively), followed by T4 treatment (33.5 g and 5.3 g respectively). Least wet weight was observed in T2 treatment (28.2 g) and least dry weight was observed in T3 treatment (4.2 g). Plants with T1 treatment resulted in wilting and death in Sitara variety as well (Figure 29).



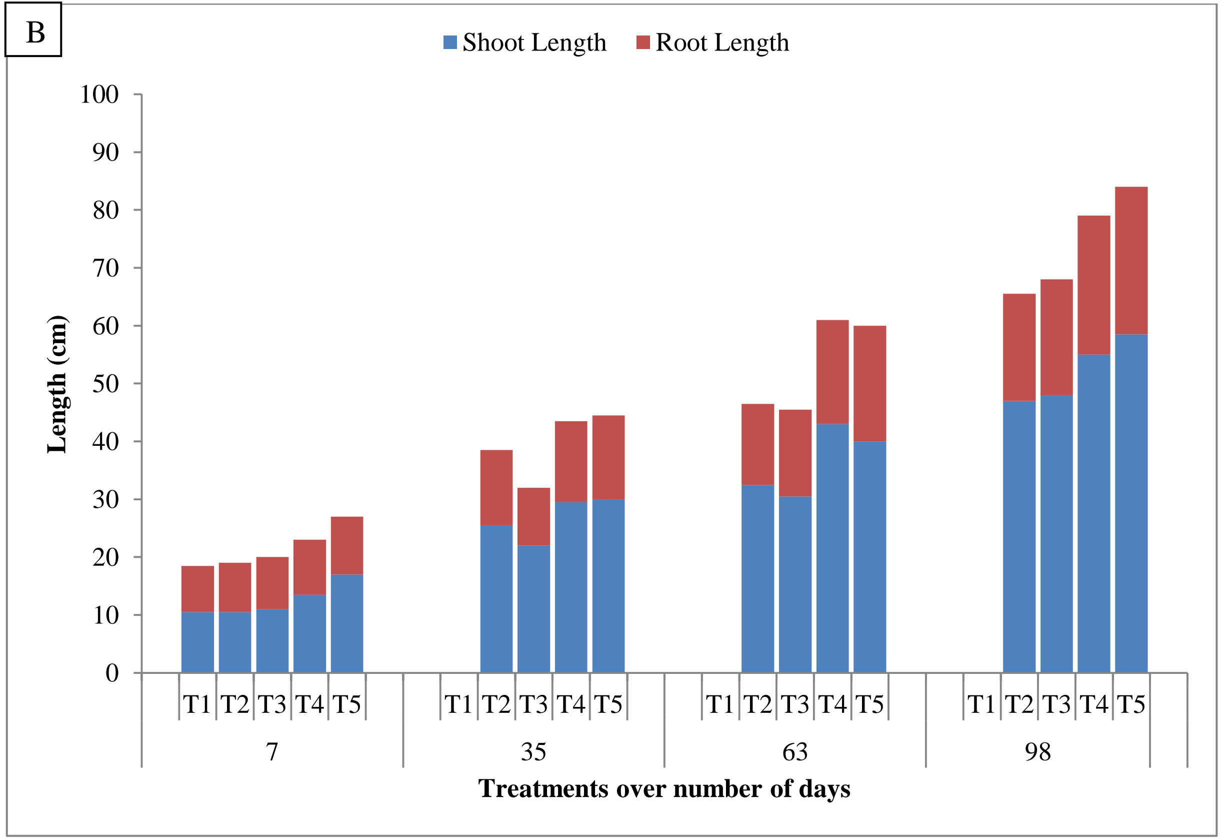
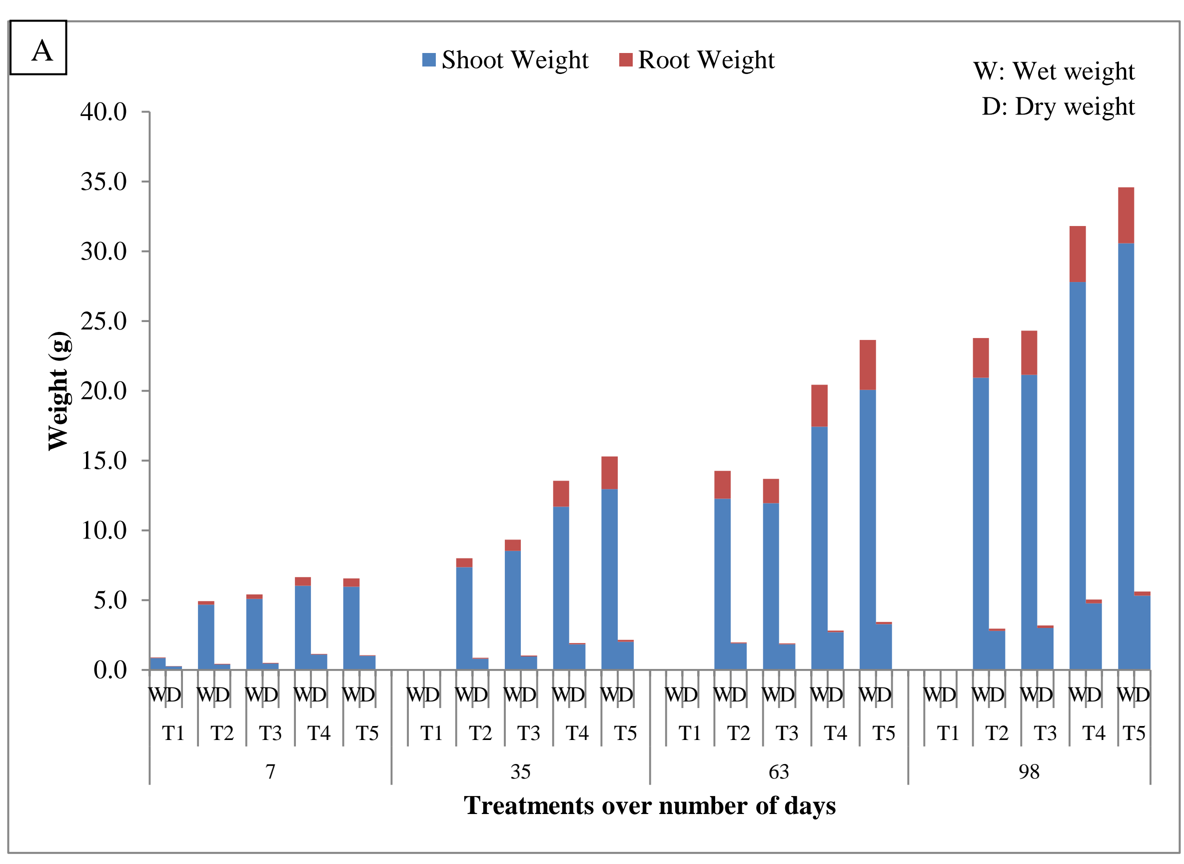


Figure 28: Graph of total length of Khola (A) and Sitara (B) chilli plants over a period of 98 days



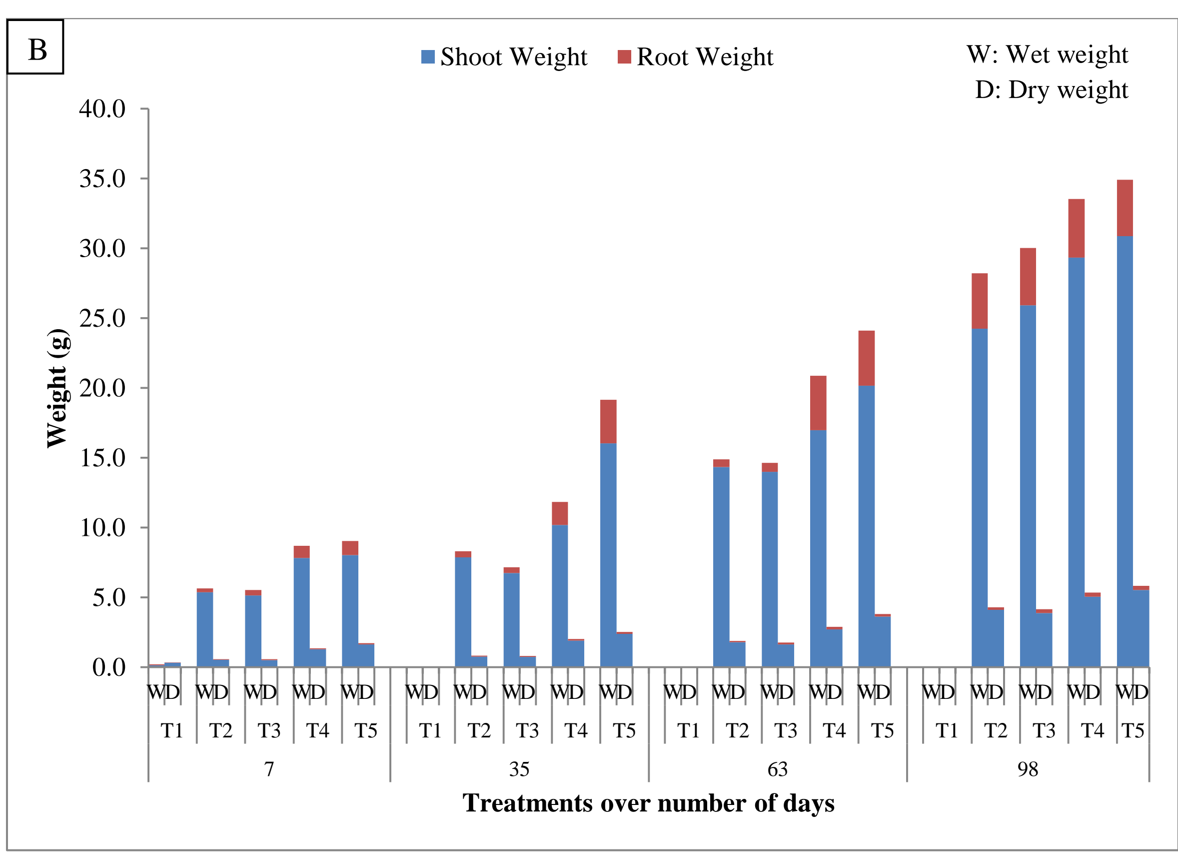
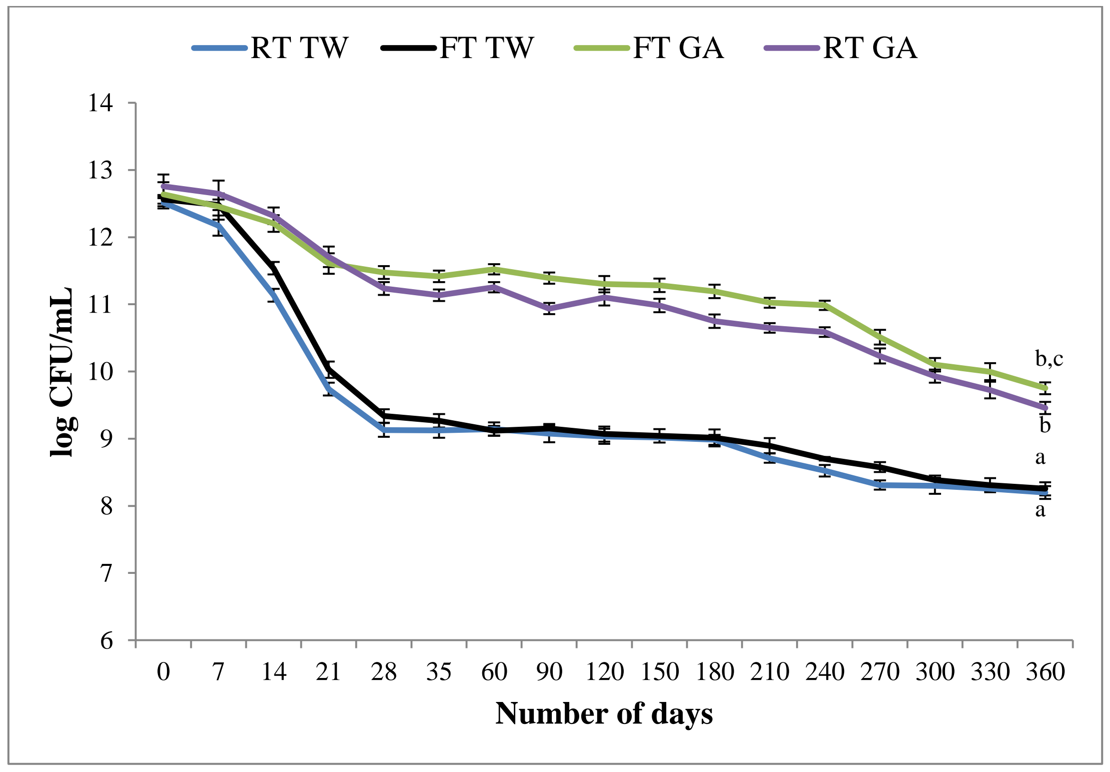


Figure 29: Graph of total wet and dry weight of Khola (A) and Sitara (B) chilli plants over a period of 98 days

4.18 Shelf life and viability of MPSK 23

MPSK 23 was found to be viable at both the temperatures (FT: 4 ± 2 ºC and RT: 28 ± 2 °C) without varying significantly ($p > 0.05$). However a higher count was observed when the cells were activated by re-suspending them in 1 % GA as compared to tap water (Figure 30). Although at the end of 12 months, there was a decrease in viable count as compared to initial, the cells of MPSK 23 still remained viable at both the above mentioned temperatures (RT TW: 8.2 logCFU/mL, FT TW 8.26 logCFU/mL, FT GA 9.75 logCFU/mL and RT GA 9.46 logCFU/mL).



Different letters represent statistical significance for respective days of each treatment (ANOVA, $p<0.05$, n=3), followed by Duncan's Multiple Range Test (DMRT) post hoc test.

Figure 30: Viability of MPSK 23 stored at room temperature (RT, 28 ± 2 °C) and refrigerator temperature (FT, 4 ± 2 °C) after resuspending in tap water (TW) and 1 % Gum acacia (GA)

Chapter 5

Discussion

In the last century, the world population reached a size three times greater than any previous value across the whole history of humanity. To cope with the rising request for food, current agricultural practices are based on the wide use of fertilizers and pesticides. As a result, agrochemical multinationals have gradually acquired control of the global food production. In India, the agriculture sector employs nearly half of the workforce in the country and contributes to 17.5% of the GDP (Deshpande 2017). To guarantee country wide access to safe and economical food in sufficient quantity to the country's ever increasing population, agricultural production must also increase. Along with the food grain production, India is also the largest producer of 1.7 M tonnes of Chilli (*Capsicum annuum* L.) spice in the world, followed by Thailand and mainland China (FAOSTAT 2020). India also ranks first in the world in terms of consumption and export of this commercial spice. The variable climatic conditions in India with respect to the different ecological zones, makes this spice crop susceptible to diseases by various microbial pathogens. In the light of present day constraints on plant disease control practices, the losses incurred in agriculture due to plant diseases, sums up to around 16% globally, leading to the increase in the prices of agricultural commodities (Oerke 2006).

5.1 Wilt and Rot causing fungal pathogens of Chilli plants and their control

Chilli plants are susceptible to several diseases and pests, which become major constraints in its production. Most devastating are fungal pathogens causing plant wilt and rot which considerably lower the yield of chilli crops annually (Khan et al. 2018). In our study three fungal strains namely *Fusarium solani, Macrophomina phaseolina* and *Fusarium oxysporum* were isolated from the diseased chilli plants exhibiting rotting and wilting symptoms. *F. solani, M. phaseolina* and *F. oxysporum* are known phytopathogens with several reports proving its pathogenicity to *Capsicum annuum* L. causing wilt and rot in plants (Hussain et al. 2013; Gurung et al. 2016; Polapally et al. 2022). Apart from these *Fusarium* spp., *F. pallidoroseum* also lead to the wilting of the chilli plant when infested (Wani et al. 2012, Muzaffar et al. 2021). Previously Naik et al. (2008) and Tembhurne et al. (2017) isolated *F. solani* from infected plants of chilli from wilted fields. This pathogenic

fungus was isolated from the roots of the diseased plants, which was also followed in this study. In a study by Ismail et al. (2017), *F. solani* and *F. oxysporum* were isolated from stem and root whereas *Fusarium verticillioide* and *Fusarium subglutinans* were isolated exclusively from the stem of the wilted chilli plants. They also isolated *M. phaseolina* from the roots of the diseased plant samples. Another study isolated and mycologically identified (based on cultural; morphological and microscopical characteristics) causative agents of wilting and rot diseases in chilli plant as *R. solani*, *F. solani*, *F. oxysporum*, *Fusarium sambucinum* and *M. phaseolina* (Madbouly & Abdelbacki 2017). Similarly Güney & Güldür (2018) isolated *M. phaseolina, F. oxysporum* and *F. solani*; Dewi et al. (2021) isolated *F. oxysporum* and Malik et al. (2022) also isolated *F. oxysporum* from the infected chilli plants. Chilli wilt disease caused by various fungal species like *F. solani, F. pallidoroseum* and *F. oxysporum* are known to effect 90 % of yield in India (Singh and Singh 2004; Madhavi et al. 2006). Similarly in the state of Goa, India we also observe the destruction of the chilli crop majorly by *F. solani* which results in phenomenal decrease in the production of the local as well as commercial chilli cultivars. Another root rot-causing saprophytic pathogen *Rhizoctonia solani*, causes root rot in chilli plant at both the seedling stage under greenhouse conditions and in the main field (Sanogo 2003, Mannai et al. 2018). Fungus belonging to genus *Pythium* shows initial damping off symptoms in chilli seeds which fail to germinate or rot in young chilli seedlings, leading to wilt and eventual death (Khan et al. 2003, Mishra et al. 2013). Therefore in the present study *Fusarium solani, Macrophomina phaseolina*, *Fusarium oxysporum, Fusarium pallidoroseum, Rhizoctonia solani and Pythium aphanidermatum* were targeted as test pathogens. Since wilting is one of the gravest diseases in chilli cultivation and is generally caused by *F. solani,* this was the first species which we isolated from the diseased chilli plants showing wilting symptoms in fields of Goa. Subsequent to isolation, we infected Sitara and Khola chilli cultivars with the strain and succeeded in re-isolating this pathogen from the diseased plants. Further the pathogenicity of *F. solani* was confirmed after staining the fungus and identifying it mycologically thus proving to be pathogenic to above mentioned cultivars of chilli in accordance with the Koch's Postulates of pathogenicity (Bhunjun et al. 2021).

A fast solution to the treatment of a sudden outbreak of crop diseases is generally tackled with chemical agents to obtain immediate results. For any pest problem, chemical agents are usually preferred over organic ones as they are cheaper, readily available, broad-spectrum, persistent and have longer shelf life than the later. However due to such properties of persistency and broad spectrum activity, the indiscriminate use of synthetic pesticides has

resulted in serious biological and ecological problems (Whipps 2001; Muthukumar et al. 2008). However to reduce the dependency on chemical control, the use of beneficial microorganisms termed as biocontrol agents (BCAs) which are antagonistic to pest pathogens has been promoted (Marian & Shimizu 2019).

While competing for living space and nutrients, microorganisms from hypersaline environments are also known to produce various bioactive metabolites, which can inhibit the growth of other microbes in the vicinity. These metabolites have potential usages in diverse fields such as food technology, feed additives, chemical industries and agro-economic sector (Sadfi-Zouaoui et al. 2008; Atanasova et al. 2013). Among the various halophilic niches, salt pan ecosystems have not been exploited to their full potential as yet. Halophilic and halotolerant microorganisms from salt pans have also been reported previously to be a source of several hydrolytic enzymes and bioactive compounds having antimicrobial action (Kamat & Kerkar, 2011; Ballav et al. 2015). Keeping this as the central point of our study, we isolated and screened bacteria from three different salt pans of Goa, India for their antifungal activity against fungal pathogens causing wilt and rot in chilli plants. Out of 197 bacteria screened (isolated from water, sediment and biofilm samples), 22 isolates showed activity against six different pathogens namely *F. solani, F. oxysporum, F. pallidoroseum, M. phaseolina, P. aphanidermatum* and *R. solani.* Previously Chen et al. (2010) have shown the antagonistic ability of moderately halophilic bacteria isolated from the Weihai Solar Saltern (China) against the phytopathogens *Verticillium dahliae, F. oxysporum, Colletotrichum gloeosporioides* and *Alternaria solani*. Another study has revealed the biocontrol of *M. phaseolina* by a *Bacillus* spp. isolated from salt-pan rhizosphere of the nurse plant *J. sabina* in Spain (Castaldi et al. 2021). To our knowledge this is the first study of the antifungal activity of salt pan bacteria against *F. solani, F. oxysporum, F. pallidoroseum, M. phaseolina, P. aphanidermatum* and *R. solani*.

5.2 Mode of antagonism exhibited by the bacteria against fungal pathogens

In the present study, among the 10 salt pan bacteria tested, volatile metabolites released by three bacterial isolates *viz*. BGUM 14B, MPSK 9 and ABSK 9 showed inhibition of the fungal pathogens *F. solani, R. solani* and *M. phaseolina* by inverted plate method. Many marine bacteria are known to produce volatile organic compounds (VOCs) with antimicrobial activities. The ability of the bacteria to inhibit fungal pathogens via VOCs can be traced back

to 90's when Fiddaman and Rossall (1993) isolated a strain of *Bacillus subtilis* producing an antibiotic metabolite and a volatile compound(s) which was antifungal to *R. solani* and *Pythium ultimum.* Jayaswal et al. (1993) also showed inhibition of *Exserohilum turcicum, Diplodia maydis, Fusarium moniliforme, Fusarium roseum, M. phaseolina*, *Phytophthora megasperma var. sojae*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* by two strains of *Pseudomonas cepacia* via volatile metabolite production. Tenorio-Salgado et al. (2013) showed the fungistatic and fungicidal activities of volatile compounds produced by *Burkholderia tropica* against *Fusarium culmorum*, *F. oxysporum*, *S. rolffsi* and *Colletotrichum gloesporioides*. Another study revealed that the *Bacillus* spp. S18, A19, and A21 produced volatile compounds that reduced *F. solani* mycelial growth (Gutiérrez-Santa Ana et al. 2020). From our study, remaining seven salt pan isolates out of ten which were MPSK 14, MPSK 22, MPSK 23 MPSK 109, MPSK 186, MPSK 20 and SK 473 showed inhibition of *F. solani, R. solani* and *M. phaseolina* by the production of diffusible metabolites. A study by Al-Ani et al. (2012) revealed the activity of *Rhizobium japonicum* against the soil-borne pathogens *F. solani* and *M. phaseolina* by culture medium by well diffusion method. Using the same method Khedher et al. (2015) proved the efficacy of *Bacillus subtilis* V26 as a biological control agent against *R. solani*. Thus this method has proved to be an efficient test for detection of diffusible antibiotics produced by antagonistic cultures against the pathogens, which was also followed in the current study.

Among the different kinds of antifungal compounds produced by bacteria to compete with other microorganisms in resource-limited environments, lipopeptides are a potent form which causes less resistance, are friendlier to the environment and are broad spectrum in nature (Chen et al. 2017). When screened for the presence of antifungal lipopeptide producing genes, bacterial isolates MPSK 22 and MPSK 23 showed the amplification of *Lic*A and *Lic*C genes of lichenysin family and *Fen*C gene of fengycin family; whereas MPSK 186 showed presence of *Fen*B gene of fengycin family. A similar screening method using specific polymerase chain reaction (PCR) primers detected the presence of the *Fen*D gene of the fengycin operon in *B. subtilis* strain DFH08 which was a *Fusarium graminearum* antagonist (Ramarathnam et al. 2007). Another study demonstrated the presence of different lipopeptide isoforms belonging to surfactin, iturin, and fengycin families in *B. subtilis* SPB1 with strong antifungal activity against *R. solani* and *Rhizoctonia bataticola* (Mnif et al. 2016). A study by Wu et al. (2019c) reported that *B. subtilis* SL-44 produced antifungal lipopeptides surfactin, iturin and fengycin that inhibited or damaged the mycelial growth of *R. solani* in chilli pepper against the wilt

disease which was detected by similar technique. The study of Guo et al. (2022) identified lichenysin lipopeptide *in Bacillus altitudinis* Q7 against *Alternaria alternata* pathogen.

Many bacteria secrete lytic enzymes into their environment as a mean to generate food sources from polysaccharides or to gain a competitive advantage in their microenvironment by inhibiting or parasitizing their neighbors (Klock et al 2002). Salt pan bacteria SK 473, MPSK 14, MPSK 20, MPSK 22, MPSK 23, MPSK 109 and MPSK 186 isolated in this study tested positive for the production of lytic enzymes amylase, protease, lipase and cellulase. In addition to this, MPSK 22, MPSK 23 and MPSK 186 also showed production of chitinase and β-glucanase enzyme. Various bacterial strains have shown to excrete chitinases and glucanases, enzymes that degrade the hyphae of various fungi by digesting the principal components of fungal cell walls (chitins and glucans) and the products of digestion are used as energy sources. This enzymatic digestion of the fungal pathogens by chitinases and glucanases presents an effective method for the biological control of fungal pathogens (Garbeva et al 2004; Arora et al. 2007). Other enzymes like amylase, protease and lipase also take part in the enzymatic dissolution of cell walls leading to the loss of fungal protoplasm and thus are a part of mechanisms involved in BCA's activity (Kumari et al. 2021). Cellulase is also a cell wall degrading enzyme which helps in the inhibition of Oomycetes whose cell wall is made up of cellulose. Thus the production of lytic enzymes by BCA is of major advantage in the inhibition of fungal pathogens. In a study by Sindhu & Dadarwal (2001), *Pseudomonas* strains isolated from the rhizosphere of chickpea and green gram were screened for the production of chitinases and cellulases. Five *Pseudomonas* strains were found to produce appreciable amounts of both enzymes in culture-free supernatants and showed growth inhibition of *P. aphanidermatum* and *R. solani*. Suryadi et al. (2014) also reported promising biocontrol activity of *Pseudomonas aeruginosa* against *Pyricularia oryzae* and *R. solani* by the virtue of chitinase and glucanase produced by the isolate. Kumari et al. (2021) isolated five bacterial strains belonging to genus *Bacillus* from rhizospheric soils of rice which showed inhibition of plant fungal pathogen *S. rolfsii*. All the five bacterial isolates showed positive results for amylase, protease, and lipase production and two isolates showed a positive chitinase activity.

Further in this study all the bacterial isolates except MPSK 20 showed production of ammonia. Bacteria can emit ammonia or urease-producing bacteria can catalyse the conversion of urea to ammonia (urea, as a nitrogenous fertilizer, is often applied near the crop root in agriculture) which has fungistatic action against mycopathogens in soil (Liu et al.

2021). Back in 1988, Howell et al. presented data which concluded that the compound produced by *Enterobacter cloacae* which inhibited fungal pathogens *Pythium ultimum* and *R. solani* was ammonia. Recently ammonia-mediated fungistasis was observed *in-vitro* when the bacterial isolate *Lysinibacillus capsici* was grown on a protein-rich medium leading to the inhibition of *R. solani* (Vlassi et al. 2020). Thus the ammonia production by our isolates could be an additional property contributing to the antifungal activity exhibited.

When tested for plant growth promoting attributes, all selected isolates in this study showed production of plant growth hormone Indole-3-acetic acid (IAA) which is an important phytohormone increasing the root length and surface area, and in the process increases the level of root exudates available for uptake by plants (Ali et al. 2010). These isolates also tested positive for the production of enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. ACC deaminase acts by degrading ACC, the precursor of ethylene resulting in the production of α-ketobutyrate and ammonia, which prevents an excessive increase in the synthesis of ethylene under various stress conditions and is one of the most efficient mechanisms to induce plant tolerance to stress (del Carmen Orozco-Mosqueda et al. 2020). Further these isolates also exhibited the ability to fix atmospheric nitrogen which helps them convert nitrogen gas in the atmosphere to ammonia thus making it suitable for plant assimilation (Olanrewaju et al. 2017). Out of the 10 bacterial isolates studied, ABSK 9 exhibited phosphate solubilisation and MPSK 9 exhibited silicate solubilisation. In the case of zinc solubilisation, all the isolates except BGUM 14B and ABSK 9 solubilised the insoluble zinc from the medium. The micronutrients like P, Zn, Fe, Si and Mn are found to be deficient in most soils (Alloway 2004). Phosphorus is the most important key element in the nutrition of plants, next to nitrogen as it plays an important role in virtually all major metabolic processes in plants including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration. Plants are unable to utilize the phosphate present in the soil because 95-99 % phosphates present are in the insoluble, immobilized, and precipitated form. Thus the ability of the bacteria to turn the unavailable forms of phosphorus into soluble forms; helps in making it available for plants to assimilate (Khan et al. 2010). Similarly Zn and Si solubilisation by microorganisms in this context proves to be beneficial and economical in agricultural applications. Previously *Bacillus* sp. BPR7 isolated from the rhizosphere of common bean produced IAA, ACC deaminase, lytic enzymes and solubilized various sources of organic and inorganic phosphates as well as potassium and zinc. This strain strongly inhibited the growth of several phytopathogens such as *M. phaseolina*, *F. oxysporum, F. solani, S. sclerotiorum, R. solani* and *Colletotricum* sp. *in-vitro* (Kumar et al. 2012).

Naureen et al. (2017) deciphered the plant growth promotion and antifungal capability of the test bacterium *Lysinibacillus sphaericus* ZA9 which was found to produce high quantity of IAA and was also capable of solubilizing silicates, phosphates and potassium. *L. sphaericus* ZA9 and its cell free culture supernatant showed varied antagonistic behaviour against *A. alternata, Curvularia lunata, Aspergillus* sp., *Sclerotinia* sp., *Bipolaris spicifera* and *Trichophyton* sp. Another study by Verma & White (2018) showed the role of *Bacillus amyloliquefaciens* in modulating millet seedling development by production of IAA, providing nutrients to seedlings by phosphate solubilisation and reducing pathogen damage to seedlings caused by *F. oxysporum, Curvularia* sp., *Alternaria* sp. and *Sclerotinia homoeocarpa*. Kaur & Manhas (2022) showed the influence of *Streptomyces hydrogenans* DH16 on growth of pea seedlings which significantly enhanced the growth of the seedling via production of IAA and ACC deaminase. This streptomycete also demonstrated *in-vitro* ability to colonize plant roots, fix atmospheric nitrogen and inhibit fungal phytopathogens *Alternaria solani* and *Pyricularia oryzae*.

Thus the plant growth promoting attributes exhibited by the isolates in this study could potentially add to the biocontrol mechanism (volatile and diffusible metabolite, lytic enzymes and ammonia production) and promote growth of the plants under biotic stress condition, thus exhibiting a mixed path antagonism.

Microbial strains with the ability to produce siderophores with high affinity to iron play an important role in disease suppression through competition for iron with the pathogens (Ahmed & Holmström 2014). In this study siderophore production was shown by three isolates namely ABSK 9, MPSK 109 and MPSK 186. *Pseudomonas aureofaciens* AR 1 isolated in the study by Chaiharn et al. (2009) showed the presence of a hydroxamate-type siderophore which was primarily responsible for an *in-vitro* antagonistic effect to *Alternaria* sp., *F. oxysporum* and *P. oryzae.* Yu et al. (2011) carried out a study to check the ability of siderophore producing *B. subtilis* to inhibit the wilt caused by *F. oxysporum* in pepper. Iron supplementation in pot trials reduced this biocontrol effect of the BCA which shows that when there is limited availability of iron in the environment the BCA competes with the pathogen for it, thus inhibiting the pathogen. Exopolysaccharide (EPS) production is another good example of competition between BCA and pathogen for niche occupation. EPS formation results in colonization of the roots of the host by the BCA thus making it unavailable to the pathogen for any infection. It also helps the bacteria to survive in inhospitable environments for a longer time by forming biofilms and to endure

environmentally stressful conditions. Further it also improves the permeability by increasing the soil aggregation and maintaining higher water potential around the roots, thereby increasing the nutrient uptake by plants and protecting them from drought stress (Silambarasan et al. 2019). In this study all the isolates except BGUM 14B and MPSK 9 showed the production of EPS. Strains of *B. subtilis* that exhibited high levels of biocontrol efficacy on tomato plants against the plant pathogen *Ralstonia solanacearum* were able to form robust biofilms both in defined medium and on tomato plant roots (Chen et al. 2013). Chlebek et al. (2020) attributed the biocontrol activity of *Pseudomonas fluorescens* BRZ63 against *R. solani*, *Colletotrichum dematium*, *S, sclerotiorum* and *Fusarium avenaceum* due to its abilities of exopolysaccharide production, auto aggregation, and biofilm formation. Additionally this isolate also showed siderophore, IAA, ACC deaminase and ammonia production as well as phosphate solubilisation. The production of siderophore and EPS by the salt pan bacteria indicated that these isolates might also inhibit the fungal pathogens via indirect type of antagonism by competing for nutrition and occupation of niche.

Further interaction of the slat pan isolates MPSK 22, MPSK 23 and MPSK 186 with the fungal pathogens, revealed the deformation in the fungal hyphae due to hyperparasitism. Hyperparasitism typically involves the interaction of the BCA with the fungal pathogen followed by the production of cell wall-degrading enzymes or antifungal metabolites and finally penetration, deformation or degradation of the mycelia (Köhl et al. 2019). The light microscopy of *F. solani* revealed granulation of mycelia and SEM images showed disintegrated and empty mycelia after interacting with the bacterial isolates, which was not observed in control. In case of *R. solani*, light microscopy revealed abnormal swelling of the fungal hyphae and SEM images displayed a curling and thinning pattern. Both light microscopy and SEM analysis of *M. phaseolina* revealed the curling of the test hyphal tips. These hyphal deformations indicated that the inhibition of the above mentioned fungus by the hypersaline bacteria could be because of a direct mode of antagonism, as these isolates also tested positive for the production of lytic enzymes and antifungal metabolites previously. Boukaew & Prasertsan (2014) showed that in the presence of the culture filtrate of *Streptomyces philanthi* RM-1-138 there were pronounced morphological alterations in the hyphae of *R. solani* which involved reduced apical growth, curling of their hyphal tips and irregular distortions in the fungal hyphae. Through microscopic studies, Khedher et al. (2015) showed the hyphal deformation and swelling of *R. solani* when treated with culture supernatant of *B. subtilis* V26. Same study also reported the enlargement of cytoplasmic vacuoles, leaking out of protoplasm and the cracking of the mycelia. In the study of Tewari &

Arora (2016), post interaction abnormalities in fungal mycelium of *M. phaseolina* due to *Pseudomonas* sp. PF17 were visible in the form of halo formation, mycelial deformities and hyphal tip degradation. Digestion of fungal cell wall along with shrivelling and curling of mycelium was also pointed out by the authors. In another study, treatment by *Bacillus vallismortis* BV23 culture supernatant resulted in coarser hyphae, induced cytoplasmic granulation, and increased cell membrane permeability of *Fusarium graminearum*, causing cytoplasm leakage (Li et al. 2019). Extracellular metabolites from strain *B. amyloliquefaciens* QSB-6 showed a strong inhibitory effect on *Fusarium* hyphal growth and spore germination, causing irregular swelling, atrophy, rupture, and cytoplasmic leakage of fungal hyphae (Duan et al. 2021). These deformation patterns correspond to those reported in our study.

Finally the capability of the antagonist MPSK 23 to regulate the defense mechanisms in host chilli plants after exposure to the pathogenic *F. solani* fungus was studied *in-vivo*. Induced defense mechanisms involve the production of reactive oxygen species, phytoalexins, phenolic compounds, or enormous number of enzymes involved in plant defense, like polyphenol oxidase, β-1, 3-glucanase, chitinase, phenylalanine ammonia lyase, peroxidase which get stimulated due to BCA application after the pathogenic infection (Kaur et al. 2022). The phenylpropanoid pathway in plants is an important adaptation that enables plant defence against abiotic and biotic stresses such as pathogen infection, wounding, nutrient depletion, UV irradiation, and extreme temperatures. Phenylpropanoids are derived from cinnamic acid, which is formed from the deamination of phenylalanine by Phenylalanine ammonia-lyase (PAL). PAL plays an important role in plant defence and is also involved in the biosynthesis of salicylic acid (SA), an essential signal involved in plant systemic resistance (Kim & Hwang, 2014). Expression of oxidative peroxidases (PO) has also been reported as active or induced defence responses and is found to be increased after pathogen attack in plants. They are expressed to limit the cellular spreading of the infection through the establishment of structural barriers or the generation of highly toxic environments by massively producing reactive oxygen species (ROS) and reactive nitrogen species (RNS) and leading to programmed cell death (Almagro et al. 2009). Apart from these a number of simple and complex phenolic compounds also accumulate in plant tissues against microbial pathogens. The synthesis, release and accumulation of phenols are central to many defence strategies employed by plants against microbial invaders (Bhattacharya et al. 2010). In many plant tissues, increased abundance of Polyphenol oxidase (PPO) transcripts in response to infection suggest that PPO genes are induced as part of a general defence response in plants. PPO is known to catalyse the oxidation of monophenols and/or o-diphenols to o-quinones. These o-

quinone–protein complexes, participate in blocking invading pathogens (Boeckx et al. 2015). In this study, an increase in PAL, PO and PPO activity in leaves of plants were observed in all treatments (T1: Buffer Control PBS; T2: Biological Control *Trichoderma*; T3: Chemical Control Carbendazim 12 % + Mancozeb 63 % and T4: Test culture MPSK 23) after the challenge with *F. solani*. However in the case of T1, the activities remained steady after the initial increase up to 15 days and eventually lead to the death of the plant. Maximum accumulation of the defence enzymes was recorded for T4 treatment. Such trend was observed in both the Khola and Sitara varieties of chilli. Total phenolic content 15 days after *F. solani* exposure, in the plants treated with MPSK 23 (T4) was also higher in both the varieties as compared to that of PBS control (T1). This increase in the total phenolic compounds and defense related enzymes can be attributed to the application of MPSK 23 which might have played a role in the induction of resistance in plants after pathogen challenge. Kavitha et al. (2005) proved the effectiveness of the pre-treatment of *B. subtilis* CBE4 and *Pseudomonas chlororaphis* BCA+ in chilli plants challenged with *P. aphanidermatum* which showed higher activities of PAL, PO, PPO and, β-1,3 glucanase. In another study, Sundaramoorthy et al. (2012) also proved the effectiveness of *B. subtilis* EPCO16 and EPC5 and *P. fluorescens* Pf1 singly and in combination, in controlling the chilli *Fusarium* wilt disease by ISR as evidenced by enhanced activities of PO, PPO, PAL, β-1, 3-glucanase, chitinase and phenols. The defense-related enzyme activities including PO, catalase, superoxide dismutase, PPO, and PAL in pepper challenged with *R. solani* was also reported to be improved significantly when treated once again with the *B. subtilis* SL-44 strain (Wu et al. 2019b). Well in another study by Jayapala et al. (2019) the ISR mediated by a *Bacillus* sp. BSp.3/aM was due to the upregulation of defense-related enzymes (PAL, PO, PPO, lipoxygenase and chitinase) and by the accumulation of phenolic compounds against anthracnose disease in chilli. Recently Ravinder et al. (2022) showed the induction of enzymes PO, PPO and PAL by *Streptomyces puniceus* RHPR9 in chilli plants when challenged with *M. phaseolina*. This thus pointed out that the salt pan bacterium MPSK 23 exhibited all three modes of antagonism against the fungal pathogens which may be expressed sequentially, concurrently or synergistically.

To summarize, among the ten hypersaline bacteria BGUM 14B, MPSK 9 and ABSK 9 showed mixed path antagonism via production of volatile metabolites, ammonia and plant growth promoting attributes like IAA production, ACC deaminase production and nitrogen fixation. Additionally ABSK 9 also solubilized phosphate mineral and MPSK 9 solubilized silicate. Under indirect antagonism, among the three above mentioned isolates, only ABSK 9

showed siderophore and EPS production. The remaining seven hypersaline bacteria *viz.* MPSK 20, MPSK 22, MPSK 23, MPSK 186, MPSK 109, MPSK 14 and SK 473 exhibited mixed path antagonism via the production of diffusible metabolites, lytic enzymes and plant growth promoting attributes like IAA production, ACC deaminase production, nitrogen fixation and zinc solubilisation. Apart from these metabolites, all seven isolates except MPSK 20 also did show ammonia production. All these seven isolates produced EPS and additionally, isolate MPSK 109 and MPSK 186 showed siderophore production being an example of indirect antagonism. MPSK 22, MPSK 23 and MPSK 186 also showed deformation in fungal mycelia inferring the mechanism to be a type of direct antagonism which may be due to their ability to produce antifungal lipopeptides.

5.3 *Bacillus* spp. as biocontrol agents and its safety evaluation

Several *Bacillus* spp. from diverse habitats have been reported as potential biocontrol agents against phytopathogens affecting chilli plants, however none of them are from Goan salt pans. *Bacillus* species offers several advantages over other organisms as they produce endospores, replicate rapidly, synthesize several bioactive metabolites and enzymes and can tolerate extremes in pH, temperature and osmotic conditions (El-Bendary et al. 2016). The three antagonistic salt pan isolates in the present study i.e. MPSK 22, MPSK 23 and MPSK 186 showed similarity to *Bacillus licheniformis, Bacillus paralicheniformis* and *Bacillus subtilis subsp. inaquosorum. Bacilllus licheniformis* have been studied previously as potential antifungal agent against various pathogens (Tendulkar et al. 2007; Gomaa 2012; Jeong et al. 2017; Li et al. 2020). Plant growth promoting (PGP) attributes such as IAA, ammonia and exopolysaccharide production, nitrogen fixation, ACC deaminase activity, Zn solubilisation of *B. licheniformis* have also been previously reported by various groups (Chookietwattana & Maneewan 2012; Goswami et al. 2014; Singh & Jha 2016; James et al. 2023). Production of various hydrolytic enzymes such as glucanase, chitinase, amylase, protease, cellulase and lipase has also been studied (Lim & Kim 2009; Nabti et al. 2013; Mohammad et al. 2017; Won et al. 2019). All these attributes coincide with the findings in the present study. Previous studies by Wang et al. (2017), Pylro et al. (2019), Ramírez-Cariño et al. (2020), Jinal et al. (2020) and Kannan et al. (2021) have also shown antifungal activity of *B. paralicheniformis* against phytopathogens. However this is the first report of a salt pan *B. paralicheniformis* strain MPSK 23 to be antagonistic to *F. pallidoroseum, M. phaseolina* and *P. aphanidermatum* which is not been reported before. This strain further exhibited IAA, ammonia and exopolysaccharide production and the ability to fix atmospheric nitrogen.

Previous studies by Annapurna et al. (2018), Valenzuela-Ruiz et al. (2019) and Abadi et al. (2020) also reported similar results for the above mentioned attributes by different strains of *B. paralicheniformis*. Additionally *B. paralicheniformis* strain MPSK 23 exhibited ACC deaminase activity and Zn solubilisation which are important properties of plant growth promoting bacteria. A previous study by Roslan et al. (2021) also reports the production of protease, cellulase, lipase and amylase by a *B. paralicheniformis* strain; however no study shows production of chitinase and glucanase. Our study also reveals for the first time the potential of a salt pan *Bacillus subtilis subsp. inaquosorum* strain MPSK 186 in being a BCA against *F. pallidoroseum, P. aphanidermatum, M. phaseolina* and *R. solani*. Previous reports have shown its inhibitory activity against other various fungal pathogens such as *Alternaria alternata, Aspergillus niger, Geotrichum candidum, Sordaria fimicola, Fusarium oxysporum* and *Sporisorium scitamineum* (Knight et al. 2018; Syed et al. 2020; Taghian et al. 2021). This *Bacillus* strain is also known to be PGP bacteria and to produce enzymes such as glucanase, cellulase, amylase, lipase and chitinase (Khande et al. 2017; Shah et al. 2017; Talebi Atouei et al. 2019; Cheng et al. 2020). In addition *Bacillus subtilis subsp. inaquosorum* strain MPSK 186, used in the current study has shown siderophore production, atmospheric nitrogen fixation and ACC deaminase activity which has not been reported previously.

Further, the three isolates MPSK 22, MPSK 23 and MPSK 186 showed no haemolysis (γ-haemolysis) of human erythrocytes, thus proving its safety in *in-vivo* applications (Kim et al. 2018). However, a further assessment in ascertaining the safety at a cellular and molecular level needs to be carried out. The results of antibiotic sensitivity test indicated the susceptibility of the isolates to > 50 % of the antibiotics tested. All the isolates exhibited resistance to Cephotaxime which is known to be partially active against gram positive bacteria hence showing partial inhibition of these isolates (Carmine et al. 1983). Similarly neomycin is also known to be more active against gram negative bacteria (Castle et al. 2007, Krieger et al. 2010). Antibiotics co-trimoxazole and nitrofurantoin are active against uropathogens which make the enzyme urease (Tabibian et al. 2008). As MPSK 186 was not positive for urease production it exhibited resistance to the above antibiotics. However all the cultures showed strong susceptibility to broad spectrum antibiotics like Tetracycline, Chloramphenicol, and Ampicillin: Sulbactam; which can be thus used in case of any emergency situation.

5.4 *In-vitro* germination tests with the salt pan *Bacillus* spp.

The effect of salt pan isolates on *in-vitro* seed germination was tested before proceeding with the pot experiments. The three *Bacillus* isolates *viz.* MPSK 22, MPSK 23 and MPSK 186 were examined for their ability to promote the germination of chilli seeds of Khola and Sitara variety, previously infected with the spores of *F. solani*. The seeds were coated with the bacterial cells, 1 % Gum acacia biopolymer was selected as the adjuvant. In a study by Dawar et al. (2008), 2 % gum arabic (also called gum acacia) solution with microbial antagonists *viz, Bacillus thuringiensis, Rhizobium meliloti, A. niger* and *Trichoderma harzianum* showed more promising results in increasing the shoot length, shoot weight, root length, root weight and in reducing infection of root rot fungi *viz*., *M. phaseolina, R. solani* and *Fusarium* spp., on Okra. Various other studies have also used gum acacia as a biopolymer for coating the biocontrol agent onto seeds for inhibiting phytopathogens (Pandey et al. 2011; Chin et al. 2022b; Zhang et al. 2022).

MPSK 23 bioprimed seeds of both varieties showed maximum germination percentage (100 %) as compared to the other two bioagents. Results of the study carried out by Hyder et al. (2020) showed the improvement in the chilli seed germination by rhizobacterial *Pseudomonas* and *Bacillus* spp. seed treatment against *Phytophthora capsici* without posing any negative impact. In our study; as compared to the control seeds (PBS + Gum acacia), the bacterial treatments (MPSK 22, MPSK 23 and MPSK 186) showed better germination energy in the seeds of both the varieties of chilli which infers that the bacterial treatments promote germination of seeds. This was also supported by the data obtained from mean germination time which showed that the Khola seeds primed with MPSK 23 germinated on the 9th day, seeds primed with MPSK 22 and MPSK 186 germinated on the 10th day, whereas control seeds germinated much later on the 15th day. A similar trend was seen in Sitara variety. The total length of the seedlings that emerged from the seeds coated with MPSK 23 was also higher than that of other bacterial isolates and control. Patel et al. (2017) demonstrated higher root and shoot lengths of the chilli seedlings bacterized with *B. subtilis* and *Alcaligenes* sp. as compared to the control. Finally the seed vigour index in seeds coated with MPSK 23 was calculated which was found to be higher than that of MPSK 22 or MPSK 186 and the control. The seed vigour index is the sum total of those properties of the seed which determine the level of activity and performance of the seed during germination and seedling emergence. A study by Gowtham et al. (2018) demonstrated the highest seed germination and seedling vigor of chilli seeds treated with *B. amyloliquefaciens* followed by *Burkholderia cepacia* and

Providencia rettgeri protecting them against chilli anthracnose disease. Seed bacterization of *C. annuum* L. with strain *Bacillus* sp. LBF-01 also significantly increased the seed germination and vigour index in comparison to the untreated control conferring greater disease protection against *F. oxysporum* (Chowdhury et al. 2020). Thus it can be concluded that the biopriming of the chilli seeds with BCAs MPSK 22, MPSK 23 and MPSK 186 promoted germination and growth of the seedling even after exposing it to the plant pathogen *F. solani*. Among the three salt pan isolates MPSK 23 treatment showed the highest germination percentage, germination energy, total seedling length and vigour Index. The treatment also reduced mean germination time as compared to control non treated seeds of both the varieties. Bacterial isolate MPSK 23 was thus selected for the *in-vivo* trials.

5.5 *In-vivo* trials with *Bacillus* strain MPSK 23 as a biocontrol agent against *F. solani*

Before starting with the pot trials, the salt pan isolate *Bacillus paralicheniformis* strain MPSK 23 was assessed for its ability to tolerate extreme conditions in temperature, pH and salinity and replicate in soil. Soil is a dynamic system that responds to environmental changes. Hence it is very important for a BCA to be able to thrive in the constantly evolving soil dynamics and its extreme conditions. Isolate MPSK 23 exhibited tolerance towards a wide range of temperatures (10 – 50 °C), pH (6 - 12) and NaCl concentration (0 – 16 %). It also showed growth and replication in the soil for a longer period thus decreasing the number of applications required in an actual field scenario. Further 10 mL of 10^{10} CFU/mL cells of MPSK 23 showed >90 % fungal mycelia inhibition of *F. solani* as compared to the control, thus this cell count was selected for the pot trials. Landa et al. (2001) used 10^{10} CFU/mL of seventeen *Bacillus* spp., three *Paenibacillus* spp., two *Pseudomonas fluorescens* isolates and one *Stenotrophomonas maltophilia* isolate for seed treatment and 10 mL of the cell suspension for the soil treatment to inhibit *Fusarium* Wilt of Chickpea. In another study Maisuria et al. (2008) demonstrated biological control of *Fusarium* wilt of pigeon pea by 10^{10} CFU/mL cells of *Pantoea dispersa* in a field assessment. Recently Ramírez et al. (2022) isolated *Bacillus cereus* MH778713 which elicited tomato plant protection against *F. oxysporum* at the cell count of 10^{10} CFU/mL.

At first, the assessment of disease (wilting) caused by the *F. solani* pathogen in chilli plants was carried out. Incidence of disease was examined in the chilli seedlings which were first exposed to the *F. solani* spores and then consequently treated using various treatments (T1:

Buffer Control PBS; T2: Biological Control *Trichoderma*; T3: Chemical Control Carbendazim 12 % + Mancozeb 63 % and T4: Test culture MPSK 23). Disease incidence gave us the number of plant units that were diseased (in this case wilting of leaves) in relation to the total number of the units examined. *In-vivo* bacterial inoculation of chilli seedlings with biocontrol agent MPSK 23 caused a complete reduction in disease incidence in both the varieties after 35 days from transplantation. This also meant that it showed the highest level of disease suppression (100 %) as no wilting was observed in this treatment group (T4). A pot culture experiment was set up by Yu et al. (2011) to study the effects of *B. subtilis* CAS15 on *F. oxysporum* disease development and growth of pepper plant. *B. subtilis* CAS15 reduced the incidence of *Fusarium* wilt in pepper significantly by 12.5 – 56.9 % in greenhouse experiments. Sundaramoorthy et al. (2012) has reported the reduction of the *Fusarium* wilt incidence (by 17 – 29.75 %) compared to untreated plants in chilli plants by the talc-based bio-formulation of *P. fluorescens* Pf1 and *B. subtilis* EPCO16 and EPC5 strains individually or in combination. In another study Narayanan et al. (2015) reported 66.3 %, 48.8 % and 57.6 % reduction of the disease incidence caused by *F. solani* as compared to the control when *P. fluorescens*, *B. subtilis* and *Trichoderma viride* was applied to the soil in a pot study. However these suppressions are quiet lower in comparison to the suppression percentage reported in this study. Yu et al. (2011) also did report the control effect of the *B. subtilis* treatment on the *Fusarium* wilt in the chilli plant. Control effect or the protective value depicts the ability of the treatment to protect the host plant against the disease causing pathogen. Higher the protective value better is the biocontrol ability. In this study MPSK 23 treatment showed the highest protective value, followed by chemical treatment and biological treatment against the wilting caused by *F. solani* in both Sitara and Khola varieties. Further the severity of the wilting in the infected crops was also studied by counting the number of leaves which showed the symptoms. At the end of 15 days, highest disease severity was observed in T1 treatment (93.33 % in Khola and 95.56 % in Sitara). In T4 treatment group plants did not show any wilting symptoms over the period of study, thus the disease severity was negligible in both the varieties. In the pot experiments carried out by Al-Fadhal et al. (2019), it was found that *B. subtilis and P. fluorescence* were very effective in reducing the severity of disease in cucumber seedlings caused by *F. solani* or *R. solani*, wherein *B. subtilis* showed negligible disease severity against both the pathogens. Miguel-Ferrer et al. (2021) showed lowered wilting severity in Miahuateco chilli crop after treatment with *Trichoderma* spp. In similar study by Hussain et al. (2016) showed 76.4 % disease severity in chilli plants treated with *F. solani* without any biocontrol agent, whereas disease severity was 9.9 % when the plants were treated with *Paecilomyces* sp. Application of *Paecilomyces* sp. also positively

affected the yield and the biomass i.e. fresh and dry shoot weight, fresh and dry root weight and plant height of chilli plants. In a report of Raghu et al. (2018), integrated management *Fusarium* wilt with chemical, biocontrol agent and soil amendment recorded highest root length, shoot length, fresh root weight, dry root weight, fresh shoot weight and dry shoot weight in Sitara and Atirikta varieties of chilli plant. Report by Abd-El-Khair et al. (2019) concluded that the *Trichoderma* spp. combined with thiophanate-methyl increased the vegetative growth parameters of bean plants in terms of root length (cm), shoot length (cm), number of leaves/plant, plant fresh weight (g), and plant dry weight (g). In this study MPSK 23 treated plants also showed better foliage and increased the total height of chilli plant which was comparable to that of healthy plant. Also the length and weight (dry and wet) of both root and shoot also increased with MPSK 23 application. This confirmed the biocontrol ability and the growth promoting ability of the salt pan isolate *Bacillus paralicheniformis* strain MPSK 23 against *F. solani* in chilli plants of Khola and Sitara variety.

The results from this study strengthens the application of the salt tolerant bacteria exhibiting plant growth promoting and broad spectrum antifungal activities as an ideal candidate for sustainable growth promotion as well as biological control. Thus the *Bacillus paralicheniformis* strain MPSK 23 used in the present study isolated from Salt pan of Goa has an integrated application in the control of disease and nutrient management strategies in saline soils as well as regular fields in Goa where chillies are grown. This culture can also be further tested for its activity against various other fungal pathogens infecting an array of agricultural crops.

Summary

1. A total of 89 morphologically variant bacteria were isolated from water and sediment samples of Batim, Agarwado and Ribandar salt pans of Goa, and 108 other bacteria were used from the existing culture collection.
2. Fungal isolates (14) were obtained from the diseased chilli plants from fields of Bicholim, Saligao, Valpoi, Margaon and Khola, out of which three *viz. Fusarium solani, Fusarium oxysporum* and *Macrophomina phaseolina* were chilli plant pathogen.
3. A total of 76 bacteria out of 197 showed inhibitory activities against at least one of the six pathogens namely *Fusarium solani, Fusarium oxysporum, Fusarium pallidoroseum, Rhizoctonia solani, Macrophomina phaseolina* and *Pythium aphanidermatum*. Out of 76, 22 bacterial isolates showed inhibitory activity to all six fungal pathogens.
4. MPSK 22, MPSK 23 and MPSK 186 showed similarity to *Bacillus licheniformis, Bacillus paralicheniformis and Bacillus subtilis subsp. inaquosorum* respectively.
5. MPSK 22, MPSK 23 and MPSK 186 exhibited mixed path antagonism to fungal pathogens via production of diffusible metabolites (antifungal lipopeptides), lytic enzymes (amylase, protease, lipase, chitinase, glucanase and cellulase) and ammonia. They also exhibited plant growth promoting attributes like IAA production, ACC deaminase production, atmospheric nitrogen fixation and zinc solubilisation. All the three isolates produced EPS and additionally isolate MPSK 186 showed siderophore production; thus being an example of indirect antagonism. MPSK 22, MPSK 23 and MPSK 186 further showed hyphal deformations inferring that the fungal inhibition could be due to the direct antagonism.
6. Lichenysin and Fengycin genes were detected in MPSK 22 and MPSK 23 and only Fengycin gene was detected in case of MPSK 186 with PCR analysis, which are coding genes for the production of two broad spectrum antibiotics.
7. These isolates exhibited gamma hemolysis on human blood agar which describes their non-toxicity towards human blood cells.
8. MPSK 186 showed susceptibility to β-lactam, Chloramphenicol, Fluoroquinolones, Tetracycline, Quinolone and Macrolide class of antibiotics. MPSK 22 and MPSK 23 also showed susceptibility to the above antibiotics and in addition it was susceptible to Aminoglycoside, Lincomycin, Sulfonamides and Nitrofuran class of antibiotics.

9. Gum Acacia (1 %) was used for coating the bacterial cells of the three isolates on chilli seed cultivars of Khola and Sitara for studying the germination parameters.
10. MPSK 23 treatments showed increased germination percentage (%), germination energy, total seedling length and higher vigour Index of the chilli. The treatment also reduced the mean germination time as compared to control non treated seeds of both the varieties.
11. Isolate MPSK 23 exhibited tolerance to a wide range of temperature (10 – 50 °C), pH (6 - 12) and NaCl concentration (0 – 16 %). It also showed growth and replication in the soil for longer period.
12. *In-vivo* bacterial inoculation of chilli seedlings with MPSK 23 caused a complete suppression of wilt incidence in both the varieties of chillies.
13. It was observed that out of 3 treatments, the isolate MPSK 23 exhibited the highest protective value (100 %) in both the varieties of chillies as compared to chemical and biological treatments.
14. Application of MPSK 23 also showed increase in activity of defense enzymes Phenylalanine ammonia-lyase (PAL), Peroxidase (PO), Polyphenol oxidase (PPO) and Total phenol content (TPC) in the plants.
15. The MPSK 23 treated plants had better foliage and increase in the total height of chilli plants when compared to that of healthy plants.
16. Length and weight (dry and wet) of both root and shoot also increased with MPSK 23 application.
17. Lyophilized cells of MPSK 23 remained viable over the study period of 12 months at both refrigerated 4 ± 2 ℃ and room temperature 28 ± 2 °C, which infers the long term stability of the bacterial stain.
18. Application of cells of MPSK 23 with 1 % Gum Acacia was found to be an effective way of treatment application.

Thus we summarize that the hypersaline environment like salt pan ecosystem harbor *Bacillus* spp. that can be effectively used as growth promoters and biocontrol agents against chilli pathogenic fungi *viz. F. solani, F. oxysporum, F. pallidoroseum, M. phaseolina, P. aphanidermatum* and *R. solani* against wilting and rotting

Conclusion

This study thus elaborates the properties and the potential application of *Bacillus* spp. isolated from salt pan ecosystem in the control of fungal pathogens like *F. solani, F. pallidoroseum, F. oxysporum, P. aphanidermatum, M. phaseolina* and *R. solani* which causes wilt and rot in chilli plants. These bacteria not only suppress the disease in chilli plants, but can also enhance its growth thus proving to be effective biocontrol agents.

Bibliography

1. Abadi, V. A. J. M., Sepehri, M., Rahmani, H. A., Zarei, M., Ronaghi, A., Taghavi, S. M., & Shamshiripour, M. (2020). Role of dominant phyllosphere bacteria with plant growth–promoting characteristics on growth and nutrition of maize (*Zea mays* L.). Journal of Soil Science and Plant Nutrition, 20(4), 2348-2363.
2. Abawi, G. S., & Corrales, P. (1990). Seed transmission and effects of fungicide seed treatments against *Macrophomina phaseolina* in dry edible beans. Turrialba Volumen 40.
3. Abd-El-Khair, H., Elshahawy, I. E., & Haggag, H. E. (2019). Field application of *Trichoderma* spp. combined with thiophanate-methyl for controlling *Fusarium solani* and *Fusarium oxysporum* in dry bean. Bulletin of the National Research Centre, 43(1), 1-9.
4. Abeysinghe, S. (2009). Efficacy of combined use of biocontrol agents on control of *Sclerotium rolfsii* and *Rhizoctonia solani* of *Capsicum annuum*. Archives of Phytopathology and Plant Protection, 42(3), 221–227.
5. Acea, M. J., Moore, C. R., & Alexander, M. (1988). Survival and growth of bacteria introduced into soil. Soil Biology and Biochemistry, 20(4), 509-515.
6. Adedire, O.M., Pitan, A., Farinu, A.O. & Ogundipe, W.F. (2019). The biocontrol of soil transmitted *Cercospora capsici* with *Lactobacillus plantarum*. Journal of Advanced and Applied Microbiology, 21, 1–8.
7. Agrifarming (2014) Retrieved from: Chilli Farming; Pepper Farming - A Full Guide | Agri Farming on 9th December 2022.
8. Ahmed, E., & Holmström, S. J. (2014). Siderophores in environmental research: roles and applications. Microbial biotechnology, 7(3), 196-208.
9. Ahmed, W., Zhou, G., Yang, J., Munir, S., Ahmed, A., Liu, Q., Zhao, Z & Ji, G. (2022). *Bacillus amyloliquefaciens* WS-10 as a potential plant growth-promoter and biocontrol agent for bacterial wilt disease of flue-cured tobacco. Egyptian Journal of Biological Pest Control, 32(1), 1-14.
10. Akgül, D.S. & Mirik, M. (2008). Biocontrol of *Phytophthora capsici* on pepper plants by *Bacillus megaterium* strains. Journal of Plant Pathology, 1, 29–34.
11. Aktar, W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology, 2(1), 1-12.

12. Alaa Fathalla, A. M. & El-Sharkawy, E. E. (2020). Biological control using *Streptomyces* sp. Kp109810 and different genotypes of pepper (*Capsicum annuum* L.) on root rot diseases. Journal of Applied Plant Protection. 9(1), 25–37.
13. Al-Ani, R. A., Adhab, M. A., Mahdi, M. H., & Abood, H. M. (2012). *Rhizobium japonicum* as a biocontrol agent of soybean root rot disease caused by *Fusarium solani* and *Macrophomina phaseolina*. Plant Protection Science, 48(4), 149-155.
14. Al-Fadhal, F. A., AL-Abedy, A. N., & Al Khafaji, D. A. (2019). Isolation and molecular identification of *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber (*Cucumis sativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis*. Egyptian Journal of Biological Pest Control, 29(1), 1-11.
15. Ali, B., Sabri, A. N., & Hasnain, S. (2010). Rhizobacterial potential to alter auxin content and growth of *Vigna radiata* (L.). World Journal of Microbiology and Biotechnology, 26(8), 1379-1384.
16. Alloway, B. J. (2004). *Zinc in soils and crop nutrition. International Zinc Association Communications*, IZA Publications, Brussels.
17. Almagro, L., Gómez Ros, L. V., Belchi-Navarro, S., Bru, R., Ros Barceló, A., & Pedreño, M. A. (2009). Class III peroxidases in plant defence reactions. Journal of Experimental Botany, 60(2), 377-390.
18. Al-Rawahi, A.K. & Hancock, J.G. (1998). Parasitism and biological control of *Verticillium dahliae* by *Pythium oligandrum*. Plant Diseases, 82(10), 1100–1106.
19. Anand, T., Chandrasekaran, A., Kuttalam, S., Senthilraja, G. & Samiyappan, R. (2010). Integrated control of fruit rot and powdery mildew of chilli using the biocontrol agent *Pseudomonas fuorescens* and a chemical fungicide. Biological Control, 52(1), 1–7.
20. Annapurna, K., Govindasamy, V., Sharma, M., Ghosh, A., & Chikara, S. K. (2018). Whole genome shotgun sequence of *Bacillus paralicheniformis* strain KMS 80, a rhizobacterial endophyte isolated from rice (*Oryza sativa* L.). 3 Biotech, **8**(5), 1- 4.
21. Arfarita, N., Hidayati, N., Rosyidah, A., Machfudz, M., & Higuchi, T. (2016). Exploration of indigenous soil bacteria producing-exopolysaccharides for stabilizing of aggregates land potential as biofertilizer. Journal of Degraded and Mining Lands Management, 4(1), 697-702.
22. Arora, H., Sharma, A., Sharma, S., Haron, F. F., Gafur, A., Sayyed, R. Z., & Datta, R. (2021). *Pythium* Damping-Off and Root Rot of *Capsicum annuum* L.: Impacts, Diagnosis, and Management. Microorganisms, 9(4), 823.

23. Arora, N. K., Kim, M. J., Kang, S. C., & Maheshwari, D. K. (2007). Role of chitinase and β-1, 3-glucanase activities produced by a fluorescent pseudomonad and *in vitro* inhibition of *Phytophthora capsici* and *Rhizoctonia solani*. Canadian Journal of Microbiology, 53(2), 207-212.
24. Arul Jose, P., Satheeja Santhi, V., & Jebakumar, S. R. (2011). Phylogenetic-affiliation, antimicrobial potential and PKS gene sequence analysis of moderately halophilic *Streptomyces* sp. inhabiting an Indian saltpan. Journal of Basic Microbiology, 51(4), 348-356.
25. Ashwini, N., & Srividya, S. (2014). Potentiality of *Bacillus subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichum gloeosporioides* OGC1. 3 Biotech ,4(2), 127–136.
26. Atanasova, N. S., Pietilä, M. K., & Oksanen, H. M. (2013). Diverse antimicrobial interactions of halophilic archaea and bacteria extend over geographical distances and cross the domain barrier. Microbiology Open, 2(5), 811-825.
27. Athukorala, S. N., Fernando, W. D., & Rashid, K. Y. (2009). Identification of antifungal antibiotics of *Bacillus* species isolated from different microhabitats using polymerase chain reaction and MALDI-TOF mass spectrometry. Canadian Journal of Microbiology, 55(9), 1021-1032.
28. Babu, B. K., Saxena, A. K., Srivastava, A. K., & Arora, D. K. (2007). Identification and detection of *Macrophomina phaseolina* by using species-specific oligonucleotide primers and probe. Mycologia, 99(6), 797-803.
29. Bale, J. S., Van Lenteren, J. C., & Bigler, F. (2008). Biological control and sustainable food production. Philosophical Transactions of the Royal Society B: Biological Sciences, 363(1492), 761-776.
30. Ballav, S., Kerkar, S., Thomas, S., & Augustine, N. (2015). Halophilic and halotolerant actinomycetes from a marine saltern of Goa, India producing anti-bacterial metabolites. Journal of Bioscience and Bioengineering, 119(3), 323-330.
31. Bartakke, A. (2018). *Formulation of a biofertilizer for salt tolerant rice grown in khazan soils, using salt pan bacteria* (Doctoral dissertation, Goa University).
32. Bashir, M. R., Atiq, M., Sajid, M., Mohsan, M., Abbas, W., Alam, M. W., & Bashair, M. (2018). Antifungal exploitation of fungicides against *Fusarium oxysporum f. sp. capsici* causing *Fusarium* wilt of chilli pepper in Pakistan. Environmental Science and Pollution Research, 25(7), 6797-6801.

33. Beneduzi, A., Ambrosini, A., & Passaglia, L. M. (2012). Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genetics and molecular biology, 35, 1044-1051.
34. Berrada, I., Benkhemmar, O., Swings, J., Bendaou, N., & Amar, M. (2012). Selection of halophilic bacteria for biological control of tomato gray mould caused by *Botrytis cinerea*. Phytopathologia Mediterranea, 625-630.
35. Bhambure, A. B., Mahajan, G. R., & Kerkar, S. (2018). Salt tolerant bacterial inoculants as promoters of rice growth and microbial activity in coastal saline soil. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 88(4), 1531-1538.
36. Bhat, M. N., Mesta, R., Yenjerappa, S. T., Tatagar, M. H., Sardana, H. R., Singh, D., Vennila, S., Sabir, N. & Ahmad, M. (2016). Biological control of *Fusarium* wilt of chillies using *Trichoderma* spp. Indian Journal of Horticulture, 73(1), 74-77.
37. Bhattacharya, A., Sood, P., & Citovsky, V. (2010). The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. Molecular Plant Pathology, 11(5), 705-719.
38. Bhunjun, C. S., Phillips, A. J., Jayawardena, R. S., Promputtha, I., & Hyde, K. D. (2021). Importance of molecular data to identify fungal plant pathogens and guidelines for pathogenicity testing based on Koch's Postulates. Pathogens, 10(9), 1096.
39. Blum, J. S., Kulp, T. R., Han, S., Lanoil, B., Saltikov, C. W., Stolz, J. F., Miller, L. G. & Oremland, R. S. (2012). *Desulfohalophilus alkaliarsenatis* gen. nov., sp. nov., an extremely halophilic sulfate-and arsenate-respiring bacterium from Searles Lake, California. Extremophiles, 16(5), 727-742.
40. Boeckx, T., Winters, A. L., Webb, K. J., & Kingston-Smith, A. H. (2015). Polyphenol oxidase in leaves: is there any significance to the chloroplastic localization?. Journal of Experimental Botany, 66(12), 3571-3579.
41. Bosland, P. W., Votava, E. J. & Votava, E. M. (2012). *Peppers: vegetable and spice capsicums*, second ed. Cambridge, England: CABI.
42. Boukaew, S., & Prasertsan, P. (2014). Suppression of rice sheath blight disease using a heat stable culture filtrate from *Streptomyces philanthi* RM-1-138. Crop Protection, 61, 1-10.
43. Boukaew, S., Petlamul, W., Bunkrongcheap, R., Chookaew, T., Kabbua, T., Thippated, A., & Prasertsan, P. (2018). Fumigant activity of volatile compounds of *Streptomyces philanthi* RM-1-138 and pure chemicals (acetophenone and phenylethyl alcohol) against anthracnose pathogen in postharvest chili fruit. Crop Protection, 103, 1–8.

44. Bowers, J. H., & Locke, J. C. (2000). Effect of botanical extracts on soil populations of *Fusarium* and other soilborne pathogens. Plant Disease, 6, 46-49.
45. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2), 248-254.
46. Brand, M., Messika, Y., Elad, Y., David, D.R., & Sztejnberg, A. (2009). Spray treatments combined with climate modification for the management of *Leveillula taurica* in sweet pepper. European Journal of Plant Pathology, 124(2), 309–329.
47. Bratbak, G. (2018). Microscope methods for measuring bacterial biovolume: epifluorescence microscopy, scanning electron microscopy, and transmission electron microscopy. In *Handbook of methods in aquatic microbial ecology* (pp. 309-317). CRC Press.
48. Brauer, V. S., Rezende, C. P., Pessoni, A. M., De Paula, R. G., Rangappa, K. S., Nayaka, S. C., Gupta, V. K. & Almeida, F. (2019). Antifungal agents in agriculture: Friends and foes of public health. Biomolecules, 9(10), 521.
49. Brueske, C. H. (1980). Phenylalanine ammonia lyase activity in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita*. Physiological Plant Pathology, 16(3), 409-414.
50. Carmichael, J. K. (1991). Treatment of herpes zoster and postherpetic neuralgia. American Family Physician, 44(1), 203-10.
51. Carmine, A. A., Brogden, R. N., Heel, R. C., Speight, T. M., & Avery, G. S. (1983). Cefotaxime. Drugs, 25(3), 223-289.
52. Castaldi, S., Petrillo, C., Donadio, G., Piaz, F. D., Cimmino, A., Masi, M., Evidente, A., & Isticato, R. (2021). Plant Growth Promotion Function of *Bacillus* sp. Strains Isolated from Salt-Pan Rhizosphere and Their Biocontrol Potential against *Macrophomina phaseolina*. International Journal of Molecular Sciences, 22(7), 3324.
53. Castle, S. S. (2007). *xPharm: The comprehensive pharmacology reference* (pp.905-909), Elsevier, Netherland.
54. Chacón-López, A., Guardado-Valdivia, L., Bañuelos-González, M., López-García, U., Montalvo-González, E., Arvizu-Gómez, J., Stoll, A., & Aguilera, S. (2021). Effect of Metabolites Produced by *Bacillus atrophaeus* and *Brevibacterium frigoritolerans* Strains on Postharvest Biocontrol of *Alternaria alternata* in Tomato (*Solanum lycopersicum* L.). Biocontrol Science, 26(2), 67-74.

55. Chaiharn, M., Chunhaleuchanon, S., & Lumyong, S. (2009). Screening siderophore producing bacteria as potential biological control agent for fungal rice pathogens in Thailand. World Journal of Microbiology and Biotechnology, 25(11), 1919-1928.
56. Chanchaichaovivat, A., Ruenwongsa, P., & Panijpan, B. (2007). Screening and identifcation of yeast strains from fruits and vegetables: Potential for biological control of postharvest chilli anthracnose (*Colletotrichum capsici*). Biological Control, 42(3), 326–335.
57. Chandrakala, C., Voleti, S. R., Bandeppa, S., Sunil Kumar, N., & Latha, P. C. (2019). Silicate solubilization and plant growth promoting potential of *Rhizobium* sp. isolated from rice rhizosphere. Silicon, 11(6), 2895-2906.
58. Chang, C. I., Liu, W. Y., & Shyu, C. Z. (2000). Use of prawn blood agar hemolysis to screen for bacteria pathogenic to cultured tiger prawns *Penaeus monodon*. Diseases of Aquatic Organisms, 43(2), 153-157.
59. Chaudhari, R., Hajoori, M., Suthar, M., Desai, S., & Bharthana-Vesu, S. (2017). Isolation, screening and characterization of marine bacteria for exopolysaccharide production. Bioscience Discovery, 8, 643-9.
60. Chemeltorit, P.P., Mutaqin, K.H., & Widodo, W. (2017). Combining *Trichoderma hamatum* THSW13 and *Pseudomonas aeruginosa* BJ10–86: a synergistic chili pepper seed treatment for *Phytophthora capsici* infested soil. European Journal of Plant Pathology, 147(1), 157–166.
61. Chen, L., Wang, G., Bu, T., Zhang, Y., Wang, Y., Liu, M., & Lin, X. (2010). Phylogenetic analysis and screening of antimicrobial and cytotoxic activities of moderately halophilic bacteria isolated from the Weihai Solar Saltern (China). World Journal of Microbiology and Biotechnology, 26(5), 879-888.
62. Chen, W. M., Sheu, F. S., & Sheu, S. Y. (2011). Novel L-amino acid oxidase with algicidal activity against toxic cyanobacterium *Microcystis aeruginosa* synthesized by a bacterium *Aquimarina* sp. Enzyme and Microbial Technology, 49(4), 372-379.
63. Chen, Y., Liu, S. A., Mou, H., Ma, Y., Li, M., & Hu, X. (2017). Characterization of lipopeptide biosurfactants produced by *Bacillus licheniformis* MB01 from marine sediments. Frontiers in Microbiology, 8, 871.
64. Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R., & Guo, J. H. (2013). Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. Environmental Microbiology, 15(3), 848-864.

65. Cheng, T. H., Ismail, N., Kamaruding, N., Saidin, J., & Danish-Daniel, M. (2020). Industrial enzymes-producing marine bacteria from marine resources. Biotechnology Reports, 27, e00482.
66. Chin, J. M., Lim, Y. Y., & Ting, A. S. Y. (2022b). Biopriming chilli seeds with *Trichoderma asperellum*: A study on biopolymer compatibility with seed and biocontrol agent for disease suppression. Biological Control, 165, 104819.
67. Chin, J. M., Lim, Y. Y., & Ting, A. S. Y. (2022a). Biopriming *Pseudomonas fluorescens* to vegetable seeds with biopolymers to promote coating efficacy, seed germination and disease suppression. Journal of the Saudi Society of Agricultural Sciences, 21 (8), 493-505.
68. Chlebek, D., Pinski, A., Żur, J., Michalska, J., & Hupert-Kocurek, K. (2020). Genome mining and evaluation of the biocontrol potential of *Pseudomonas fluorescens* BRZ63, a new endophyte of oilseed rape (*Brassica napus* L.) against fungal pathogens. International journal of Molecular Sciences, 21(22), 8740.
69. Chookietwattana, K., & Maneewan, K. (2012). Selection of efficient salt-tolerant bacteria containing ACC deaminase for promotion of tomato growth under salinity stress. Soil & Environment, 31(1), 30-36.
70. Chowdhury, S. K., Majumdar, S., & Mandal, V. (2020). Application of *Bacillus* sp. LBF-01 in *Capsicum annuum* plant reduces the fungicide use against *Fusarium oxysporum*. Biocatalysis and Agricultural Biotechnology, 27, 101714.
71. Claus, D. (1986). The genus *Bacillus*. Bergey's manual of systematic bacteriology, 2, 1105-1139.
72. Cochrane, S. A., & Vederas, J. C. (2016). Lipopeptides from *Bacillus* and *Paenibacillus* spp.: a gold mine of antibiotic candidates. Medicinal Research Reviews, 36(1), 4-31.
73. Coico, R. (1997). Gram staining. Current Protocols in Immunology, 23(1), A-3O.
74. Coker, J. A. (2016). Extremophiles and biotechnology: current uses and prospects. F1000Research, 5.
75. Coronado, M. J., Vargas, C., Hofemeister, J., Ventosa, A., & Nieto, J. J. (2000). Production and biochemical characterization of an α-amylase from the moderate halophile *Halomonas meridiana*. FEMS microbiology letters, 183(1), 67-71.
76. Couto, C. R. D. A., Alvarez, V. M., Marques, J. M., Jurelevicius, D. D. A., & Seldin, L. (2015). Exploiting the aerobic endospore-forming bacterial diversity in saline and hypersaline environments for biosurfactant production. BMC Microbiology, 15(1), 1-17.

77. Crowley, D. E. (2006). Microbial siderophores in the plant rhizosphere. In *Iron nutrition in plants and rhizospheric microorganisms* (pp. 169-198). Springer, Dordrecht.
78. Dahiya, P., Kaushik, R., & Sindhu, A. (2020). An Introduction to Plant Growth Promoting Rhizobacteria, Antifungal Metabolites Biosynthesis using PRPR with reference to *Pseudomonas* species and It's other characteristics like Antagonistic and Biocontrolling properties. International Research Journal on Advanced Science Hub, 2, 95-100.
79. Daliakopoulos, I. N., Tsanis, I. K., Koutroulis, A., Kourgialas, N. N., Varouchakis, A. E., Karatzas, G. P., & Ritsema, C. J. (2016). The threat of soil salinity: A European scale review. Science of the Total Environment, 573, 727-739.
80. Dar, G. H., Mir, G. H., Rashid, H., Dar, W. A., & Majeed, M. (2015). Evaluation of microbial antagonists for the management of wilt/root rot and damping-off diseases in chilli (*Capsicum annuum*). Vegetos, 28(4), 102-110.
81. DasSarma, S., DasSarma, P., Laye, V. J., & Schwieterman, E. W. (2020). Extremophilic models for astrobiology: haloarchaeal survival strategies and pigments for remote sensing. Extremophiles, 24(1), 31-41.
82. Dawar, S., Hayat, S., Anis, M., & Zaki, M. J. (2008). Effect of seed coating material in the efficacy of microbial antagonists for the control of root rot fungi on okra and sunflower. Pakistan Journal of Botany, 40(3), 1269-1278.
83. de Lourdes Moreno, M., Pérez, D., García, M. T., & Mellado, E. (2013). Halophilic bacteria as a source of novel hydrolytic enzymes. Life, 3(1), 38-51.
84. De, K.K. (2013). *Plant Tissue Culture*. Kolkata, India: New Central Book Agency (P) Ltd.
85. del Carmen Orozco-Mosqueda, M., Glick, B. R., & Santoyo, G. (2020). ACC deaminase in plant growth-promoting bacteria (PGPB): an efficient mechanism to counter salt stress in crops. Microbiological Research, 235, 126439.
86. Deshpande, T. (2017). State of agriculture in India. *PRS Legislative Research*, 53(8), 6-7.
87. Dewi, E., Rosmana, A., & Kuswinanti, T. (2021). The use of endophyte fungal isolates in controlling *Fusarium oxysporum*, the causal agent of wilt disease on chilli (*Capsicum annuum*). In *IOP Conference Series: Earth and Environmental Science* (Vol. 807, No. 2, p. 022104). IOP Publishing.
88. Dharumadurai, D., Annamalai, P., Nooruddin, T., & Saravanan, C. (2014). Isolation, characterization of antibacterial methyl substituted β-lactum compound from

Streptomyces noursei DPTD21 in saltpan soil, India. Journal of Biologically Active Products from Nature, 4(2), 71-88.

89. Donia, M., & Hamann, M. T. (2003). Marine natural products and their potential applications as anti-infective agents. The Lancet infectious diseases, 3(6), 338-348.
90. Donio, M. B. S., Viji, V. T., Jenifer, J. A., Velmurugan, S., Raman, K., Babu, M. M., & Citarasu, T. (2012). Antagonistic *Bacillus cereus* TC-1 Isolated from Solar Salt Work in Southern India. Bangladesh Journal of Microbiology, 29(1), 27-31.
91. Duan, Y., Chen, R., Zhang, R., Jiang, W., Chen, X., Yin, C., & Mao, Z. (2021). Isolation, identification, and antibacterial mechanisms of *Bacillus amyloliquefaciens* QSB-6 and its effect on plant roots. Frontiers in Microbiology, 12, 746799.
92. Dutta, B., & Bandopadhyay, R. (2022). Biotechnological potentials of halophilic microorganisms and their impact on mankind. Beni-Suef University Journal of Basic and Applied Sciences, 11(1), 1-16.
93. Echave, J., Pereira, A. G., Carpena, M., Prieto, M. Á., & Simal-Gandara, J. (2020). Capsicum seeds as a source of bioactive compounds: biological properties, extraction systems, and industrial application. In *Capsicum*. London: IntechOpen.
94. Eilenberg, J. (2006). Concepts and visions of biological control. In *An ecological and societal approach to biological control* (pp. 1-11). Springer, Dordrecht.
95. Eilenberg, J., Hajek, A., & Lomer, C. (2001). Suggestions for unifying the terminology in biological control. BioControl, 46(4), 387-400.
96. Ekefan, E.J., Jama, A., & Gowen, S.R. (2009). Potential of *Trichoderma harzianum* isolates in biocontrol of *Colletotrichum capsici* causing anthracnose of pepper (*Capsicum* spp.) in Nigeria. Journal of Applied Biosciences, 20, 1138–1145.
97. El-Bendary, M. A., Hamed, H. A., & Moharam, M. E. (2016). Potential of *Bacillus* isolates as bio-control agents against some fungal phytopathogens. Biocatalysis and Agricultural Biotechnology, 5, 173-178.
98. Enache, M., Teodosiu, G., Itoh, T., Kamekura, M., & Stan-Lotter, H. (2017). Halophilic microorganisms from man-made and natural hypersaline environments: physiology, ecology, and biotechnological potential. In *Adaption of microbial life to environmental extremes* (pp. 201-226). Springer, Cham.
99. Essghaier, B., Fardeau, M. L., Cayol, J. L., Hajlaoui, M. R., Boudabous, A., Jijakli, H., & Sadfi-Zouaoui, N. (2009). Biological control of grey mould in strawberry fruits by halophilic bacteria. Journal of Applied Microbiology, 106(3), 833-846.

100. Etebarian, H. R., Scott, E. S., & Wicks, T. J. (2000). *Trichoderma harzianum* T39 and *T. virens* DAR 74290 as potential biological control agents for *Phytophthora erythroseptica*. European Journal of Plant Pathology, 106(4), 329-337.

101. FAIFA (2016) Retrieved from: https://www.protectourlivelihood.in/ on 9th December 2022.

102. FAO, FAOSTAT (2020). Food and Agriculture Organization, Rome, Italy, retrieved from https://www.fao.org/faostat/en/#data/QCL/visualize on 17th September 2021.

103. Fernandes, M. S., & Kerkar, S. (2021). Halotolerant *Bacillus* sp. as a source of antifungal agents against major mushroom pathogens. Journal of Microbiology, Biotechnology and Food Sciences, 2021, 1125-1129.

104. Fernandes, S., Kerkar, S., Leitao, J., & Mishra, A. (2019). Probiotic Role of Salt Pan Bacteria in Enhancing the Growth of Whiteleg Shrimp, *Litopenaeus vannamei*. Probiotics and Antimicrobial Proteins, 11(4), 1309-1323.

105. Gachomo, E. W., Shonukan, O. O., & Kotchoni, S. O. (2003). The molecular initiation and subsequent acquisition of disease resistance in plants. African Journal of Biotechnology, 2(2), 26-32.

106. Garbeva, P., Van Veen, J. A., & Van Elsas, J. D. (2004). Assessment of the diversity, and antagonism towards *Rhizoctonia solani* AG3, of *Pseudomonas* species in soil from different agricultural regimes. FEMS Microbiology Ecology, 47(1), 51-64.

107. Garrido, A., Atencio, L. A., Bethancourt, R., Bethancourt, A., Guzmán, H., Gutiérrez, M., & Durant-Archibold, A. A. (2020). Antibacterial Activity of Volatile Organic Compounds Produced by the Octocoral-Associated Bacteria *Bacillus* sp. BO53 and *Pseudoalteromonas* sp. GA327. Antibiotics, 9(12), 923.

108. Gauillard, F., Richardforget, F., & Nicolas, J. (1993). New spectrophotometric assay for polyphenol oxidase activity. Analytical Biochemistry, 215(1), 59-65.

109. Giani, M., Garbayo, I., Vílchez, C., & Martínez-Espinosa, R. M. (2019). Haloarchaeal carotenoids: healthy novel compounds from extreme environments. Marine drugs, 17(9), 524.

110. Gomaa, E. Z. (2012). Chitinase production by *Bacillus thuringiensis* and *Bacillus licheniformis*: their potential in antifungal biocontrol. The Journal of Microbiology, 50(1), 103-111.

111. Gond, S. K., Bergen, M. S., Torres, M. S., & White Jr, J. F. (2015). Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. Microbiological Research, 172, 79-87.

112. Gontia-Mishra, I., Sapre, S., & Tiwari, S. (2017). Diversity of halophilic bacteria and actinobacteria from India and their biotechnological applications. Indian Journal of Geo-Marine Science, 46(08), 1575-1587.

113. Gopalakrishnan, S., Humayun, P., Kiran, B. K., Kannan, I. G. K., Vidya, M. S., Deepthi, K., & Rupela, O. (2011). Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. World Journal of Microbiology and Biotechnology, 27(6), 1313-1321.

114. Gopalakrishnan, T. R. (2007). Solanaceous Fruit Vegetables. In *Horticulture Science Series 4: Vegetable Crops*, ed. K.V. Peter, 75. New Delhi, India: New Indian Publishing Agency.

115. Goswami, D., Dhandhukia, P., Patel, P., & Thakker, J. N. (2014). Screening of PGPR from saline desert of Kutch: growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. Microbiological Research, 169(1), 66-75.

116. Gowtham, H. G., Murali, M., Singh, S. B., Lakshmeesha, T. R., Murthy, K. N., Amruthesh, K. N., & Niranjana, S. R. (2018). Plant growth promoting rhizobacteria-*Bacillus amyloliquefaciens* improves plant growth and induces resistance in chilli against anthracnose disease. Biological control, 126, 209-217.

117. Güney, İ. G., & Güldür, E. (2018). Inoculation techniques for assessing pathogenicity of *Rhizoctonia solani, Macrophomina phaseolina, Fusarium oxysporum* and *Fusarium solani* on pepper seedlings. Türkiye Tarımsal Araştırmalar Dergisi, 5(1), 1-8.

118. Guo, P., Yang, F., Ye, S., Li, J., Shen, F., & Ding, Y. (2022). Characterization of lipopeptide produced by *Bacillus altitudinis* Q7 and inhibitory effect on *Alternaria alternata*. Journal of Basic Microbiology, 63(1), 26-38.

119. Gupta, B. & Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. International Journal of Genomics, 2014, 1–18.

120. Gupta, G., Parihar, S. S., Ahirwar, N. K., Snehi, S. K., & Singh, V. (2015). Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. Microbial & Biochemical Technology, 7(2), 096-102.

121. Gupta, M., Topgyal, T., Zahoor, A., & Gupta, S. (2021). Rhizobium: Eco-friendly microbes for global food security. In *Microbial Management of Plant Stresses* (pp. 221-233). Woodhead Publishing.

122. Gurung, S. (2016). *Screening of endophytes for the control of root rot pathogens of pepper (Capsicum annum)* (Doctoral dissertation, Tennessee State University).

123. Gutiérrez-Santa Ana, A., Carrillo-Cerda, H. A., Rodriguez-Campos, J., Kirchmayr, M. R., Contreras-Ramos, S. M., & Velázquez-Fernández, J. B. (2020). Volatile emission compounds from plant growth-promoting bacteria are responsible for the antifungal activity against *F. solani*. 3 Biotech, 10(7), 292.

124. Hamedi, J., Mohammadipanah, F., & Ventosa, A. (2013). Systematic and biotechnological aspects of halophilic and halotolerant actinomycetes. Extremophiles, 17(1), 1-13.

125. Hammerschmidt, R., Nuckles, E. M., & Kuć, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiological Plant Pathology, 20(1), 73-82.

126. Hao, R., & Lu, A. (2009). Biodegradation of heavy oils by halophilic bacterium. Progress in Natural Science, 19(8), 997-1001.

127. Henciya, S., Vengateshwaran, T. D., Gokul, M. S., Dahms, H. U., & James, R. A. (2020). Antibacterial activity of halophilic bacteria against drug-resistant microbes associated with diabetic foot infections. Current Microbiology, 77(11), 3711-3723.

128. Heydari, A., & Pessarakli, M. (2010). A review on biological control of fungal plant pathogens using microbial antagonists. Journal of Biological Sciences, 10(4), 273-290.

129. Holt, J. G., Krieg, N. R., Sneath, P. H., Staley, J. T., & Williams, S. T. (1994). Bergey's Manual of determinative bacteriology.

130. Howard, L. R., Talcott, S. T., Brenes, C. H. & Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. Journal of Agricultural and Food Chemistry, 48(5), 1713-1720.

131. Howell, C. R., Beier, R. C., & Stipanovic, R. D. (1988). Production of ammonia by *Enterobacter cloacae* and its possible role in the biological control of *Pythium* pre-emergence damping-off by the bacterium. Phytopathology, 78(8), 1075-1078.

132. Hsieh, F. C., Li, M. C., Lin, T. C., & Kao, S. S. (2004). Rapid detection and characterization of surfactin-producing *Bacillus subtilis* and closely related species based on PCR. Current Microbiology, 49(3), 186-191.

133. Hussain, F., Shaukat, S. S., Abid, M., Usman, F., & Akbar, M. (2013). Pathogenicity of some important root rot fungi to the chilli crop and their biological control. International Journal of Biology and Biotechnology, 10, 101-108.

134. Hussain, I., Alam, S. S., Khan, I., Shah, B., Naeem, A., Khan, N., Ullah, W., Adnan, M., Shah, S.R.A., Junaid, K. & Iqbal, M. (2016). Medicinal plants rhizosphere

exploration for the presence of potential biocontrol fungi. Journal of Entomology and Zoology Studies, 4(3), 108-113.

135. Hyder, S., Gondal, A. S., Rizvi, Z. F., Ahmad, R., Alam, M. M., Hannan, A., Ahmed, W., Fatima, N. & Inam-ul-Haq, M. (2020). Characterization of native plant growth promoting rhizobacteria and their anti-oomycete potential against *Phytophthora capsici* affecting chilli pepper (*Capsicum annum* L.). Scientific Reports, 10(1), 1-15.
136. Ismail, M. A., Moubasher, A., El-Eraky, A. M., El-Shaer, A. H., & Gouda, H. A. (2017). Virulence of wilt pathogens against pepper cultivars in Egypt. International Journal of Technical Research & Science, 1(10), 304-314.
137. Jafari, S., Aghaei, S. S., Afifi-Sabet, H., Shams-Ghahfarokhi, M., Jahanshiri, Z., Gholami-Shabani, M., Shafiei-Darabi, S. & Razzaghi-Abyaneh, M. (2018). Exploration, antifungal and antiaflatoxigenic activity of halophilic bacteria communities from saline soils of Howze-Soltan playa in Iran. Extremophiles, 22(1), 87-98.
138. Jaihan, P., Sangdee, K., Sangdee, A. (2016). Selection of entomopathogenic fungus for biological control of chilli anthracnose disease caused by *Colletotrichum* spp. European Journal of Plant Pathology, 146(3), 551–564.
139. James, N., Umesh, M., Sarojini, S., Shanmugam, S., Nasif, O., Alharbi, S. A., Chi, N. T. L. & Brindhadevi, K. (2023). Unravelling the potential plant growth activity of halotolerant *Bacillus licheniformis* NJ04 isolated from soil and its possible use as a green bioinoculant on *Solanum lycopersicum* L. Environmental Research, 216, 114620.
140. Jayapala, N., Mallikarjunaiah, N. H., Puttaswamy, H., Gavirangappa, H., & Ramachandrappa, N. S. (2019). Rhizobacteria *Bacillus* spp. induce resistance against anthracnose disease in chili (*Capsicum annuum* L.) through activating host defense response. Egyptian Journal of Biological Pest Control, 29(1), 1-9.
141. Jayaswal, R. K., Fernandez, M., Upadhyay, R. S., Visintin, L., Kurz, M., Webb, J., & Rinehart, K. (1993). Antagonism of *Pseudomonas cepacia* against phytopathogenic fungi. Current Microbiology, 26(1), 17-22.
142. Jeong, M. H., Lee, Y. S., Cho, J. Y., Ahn, Y. S., Moon, J. H., Hyun, H. N., Cha, G.S., & Kim, K. Y. (2017). Isolation and characterization of metabolites from *Bacillus licheniformis* MH48 with antifungal activity against plant pathogens. Microbial Pathogenesis, 110, 645-653.
143. Jeyanthi, V., & Velusamy, P. (2016). Anti-methicillin resistant *Staphylococcus aureus* compound isolation from halophilic *Bacillus amyloliquefaciens* MHB1 and determination of its mode of action using electron microscope and flow cytometry analysis. Indian Journal of Microbiology, 56(2), 148-157.

144. Jinal, H. N., Sakthivel, K., & Amaresan, N. (2020). Characterisation of antagonistic *Bacillus paralicheniformis* (strain EAL) by LC–MS, antimicrobial peptide genes, and ISR determinants. Antonie Van Leeuwenhoek, 113, 1167-1177.
145. Jisha, M.S., Linu, M.S., & Sreekumar, J. (2019). Induction of systemic resistance in chilli (*Capsicum annuum* L.) by *Pseudomonas aeruginosa* against anthracnose pathogen *Colletotrichum capsici*. Journal of Tropical Agriculture, 56(2), 153–166.
146. John, J., Siva, V., Kumari, R., Arya, A., & Kumar, A. (2020). Unveiling cultivable and uncultivable halophilic bacteria inhabiting Marakkanam saltpan, India and their potential for biotechnological applications. Geomicrobiology Journal, 37(8), 691-701.
147. Jones, D. L., & Baxter, B. K. (2017). DNA repair and photoprotection: Mechanisms of overcoming environmental ultraviolet radiation exposure in halophilic archaea. Frontiers in Microbiology, 8, 1882.
148. Joo, G.J. (2005). Production of an anti-fungal substance for biological control of *Phytophthora capsici* causing *Phytophthora* blight in red-peppers by *Streptomyces halstedii*. Biotechnology Letters, 27(3), 201–205.
149. Kamat, T., & Kerkar, S. (2011). Bacteria from Salt Pans: a potential resource of antibacterial metabolites. Recent Research in Science and Technology, 3(9), 46-52.
150. Kamran, S., Shahid, I., Baig, D. N., Rizwan, M., Malik, K. A., & Mehnaz, S. (2017). Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. Frontiers in Microbiology, 8, 2593.
151. Kannan, C., Mishra, D., Rekha, G., Maruthi, P., Shaik, H., & Sundaram, R. M. (2021). Diversity analysis of antagonistic microbes against bacterial leaf and fungal sheath blight diseases of rice. Egyptian Journal of Biological Pest Control, 31(1), 1-16.
152. Karbalaei-Heidari, H. R., Shahbazi, M., & Absalan, G. (2013). Characterization of a novel organic solvent tolerant protease from a moderately halophilic bacterium and its behavior in ionic liquids. Applied Biochemistry and Biotechnology, 170(3), 573-586.
153. Karimi, E., Safaie, N., Shams-Baksh, M., & Mahmoudi, B. (2016). *Bacillus amyloliquefaciens* SB14 from rhizosphere alleviates *Rhizoctonia* damping-off disease on sugar beet. Microbiological Research, 192, 221-230.
154. Karthikeyan, P., Bhat, S. G., & Chandrasekaran, A. M. (2013). Halocin SH10 production by an extreme haloarchaeon *Natrinema* sp. BTSH10 isolated from salt pans of South India. Saudi Journal of Biological Sciences, 20(2), 205-212.
155. Kaur, S., Samota, M. K., Choudhary, M., Choudhary, M., Pandey, A. K., Sharma, A., & Thakur, J. (2022). How do plants defend themselves against pathogens-Biochemical

mechanisms and genetic interventions. Physiology and Molecular Biology of Plants, 1-20.

156. Kaur, T., & Manhas, R. K. (2022). Evaluation of ACC deaminase and indole acetic acid production by *Streptomyces hydrogenans* DH16 and its effect on plant growth promotion. Biocatalysis and Agricultural Biotechnology, 42, 102321.
157. Kavitha, K., Mathiyazhagan, S., Senthilvel, V., Nakkeeran, S., & Chandrasekar, G. (2005). Development of bioformulations of antagonistic bacteria for the management of damping off of Chilli (*Capsicum annuum* L). Archives of Phytopathology and Plant Protection, 38(1), 19-30.
158. Kayasth, M., Gera, R., Dudeja, S. S., Sharma, P. K., & Kumar, V. (2014). Studies on salinization in Haryana soils on free-living nitrogen-fixing bacterial populations and their activity. Journal of Basic Microbiology, 54(3), 170-179.
159. Keijer, J. (1996). The initial steps of the infection process in *Rhizoctonia solani*. In *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control* (pp. 149-162). Springer, Dordrecht.
160. Khan, A., Sutton, J. C., & Grodzinski, B. (2003). Effects of *Pseudomonas chlororaphis* on *Pythium aphanidermatum* and root rot in peppers grown in small-scale hydroponic troughs. Biocontrol Science and Technology, 13(6), 615-630.
161. Khan, K., Bhat, F., & Srinagar, T. (2018). Chilli Wilt Disease: A Serious problem in Chilli cultivation in India. Indian Farmer, 5(09), 988-991.
162. Khan, M. S., Zaidi, A., Ahemad, M., Oves, M., & Wani, P. A. (2010). Plant growth promotion by phosphate solubilizing fungi–current perspective. Archives of Agronomy and Soil Science, 56(1), 73-98.
163. Khande, R., Sharma, S. K., Ramesh, A., & Sharma, M. P. (2017). Zinc solubilizing *Bacillus* strains that modulate growth, yield and zinc biofortification of soybean and wheat. Rhizosphere, 4, 126-138.
164. Khedher, S. B., Kilani-Feki, O., Dammak, M., Jabnoun-Khiareddine, H., Daami-Remadi, M., & Tounsi, S. (2015). Efficacy of *Bacillus subtilis* V26 as a biological control agent against *Rhizoctonia solani* on potato. Comptes Rendus Biologies, 338(12), 784-792.
165. Kim, D. S., & Hwang, B. K. (2014). An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic acid-dependent signalling of the defence response to microbial pathogens. Journal of experimental Botany, 65(9), 2295-2306.
166. Kim, M. J., Ku, S., Kim, S. Y., Lee, H. H., Jin, H., Kang, S., Li, R., Johnston, T.V., Park,M.S., & Ji, G. E. (2018). Safety evaluations of *Bifidobacterium bifidum* BGN4

and *Bifidobacterium longum* BORI. International Journal of Molecular Sciences, 19(5), 1422.

167. Kim, P.I., Ryu, J.W., Kim, Y.H., & Chi, Y.T. (2010). Production of biosurfactant lipopeptides iturin A, fengycin, and surfactin A from *Bacillus subtilis* CMB32 for control of *Colletotrichum gloeosporioides*. Journal of Microbiology and Biotechnology, 20(1), 138–145.
168. Kim, T.S., Dutta, S., Lee, S.W., & Park, K. (2014). Endophytic bacterium *Pseudomonas fuorescens* strain EP103 was effective against *Phytophthora capsici* causing blight in chili pepper. The Korean Journal of Pesticide Science, 18(4), 422–428.
169. Klock, J., Mishra, C., & Starr, C. (2002). U.S. Patent Application No. 09/932,558.
170. Knight, C. A., Bowman, M. J., Frederick, L., Day, A., Lee, C., & Dunlap, C. A. (2018). The first report of antifungal lipopeptide production by a *Bacillus subtilis subsp. inaquosorum* strain. Microbiological Research, 216, 40-46.
171. Ko, H.S., Jin, R.D., Krishnan, H.B., Lee, S.B., & Kim, K.Y. (2009). Biocontrol ability of *Lysobacter antibioticus* HS124 against *Phytophthora* blight is mediated by the production of 4-hydroxyphenylacetic acid and several lytic enzymes. Current Microbiology, 59(6), 608–615.
172. Köhl, J., Kolnaar, R., & Ravensberg, W. J. (2019). Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Frontiers in Plant Science, 845.
173. Koul, B., Chopra, M., & Lamba, S. (2022). Microorganisms as biocontrol agents for sustainable agriculture. In *Relationship Between Microbes and the Environment for Sustainable Ecosystem Services, Volume 1* (pp. 45-68). Elsevier.
174. Krieger, R. (2010). *Hayes' handbook of pesticide toxicology* (Vol. 1). Academic press.
175. Kumar, A. P., Shruti, A., Kumar, V. S., Kumar, S. S., & Prashad, S. K. (2011). Characterization of plant growth promoting bacteria from soil of central and upper Himalayan region. International Journal of Applied Biology and Pharmaceutical Technology, 2(1), 363-369.
176. Kumar, P. (2020). GI Tag: Specificity of the products. *Times of Agriculture, 3,* 17-20. July-Issue-3.pdf (timesofagriculture.in).
177. Kumar, P., Dubey, R. C., & Maheshwari, D. K. (2012). *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiological Research, 167(8), 493-499.
178. Kumar, P., & Sharma, P. K. (2020). Soil salinity and food security in India. Frontiers in Sustainable Food Systems, 4, 533781.

179. Kumar, R.S., Ayyadurai, N., Pandiaraja, P., Reddy, A.V., Venkateswarlu, Y., Prakash, O., & Sakthivel, N. (2005). Characterization of antifungal metabolite produced by a new strain *Pseudomonas aeruginosa* PUPa3 that exhibits broad-spectrum antifungal activity and biofertilizing traits. Journal of Applied Microbiology, 98(1), 145–154.

180. Kumar, V., Menon, S., Agarwal, H., & Gopalakrishnan, D. (2017). Characterization and optimization of bacterium isolated from soil samples for the production of siderophores. Resource-Efficient Technologies, 3(4), 434-439.

181. Kumari, P., Bishnoi, S. K., & Chandra, S. (2021). Assessment of antibiosis potential of *Bacillus* sp. against the soil-borne fungal pathogen *Sclerotium rolfsii* Sacc.(*Athelia rolfsii* (Curzi) Tu & Kimbrough). Egyptian Journal of Biological Pest Control, 31(1), 1-11.

182. Kushner D.J. & Kamekura M. (1988). Physiology of halophilic eubacteria. In: *Halophilic Bacteria* (pp. 109–138). Rodriguez- Valera F, ed. Boca Raton: CRC Press.

183. Kuske, C. R., Banton, K. L., Adorada, D. L., Stark, P. C., Hill, K. K., & Jackson, P. J. (1998). Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. Applied and Environmental Microbiology, 64(7), 2463-2472.

184. Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z. & Barka, E. A. (2022). Biological control of plant pathogens: A global perspective. Microorganisms, 10(3), 596.

185. Lakshmipathy, D. T., & Kannabiran, K. (2009). A morphological, biochemical and biological studies of halophilic *Streptomyces* sp. isolated from saltpan environment. American Journal of Infectious Diseases, 5(3), 200-206.

186. Landa, B. B., Navas-Cortés, J. A., Hervás, A., & Jiménez-Díaz, R. M. (2001). Influence of temperature and inoculum density of *Fusarium oxysporum* f. sp. ciceris on suppression of *Fusarium* wilt of chickpea by rhizosphere bacteria. Phytopathology, 91(8), 807-816.

187. Landis, D. A., & Orr, D. B. (1996). *Biological control: approaches and applications*. Electronic IPM Textbook.

188. Le Borgne, S., Paniagua, D., & Vazquez-Duhalt, R. (2008). Biodegradation of organic pollutants by halophilic bacteria and archaea. Microbial Physiology, 15(2-3), 74-92.

189. Lee, K.J., Kamala-Kannan, S., Sub, H.S., Seong, C.K., & Lee, G.W. (2008). Biological control of *Phytophthora* blight in red pepper (*Capsicum annuum* L.) using *Bacillus subtilis*. World Journal of Microbiology and Biotehnology, 24(7), 1139–1145.

190. Li, Y., Feng, X., Wang, X., Zheng, L., & Liu, H. (2020). Inhibitory effects of *Bacillus licheniformis* BL06 on *Phytophthora capsici* in pepper by multiple modes of action. Biological Control, 144, 104210.
191. Li, Y., Wang, R., Liu, J., Xu, L., Ji, P., Sun, L., Pan, H., Jiang, B. & Li, L. (2019). Identification of a biocontrol agent *Bacillus vallismortis* BV23 and assessment of effects of its metabolites on *Fusarium graminearum* causing corn stalk rot. Biocontrol Science and Technology, 29(3), 263-275.
192. Liebeke, M., Brözel, V. S., Hecker, M., & Lalk, M. (2009). Chemical characterization of soil extract as growth media for the ecophysiological study of bacteria. Applied Microbiology and Biotechnology, 83(1), 161-173.
193. Lim, J. H., & Kim, S. D. (2009). Synergistic plant growth promotion by the indigenous auxins-producing PGPR *Bacillus subtilis* AH18 and *Bacillus licheniforims* K11. Journal of the Korean Society for Applied Biological Chemistry, 52(5), 531-538.
194. Lim, S. M., Yoon, M. Y., Choi, G. J., Choi, Y. H., Jang, K. S., Shin, T. S., Park, H.W., Yu, N.H., Kim, Y.H. & Kim, J. C. (2017). Diffusible and volatile antifungal compounds produced by an antagonistic *Bacillus velezensis* G341 against various phytopathogenic fungi. The Plant Pathology Journal, 33(5), 488.
195. Liu, T., Long, X., Zhou, J. P., Tian, D. W., Yang, Y. H., Zou, C. G., Xu, J.P., Mo, M.H. & Zhang, K. Q. (2021). Fungistatic Mechanism of Ammonia against Nematode-Trapping Fungus *Arthrobotrys oligospora*, and Strategy for This Fungus To Survive Ammonia. mSystems, 6(5), e00879-21.
196. López, C.G., Castellanos, L.N., Ortiz, N.A., & González, J.A. (2019). Control of powdery mildew (*Leveillula taurica*) using *Trichoderma asperellum* and *Metarhizium anisopliae* in different pepper types. Biocontrol 64(1), 77–89.
197. Madbouly, A. K., & Abdelbacki, A. M. (2017). Biocontrol of certain soilborne diseases and promotion of growth of *Capsicum annuum* using biofungicides. Pakistan Journal of Botany, 49(1), 371-378.
198. Madslien, E. H., Rønning, H. T., Lindbäck, T., Hassel, B., Andersson, M. A., & Granum, P. E. (2013). Lichenysin is produced by most *Bacillus licheniformis* strains. Journal of Applied Microbiology, 115(4), 1068-1080.
199. Maisuria, V. B., Gohel, V., Mehta, A. N., Patel, R. R., & Chhatpar, H. S. (2008). Biological control of *Fusarium* wilt of pigeonpea by *Pantoea dispersa*, a field assessment. Annals of Microbiology, 58(3), 411-419.

200. Majeed, M., Mir, G. H., Hassan, M., Mohuiddin, F. A., Paswal, S., & Farooq, S. (2018). Damping off in chilli and its biological management–A Review. International Journal of Current Microbiology and Applied Sciences, 7(04), 2175-2185.
201. Malik, N., Habib, A., Malik, M. K., Zubair, M., Ali, S., Khan, Q. A. T., Qamar, H., Iqbal, S., Amin, M., Hanif, K., Khurshid, M. R. & Mahmood, T. (2022). Use of various plant extracts to inhibit growth of *Fusarium* Wilt caused by *Fusarium oxysporum* in Chillies under laboratory conditions. Bioscience Research, 19(2), 1030-1033.
202. Mallikarjunaswamy, G. E., & Noushad, N. (2021). Probiotic rhizospheric *Bacillus* sp. from *Zingiber officinale* Rosc. displays antifungal activity against soft rot pathogen Pythium sp. Current Plant Biology, 27, 100217.
203. Manjunath, M., Prasanna, R., Nain, L., Dureja, P., Singh, R., Kumar, A., Jaggi, S., & Kaushik, B.D. (2010). Biocontrol potential of cyanobacterial metabolites against damping of disease caused by *Pythium aphanidermatum* in solanaceous vegetables. Archives of Phytopathology and Plant Protection, 43(7), 666–677.
204. Mannai, S., Jabnoun-Khiareddine, H., Nasraoui, B., & Daami-Remadi, M. (2018). *Rhizoctonia* root rot of pepper (*Capsicum annuum*): Comparative pathogenicity of causal agent and biocontrol attempt using fungal and bacterial agents. Journal of Plant Pathology and Microbiology, 9(2), 431-439.
205. Margesin, R., & Schinner, F. (2001). Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles, 5(2), 73-83.
206. Marian, M., & Shimizu, M. (2019). Improving performance of microbial biocontrol agents against plant diseases. Journal of General Plant Pathology, 85(5), 329-336.
207. Marín, A., Ferreres, F., Tomás-Barberán, F. A. & Gil, M. I. (2004). Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). Journal of Agricultural and Food Chemistry, 52(12), 3861-3869.
208. Matsufuji, H., Ishikawa, K., Nunomura, O., Chino, M. & Takeda, M. (2007). Anti-oxidant content of different coloured sweet peppers, white, green, yellow, orange and red (*Capsicum annuum L.*). International Journal of Food Science & Technology, 42(12), 1482-1488.
209. McCoy, T., Frank, D., & Beegle, D. K. (2020). Understanding Pesticide Toxicity. Retrieved from https://vtechworks.lib.vt.edu/bitstream/handle/10919/101593/ENTO-389.pdf?sequence=1&isAllowed=y on 9th December 2022.
210. Menasria, T., Monteoliva-Sánchez, M., Benammar, L., Benhadj, M., Ayachi, A., Hacène, H., Gonzalez-Paredes, A. & Aguilera, M. (2019). Culturable halophilic bacteria

inhabiting Algerian saline ecosystems: a source of promising features and potentialities. World Journal of Microbiology and Biotechnology, 35(9), 1-16.

211. Miguel-Ferrer, L., Romero-Arenas, O., Andrade-Hoyos, P., Sánchez-Morales, P., Rivera-Tapia, J. A., & Fernández-Pavía, S. P. (2021). Antifungal activity of *Trichoderma harzianum* and *T. koningiopsis* against *Fusarium solani* in seed germination and vigor of Miahuateco chili seedlings. Revista mexicana de fitopatología, 39(2), 228-247.
212. Mishra, R. K. (2013). *Diseases Of Vegetable Crops And Their Integrated Management: A Colour Handbook*. New India Publishing Agency.
213. Mmbaga, M. T., Gurung, S., & Maheshwari, A. (2018). Screening of plant endophytes as biological control agents against root rot pathogens of pepper (*Capsicum annum* L.). Journal of Plant Pathololology and Microbiology, 9(3), 1-8.
214. Mnif, I., Grau-Campistany, A., Coronel-León, J., Hammami, I., Triki, M. A., Manresa, A., & Ghribi, D. (2016). Purification and identification of *Bacillus subtilis* SPB1 lipopeptide biosurfactant exhibiting antifungal activity against *Rhizoctonia bataticola* and *Rhizoctonia solani*. Environmental Science and Pollution Research, 23(7), 6690-6699.
215. Mohammad, B. T., Al Daghistani, H. I., Jaouani, A., Abdel-Latif, S., & Kennes, C. (2017). Isolation and characterization of thermophilic bacteria from Jordanian hot springs: *Bacillus licheniformis* and *Thermomonas hydrothermalis* isolates as potential producers of thermostable enzymes. International Journal of Microbiology, 2017.
216. Mohanty, S. K., Franssen, L., & Saha, S. (2019). *The power of international value chains in the Global South*. Geneva: International Trade Centre (ITC).
217. Mojica-Marín, V., Luna-Olvera, H. A., Sandoval-Coronado, C. F., Pereyra-Alférez, B., Morales-Ramos, L. H., Hernández-Luna, C. E., & Alvarado-Gomez, O. G. (2008). Antagonistic activity of selected strains of *Bacillus thuringiensis* against *Rhizoctonia solani* of chili pepper. African Journal of Biotechnology, 7(9).
218. Munshi, N. A., & Dar, G. H. (2004). *In vitro* evaluation of some locally isolated microfungi for antagonism against mulberry fusarial blight pathogen [*Fusarium pallidoroseum* (Cook) Sacc.]. Sericologia, 44, 341-346.
219. Murugan, T., & Murugan, M. (2018). Antibacterial effect of Surface active agent produced by *Pseudomonas* sp. isolated from Saltpan. Journal of Pharmaceutical Sciences and Research, 10(1), 196-197.

220. Muzaffar, M., Bhat, Z. A., Bhat, M. A., Hussain, K., Ahanger, M. A., Hassan, M. G., Bashir, S. & Badri, Z. A. (2021). Chemical Management of Wilt Complex Disease of Chilli. Pesticide Research Journal, 33(1), 50-56.
221. Nabti, E. H., Mokrane, N., Ghoul, M., Manyani, H., Dary, M., & Megias, M. G. (2013). Isolation and characterization of two halophilic *Bacillus* (*B. licheniformis* and *Bacillus* sp.) with antifungal activity. Journal of Ecology of Health & Environment, 1(1), 13-17.
222. Naik, M., Rani, G. D., & Madhukar, H. (2008). Identification of resistant sources against wilt of chilli (*Capsicum annuum* L.) caused by *Fusarium solani* (Mart.) Sacc. Journal of Mycopathological Research, 46(1), 93-96.
223. Nakkeeran, S., Kavitha, K., Chandrasekar, G., Renukadevi, P., & Fernando, W.G. (2006). Induction of plant defence compounds by *Pseudomonas chlororaphis* PA23 and *Bacillus subtilis* BSCBE4 in controlling damping-off of hot pepper caused by *Pythium aphanidermatum*. Biocontrol Science and Technology, 16(4), 403–416.
224. Nantawanit, N., Chanchaichaovivat, A., Panijpan, B., & Ruenwongsa, P. (2010). Induction of defense response against *Colletotrichum capsici* in chili fruit by the yeast *Pichia guilliermondii* strain R13. Biological Control, 52(2), 145–152.
225. Narayanan, P., Vanitha, S., Rajalakshmi, J., Parthasarathy, S., Arunkumar, K., Nagendran, K., & Karthikeyan, G. (2015). Efficacy of biocontrol agents and fungicides in management of mulberry wilt caused by *Fusarium solani*. Journal of Biological Control, 29(2), 107-114.
226. Naureen, Z., Rehman, N. U., Hussain, H., Hussain, J., Gilani, S. A., Al Housni, S. K., Mabood, F., Khan, A.L., Farooq, S., Abbas, G. & Harrasi, A. A. (2017). Exploring the potentials of *Lysinibacillus sphaericus* ZA9 for plant growth promotion and biocontrol activities against phytopathogenic fungi. Frontiers in Microbiology, 8, 1477.
227. Nega, A. (2014). Review on concepts in biological control of plant pathogens. Journal of Biology, Agriculture and Healthcare, 4(27), 33-54.
228. Nelson, P. E., & Toussoun, T. A. (1989). Frequency and pathogenicity of *Fusarium solani* recovered from soybeans with sudden death syndrome. Plant Diseases, 73, 581-584.
229. Nguyen, X.H., Naing, K.W., Lee, Y.S., Tindwa, H., Lee, G.H., Jeong, B.K., Ro, H.M., Kim, S.J., Jung, W.J., & Kim, K.Y. (2012). Biocontrol potential of *Streptomyces griseus* H7602 against root rot disease (*Phytophthora capsici*) in pepper. The Plant Pathology Journal, 28(3), 282–289.

230. Nicholson, C. A., & Fathepure, B. Z. (2004). Biodegradation of benzene by halophilic and halotolerant bacteria under aerobic conditions. Applied and Environmental Microbiology, 70(2), 1222-1225.

231. Nyyssölä, A., Kerovuo, J., Kaukinen, P., von Weymarn, N., & Reinikainen, T. (2000). Extreme halophiles synthesize betaine from glycine by methylation. Journal of Biological Chemistry, 275(29), 22196-22201.

232. Oerke, E. C. (2006). Crop losses to pests. The Journal of Agricultural Science, 144(1), 31-43.

233. Okungbowa, F. I., & Shittu, H. O. (2012). *Fusarium* wilts: An overview. Environmental Research Journal, 6(2), 83-102.

234. Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. World Journal of Microbiology and Biotechnology, 33(11), 1-16.

235. Olatunji, T. L., & Afolayan, A. J. (2018). The suitability of chili pepper (*Capsicum annuum* L.) for alleviating human micronutrient dietary deficiencies: A review. Food science & nutrition, 6(8), 2239-2251.

236. Oren, A. (2010). Industrial and environmental applications of halophilic microorganisms. Environmental technology, 31(8-9), 825-834.

237. Oren, A. (2015). Halophilic microbial communities and their environments. Current Opinion in Biotechnology, 33, 119-124.

238. Oren, A. (2016). Life in hypersaline environments. In *Their world: a diversity of microbial environments* (pp. 301-339). Springer, Cham

239. Pal, K. K., & Gardener, M. B. (2006). Biological control of plant pathogens. The Plant Health Instructor, 1-25.

240. Pandey, R. K., Sinigh, S. R., Gupta, P. K., Goswami, B. K., Singh, D. V., & Gharde, Y. (2011). Effect of different bioformulations of *Paecilomyces lilacinus* against root-knot nematode (*Meloidogyne incognita*) infecting tomato (*Solanum esculentum*). Indian Journal of Agricultural Sciences, 81(3), 261-267.

241. Park, J.W., Balaraju, K., Kim, J.W., Lee, S.W., & Park, K. (2013). Systemic resistance and growth promotion of chili pepper induced by an antibiotic producing *Bacillus vallismortis* strain BS07. Biological Control, 65(2), 246–257.

242. Patel, S., Jinal, H. N., & Amaresan, N. (2017). Isolation and characterization of drought resistance bacteria for plant growth promoting properties and their effect on chilli (*Capsicum annuum*) seedling under salt stress. Biocatalysis and Agricultural Biotechnology, 12, 85-89.

243. Paul, N. C., Ji, S. H., Deng, J. X., & Yu, S. H. (2013). Assemblages of endophytic bacteria in chili pepper (*Capsicum annuum* L.) and their antifungal activity against phytopathogens *in vitro*. Plant Omics, 6(6), 441-448.

244. Pawaskar, M., & Kerkar, S. (2021). Microbial biocontrol agents against chilli plant pathogens over synthetic pesticides: a review. Proceedings of the Indian National Science Academy, 87(4), 578-594.

245. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Clinical and Laboratory Standards Institute, 2017.

246. Perucka, I., & Materska, M. (2007). Antioxidant vitamin contents of *Capsicum annuum* fruit extracts as affected by processing and varietal factors. Acta Scientiarum Polonorum Technologia Alimentaria, 6(4), 67-73.

247. Perveen, S., & Ghaffar, A. (1998). Use of *Paecilomyces lilacinus* in the control of *Fusarium oxysporum* root rot and *Meloidogyne javanica* root knot infection on tomato. Pakistan Journal of Nematology, 16, 71-76.

248. Peter, K. V. & Hazra, P. (2012). *Handbook of Vegetables*. Texas, USA: Studium Press LLC.

249. Petrillo, C., Castaldi, S., Lanzilli, M., Selci, M., Cordone, A., Giovannelli, D., & Isticato, R. (2021). Genomic and physiological characterization of *Bacilli* isolated from salt-pans with plant growth promoting features. Frontiers in microbiology, 12, 715678.

250. Pimentel, D. (1995). Amounts of pesticides reaching target pests: environmental impacts and ethics. Journal of Agricultural and Environmental Ethics, 8(1), 17-29.

251. Pirhadi, M., Enayatizamir, N., Motamedi, H., & Sorkheh, K. (2016). Screening of salt tolerant sugarcane endophytic bacteria with potassium and zinc for their solubilizing and antifungal activity. Bioscience Biotechnology Research Communications, 9, 530-538.

252. Płaza, G., Chojniak, J., Rudnicka, K., Paraszkiewicz, K., & Bernat, P. (2015). Detection of biosurfactants in *Bacillus* species: genes and products identification. Journal of Applied Microbiology, 119(4), 1023-1034.

253. Poornima, S. (2011). Evaluation of disease control and plant growth promotion potential of biocontrol agents on *Pisum sativum* and comparison of their activity with popular chemical control agent-carbendazim. Journal of Toxicology and Environmental Health Sciences, 3(5), 127-138.

254. Priya, E., Nagasathya, A., Steffi, P. F., & Thamilmaraiselvi, B. (2020). Characterization of Actinobacteria Isolated from Saltpan, Environment of Thondi, Ramanathapuram Dt. Research Journal of Pharmacy and Technology, 13(11), 5108-5114.

255. Pylro, V. S., Dias, A. C. F., Andreote, F. D., Varani, A. D. M., Leite, L. R., Andreote, C. C. F., Bernardo, E. R. A., & Martins, T. F. (2019). Closed genome sequence of *Bacillus paralicheniformis* strain CBMAI 1303, a bacterium applied for phytopathogen biocontrol. Microbiology Resource Announcements, 8(3), e01507-18.

256. Raghu, S., Benagi, V. I., Nargund, V. B., & Jayalakshmi, K. (2018). Effect of chemicals, bio control agents and soil amendments integrated in different management modules on plant growth, yield and incidence of *Fusarium* wilt of chilli in Karnataka. International Journal of Chemical Studies, 6, 870-877.

257. Rais, A., Jabeen, Z., Shair, F., Hafeez, F. Y., & Hassan, M. N. (2017). *Bacillus* spp., a bio-control agent enhances the activity of antioxidant defense enzymes in rice against *Pyricularia oryzae*. *PloS* one, 12(11), e0187412.

258. Ramarathnam, R., Bo, S., Chen, Y., Fernando, W. D., Xuewen, G., & De Kievit, T. (2007). Molecular and biochemical detection of fengycin-and bacillomycin D-producing *Bacillus* spp., antagonistic to fungal pathogens of canola and wheat. Canadian Journal of Microbiology, 53(7), 901-911.

259. Ramírez, V., Martínez, J., Bustillos-Cristales, M. D. R., Catañeda-Antonio, D., Munive, J. A., & Baez, A. (2022). *Bacillus cereus* MH778713 elicits tomato plant protection against *Fusarium oxysporum*. Journal of Applied Microbiology, 132(1), 470-482.

260. Ramírez-Cariño, H. F., Guadarrama-Mendoza, P. C., Sánchez-López, V., Cuervo-Parra, J. A., Ramírez-Reyes, T., Dunlap, C. A., & Valadez-Blanco, R. (2020). Biocontrol of *Alternaria alternata* and *Fusarium oxysporum* by *Trichoderma asperelloides* and *Bacillus paralicheniformis* in tomato plants. Antonie van Leeuwenhoek, 113(9), 1247-1261.

261. Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A. S., Srivastav, A.L., & Kaushal, J. (2021). An extensive review on the consequences of chemical pesticides on human health and environment. Journal of Cleaner Production, 283, 124657.

262. Ravinder, P., Manasa, M., & Hameeda, M. V. (2022). Evaluation of antifungal activity of *Streptomyces puniceus* rhpr9 against *Macrophomina phaseolina*, causative agent of charcoal rot disease in chilli (*Capsicum annuum* L.). Plant Archives, 22(2), 1-6.

263. Rini, C. R., & Sulochana, K. K. (2007). Usefulness of *Trichoderma* spp. and fluoresecent pseudomonads (*Pseudomonas fluorescens*) against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. Journal of Tropical Agriculture, 44, 79-82.

264. Romero, D., De Vicente, A., Rakotoaly, R. H., Dufour, S. E., Veening, J. W., Arrebola, E., Cazorla, F.M., Kuipers, O.P., Paquot, M., & Pérez-García, A. (2007). The iturin and

fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward *Podosphaera fusca*. Molecular Plant-Microbe Interactions, 20(4), 430-440.

265. Roshan, K., Tarafdar, A., Saurav, K., Ali, S., Lone, S. A., Pattnaik, S., Tyagi, A., Biswas, K., & Mir, Z. A. (2013). Isolation and screening of bioactive compound from actinomycetes isolated from salt pan of Marakanam district of the state Tamil Nadu, India. Elixir International Journal, 61 (2013) 16826-16831.
266. Roslan, M. A. M., Jefri, N. Q. U. A., Ramlee, N., Rahman, N. A. A., Chong, N. H. H., Bunawan, H., Bharudin, I., Kadir, M.H.A., Mohammad, M., & Razali, H. (2021). Enhancing food waste biodegradation rate in a food waste biodigester with the synergistic action of hydrolase-producing *Bacillus paralicheniformis* GRA2 and *Bacillus velezensis* TAP5 co-culture inoculation. Saudi Journal of Biological Sciences, 28(5), 3001-3012.
267. Rothschild, L. J., & Mancinelli, R. L. (2001). Life in extreme environments. Nature, 409(6823), 1092-1101.
268. Roychowdhury, R., Qaiser, T. F., Mukherjee, P., & Roy, M. (2019). Isolation and characterization of a *Pseudomonas aeruginosa* strain PGP for plant growth promotion. Proceedings of the national academy of sciences, India section b: biological sciences, 89(1), 353-360.
269. Ruangwong, O.U., Pornsuriya, C., Pitija, K., & Sunpapao, A. (2021). Biocontrol mechanisms of *Trichoderma koningiopsis* PSU3-2 against postharvest anthracnose of chili pepper. Journal of Fungi, 7(4), 276.
270. Sadfi, N., Cherif, M., Fliss, I., Boudabous, A., & Antoun, H. (2001). Evaluation of *Bacillus* isolates from salty soils and *Bacillus thuringiensis* strains for the biocontrol of *Fusarium* dry rot of potato tubers. Journal of Plant Pathology, 83,101–118.
271. Sadfi-Zouaoui, N., Essghaier, B., Hajlaoui, M. R., Fardeau, M. L., Cayaol, J. L., Ollivier, B., & Boudabous, A. (2008). Ability of moderately halophilic bacteria to control grey mould disease on tomato fruits. Journal of Phytopathology, 156(1), 42-52.
272. Sahu, P. K., Singh, S., Gupta, A. R., Gupta, A., Singh, U. B., Manzar, N., Bhowmik, A., Singh, H.V., & Saxena, A. K. (2020). Endophytic bacilli from medicinal-aromatic perennial Holy basil (*Ocimum tenuiflorum* L.) modulate plant growth promotion and induced systemic resistance against *Rhizoctonia solani* in rice (*Oryza sativa* L.). Biological Control, 150, 104353.
273. Sandani, H.B., Ranathunge, N.P., Lakshman, P.L., & Weerakoon, W.M. (2019). Biocontrol potential of five *Burkholderia* and *Pseudomonas* strains against

Colletotrichum truncatum infecting chilli pepper. Biocontrol Science and Technology, 29(8), 727–745.

274. Sanogo, S. (2003). Chile pepper and the threat of wilt diseases. Plant Health Progress, 4(1), 23.
275. Sasirekha, B., & Srividya, S. (2016). Siderophore production by *Pseudomonas aeruginosa* FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporioides* causing diseases in chilli. Agriculture and Natural Resources, 50(4), 250–256.
276. Sehrawat, A., Sindhu, S. S., & Glick, B. R. (2022). Hydrogen cyanide production by soil bacteria: Biological control of pests and promotion of plant growth in sustainable agriculture. Pedosphere, 32(1), 15-38.
277. Sekar, A., & Packyam, M. (2014). Screening, identification and antagonistic activity of halo stable *Bacillus* sp. Mk22 used as probiotic in *Penaeus monodon Fabricius*, 1798. African Journal of Food Science, 8(1), 48-53.
278. Setati, M. E. (2010). Diversity and industrial potential of hydrolase producing halophilic/ halotolerant eubacteria. African Journal of Biotechnology, 9(11), 1555-1560.
279. Shah, W., Ali, N., Ahmad, S., Qasim, M., Rahman, H., Ullah, S., Ullah, F., Khattak, B., & Khan, M. D. (2017). Molecular characterization and growth optimization of halo-tolerant amylase producing bacteria isolated from salt mines of Karak, Pakistan. Pure and Applied Biology (PAB), 6(1), 385-393.
280. Shahid, I., Han, J., Hanooq, S., Malik, K. A., Borchers, C. H., & Mehnaz, S. (2021). Profiling of metabolites of *Bacillus* spp. and their application in sustainable plant growth promotion and biocontrol. Frontiers in Sustainable Food Systems, 5, 37.
281. Shahid, S., & Khan, M. R. (2016). Biological control of root-rot on mungbean plants incited by *Macrophomina phaseolina* through microbial antagonists. Plant Pathology Journal, 15(2), 27.
282. Shet, S. A., & Garg, S. (2022). Plant Growth Promotion of *Vigna unguiculata* in Arid Sandy Soil Using Bacterial Species from Coastal Sand Dune. Agricultural Research, 1-12.
283. Sid Ahmed, A., Pérez-Sánchez, C., Egea, C., & Candela, M.E. (1999). Evaluation of *Trichoderma harzianum* for controlling root rot caused by *Phytophthora capsici* in pepper plants. Plant Pathology, 48(1), 58–65.
284. Sid, A., Ezziyyani, M., Egea-Gilabert, C., & Candela, M.E. (2003). Selecting bacterial strains for use in the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternata* in sweet pepper plants. Biologia Plantarum, 47(4), 569–574.

285. Silambarasan, S., Logeswari, P., Cornejo, P., & Kannan, V. R. (2019). Evaluation of the production of exopolysaccharide by plant growth promoting yeast *Rhodotorula* sp. strain CAH2 under abiotic stress conditions. International Journal of Biological Macromolecules, 121, 55-62.
286. Sindhu, S. S., & Dadarwal, K. R. (2001). Chitinolytic and cellulolytic *Pseudomonas* sp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* sp. Cicer in chickpea. Microbiological Research, 156(4), 353-358.
287. Singh, H. P., Parthasarathy V. A., & Prasath, D. (2009). *Horticulture Crops- Varietal Wealth.* New Delhi, India: Studium Press.
288. Singh, J., & Faull, J. L. (2020). Antagonism and biological control. In *Biocontrol of plant diseases* (pp. 167-177). CRC Press.
289. Singh, R. P., & Jha, P. N. (2016). A halotolerant bacterium *Bacillus licheniformis* HSW-16 augments induced systemic tolerance to salt stress in wheat plant (*Triticum aestivum*). Frontiers in Plant Science, 7, 1890.
290. Smith, C. J., & Perfetti, T. A. (2020). A comparison of the persistence, toxicity, and exposure to high-volume natural plant-derived and synthetic pesticides. Toxicology Research and Application, 4, 2397847320940561.
291. Smith, S. N. (2007). An overview of ecological and habitat aspects in the genus *Fusarium* with special emphasis on the soil-borne pathogenic forms. Plant Pathology Bulletin, 16, 97-120.
292. Srikhong, P., Lertmongkonthum, K., Sowanpreecha, R., & Rerngsamran, P. (2018) *Bacillus* sp. strain M10 as a potential biocontrol agent protecting chili pepper and tomato fruits from anthracnose disease caused by *Colletotrichum capsici*. BioControl 63(6), 833–842.
293. Stoytcheva, M. (Ed.). (2011). *Pesticides: formulations, effects, fate*. BoD–Books on Demand.
294. Sundaramoorthy, S., Raguchander, T., Ragupathi, N., & Samiyappan, R. (2012). Combinatorial effect of endophytic and plant growth promoting rhizobacteria against wilt disease of *Capsicum annum* L. caused by *Fusarium solani*. Biological Control, 60(1), 59-67.
295. Suprapta, D. N., Darmadi, A. A. K., & Khalimi, K. (2020). Potential antagonistic rhizobacteria to control *Colletotrichum scovillei*, the cause of anthracnose disease in chili pepper. Biodiversitas Journal of Biological Diversity, 21(6).

296. Surepeddi, S. K., & Giridhar, K. (2015). Chilli. In *Vegetables, Flowers and Plantation Crops. Vol. 3. Managing Post-harvest Quality and Losses in Horticulture Crops,* eds. K. L. Chadha and R. K. Pal, 597. New Delhi, India: Daya Publishing House.
297. Suryadi, Y., Susilowati, D. N., Lestari, P., Priyatno, T. P., Samudra, I. M., Hikmawati, N., & Mubarik, N. R. (2014). Characterization of bacterial isolates producing chitinase and glucanase for biocontrol of plant fungal pathogens. Journal of Agricultural Technology, 10(4), 983-999.
298. Suryanto, D., Patonah, S., & Munir, E. (2010). Control of *Fusarium* wilt of chili with chitinolytic bacteria. HAYATI Journal of Biosciences, 17(1), 5-8.
299. Suthindhiran, K., & Kannabiran, K. (2009). Cytotoxic and antimicrobial potential of actinomycete species *Saccharopolyspora salina* VITSDK4 isolated from the Bay of Bengal Coast of India. American Journal of Infectious Diseases, 5(2), 90-98.
300. Swain, M. R., Ray, R. C., & Nautiyal, C. S. (2008). Biocontrol efficacy of *Bacillus subtilis* strains isolated from cow dung against postharvest yam (*Dioscorea rotundata* L.) pathogens. Current Microbiology, 57(5), 407-411.
301. Syed, S., Tollamadugu, N. P., & Lian, B. (2020). *Aspergillus* and *Fusarium* control in the early stages of *Arachis hypogaea* (groundnut crop) by plant growth-promoting rhizobacteria (PGPR) consortium. Microbiological Research, 240, 126562.
302. Tabibian, J. H., Gornbein, J., Heidari, A., Dien, S. L., Lau, V. H., Chahal, P., Churchill B.M., & Haake, D. A. (2008). Uropathogens and host characteristics. Journal of Clinical Microbiology, 46(12), 3980-3986.
303. Taghian, S., Esh, A., Nagy, A. G., & El-Meneisy, A. (2021). Endophytic Bacteria for Biological Control of Sugarcane Smut Disease and Its Effect on Some Plant Growth Parameters. Egyptian-Arab Journal of Applied Sciences and Technology, 1(2), 39-53.
304. Talebi Atouei, M., Pourbabaee, A.A., & Shorafa, M. (2019). Alleviation of Salinity Stress on Some Growth Parameters of Wheat by Exopolysaccharide-Producing Bacteria. Iranian Journal of Science and Technology, Transactions A: Science, 43, 2725–2733.
305. Tembhurne, B., Belabadevi, B., Kisan, B., Tilak, I. S., Ashwathanarayana, D. S., Suvarna, N., & Naik, M. K. (2017). Molecular characterization and screening for *Fusarium (Fusarium solani)* resistance in Chilli (*Capsicum annuum* L.) genotypes. International Journal of Current Microbiology and Applied Sciences, 6(9), 1585-1597.
306. Tendulkar, S. R., Saikumari, Y. K., Patel, V., Raghotama, S., Munshi, T. K., Balaram, P., & Chattoo, B. B. (2007). Isolation, purification and characterization of an antifungal

molecule produced by *Bacillus licheniformis* BC98, and its effect on phytopathogen *Magnaporthe grisea*. Journal of Applied Microbiology, 103(6), 2331-2339.

307. Tenorio-Salgado, S., Tinoco, R., Vazquez-Duhalt, R., Caballero-Mellado, J., & Perez-Rueda, E. (2013). Identification of volatile compounds produced by the bacterium *Burkholderia tropica* that inhibit the growth of fungal pathogens. Bioengineered, 4(4), 236-243.
308. Tewari, S., & Arora, N. K. (2016). Fluorescent *Pseudomonas* sp. PF17 as an efficient plant growth regulator and biocontrol agent for sunflower crop under saline conditions. Symbiosis, 68(1), 99-108.
309. Tomah, A.A., Abd Alamer, I.S., Li, B., & Zhang, J.Z. (2020). A new species of *Trichoderma* and *gliotoxin* role: a new observation in enhancing biocontrol potential of *T. virens* against *Phytophthora capsici* on chili pepper. Biological Control, 145, 104261.
310. Usta, C. (2013). Microorganisms in biological pest control—a review (bacterial toxin application and effect of environmental factors). Current Progress in Biological Research, 13, 287-317.
311. Valenzuela-Ruiz, V., Robles-Montoya, R. I., Parra-Cota, F. I., Santoyo, G., del Carmen Orozco-Mosqueda, M., Rodríguez-Ramírez, R., & de Los Santos-Villalobos, S. (2019). Draft genome sequence of *Bacillus paralicheniformis* TRQ65, a biological control agent and plant growth-promoting bacterium isolated from wheat (*Triticum turgidum subsp. durum*) rhizosphere in the Yaqui Valley, Mexico. 3 Biotech, 9(11), 1-7.
312. Vasanthakumari R (2009). *Practical microbiology*. BI publications Pvt Ltd, New Delhi.
313. Ventosa, A., Nieto, J. J., & Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. Microbiology and Molecular Biology Reviews, 62(2), 504-544.
314. Verma, P. P., Shelake, R. M., Das, S., Sharma, P., & Kim, J. Y. (2019). Plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF): potential biological control agents of diseases and pests. In *Microbial interventions in agriculture and environment* (pp. 281-311). Springer, Singapore.
315. Verma, S. K., & White, J. F. (2018). Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in browntop millet (*Urochloa ramosa* L.). Journal of Applied Microbiology, 124(3), 764-778.
316. Vigneshwari, J., Senthilkumar, P. K., Vijayakumar, N., Gnanasekaran, A., Dineshkumar, K., Selvamani, V., & Dar, K. A. (2022). Broad spectrum of bioactive compounds from halophilic actinomycetes isolated from the Kodiyakadu saltpan of Vedaranyam-India. Materials Today: Proceedings, 59, 979-987.

317. Vijayakumar, R., Panneer Selvam, K., Muthukumar, C., Thajuddin, N., Panneerselvam, A., & Saravanamuthu, R. (2012). Antimicrobial potentiality of a halophilic strain of *Streptomyces* sp. VPTSA18 isolated from the saltpan environment of Vedaranyam, India. Annals of Microbiology, 62(3), 1039-1047.
318. Vivekanandhan, G., Savithamani, K., Hatha, A. A. M., & Lakshmanaperumalsamy, P. (2002). Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. International Journal of Food Microbiology, 76(1-2), 165-168.
319. Vlassi, A., Nesler, A., Parich, A., Puopolo, G., & Schuhmacher, R. (2020). Volatile-mediated inhibitory activity of rhizobacteria as a result of multiple factors interaction: The case of *Lysobacter capsici* AZ78. Microorganisms, 8(11), 1761.
320. Voica, D. M., Bartha, L., Banciu, H. L., & Oren, A. (2016). Heavy metal resistance in halophilic Bacteria and Archaea. FEMS Microbiology Letters, 363(14).
321. Vurukonda, S. S. K. P., Giovanardi, D., & Stefani, E. (2018). Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. International Journal of Molecular Sciences, 19(4), 952.
322. Waditee-Sirisattha, R., Kageyama, H., & Takabe, T. (2016). Halophilic microorganism resources and their applications in industrial and environmental biotechnology. AIMS Microbiology, 2(1), 42-54.
323. Wang, Y., Liu, H., Liu, K., Wang, C., Ma, H., Li, Y., Hou, Q., Liu, F., Zhang, T., Wang, H., & Du, B. (2017). Complete genome sequence of *Bacillus paralicheniformis* MDJK30, a plant growth-promoting rhizobacterium with antifungal activity. Genome Announcements, 5(25), e00577-17.
324. Wani, F. A., & Najar, A. G. (2012). Integrated Management of *Fusarium* Wilt [*Fusarium pallidoroseum* (Cooke) Sacc.] in Chilli (*Capsicum annum* L.). SKUAST Journal of Research, 14(1and2), 40-45.
325. Winkler, U. K., & Stuckmann, M. (1979). Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. Journal of Bacteriology, 138(3), 663-670.
326. Won, S. J., Kwon, J. H., Kim, D. H., & Ahn, Y. S. (2019). The effect of *Bacillus licheniformis* MH48 on control of foliar fungal diseases and growth promotion of *Camellia oleifera* seedlings in the coastal reclaimed land of Korea. Pathogens, 8(1), 6.
327. Wu, C., Shang, Z., Lemetre, C., Ternei, M. A., & Brady, S. F. (2019a). Cadasides, calcium-dependent acidic lipopeptides from the soil metagenome that are active against multidrug-resistant bacteria. Journal of the American Chemical Society, 141(9), 3910-3919.

328. Wu, S., Liu, G., Zhou, S., Sha, Z., & Sun, C. (2019b). Characterization of antifungal lipopeptide biosurfactants produced by marine bacterium *Bacillus* sp. CS30. Marine Drugs, 17(4), 199.
329. Wu, Z., Huang, Y., Li, Y., Dong, J., Liu, X., & Li, C. (2019c). Biocontrol of *Rhizoctonia solani* via induction of the defense mechanism and antimicrobial compounds produced by *Bacillus subtilis* SL-44 on pepper (*Capsicum annuum* L.). Frontiers in Microbiology, 10, 2676.
330. Yadav, M., Dubey, M.K., & Upadhyay, R.S. (2021). Systemic Resistance in Chilli Pepper against Anthracnose (Caused by *Colletotrichum truncatum*) induced by *Trichoderma harzianum, Trichoderma asperellum* and *Paenibacillus dendritiformis*. Journal of Fungi 7(4), 307.
331. Yu, X., Ai, C., Xin, L., & Zhou, G. (2011). The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. European Journal of Soil Biology, 47(2), 138-145.
332. Zagade, S. N., Deshpande, G. D., Gawade, D. B., Wadje, A. G., & Pawar, A. K. (2012). Evaluation of fungicides, bioagent and botanicals against chilli damping off against *Pythium Ultimum*. Journal of Plant Disease Sciences, *7*(1), 60-63.
333. Zehra, A., Raytekar, N. A., Meena, M., & Swapnil, P. (2021). Efficiency of microbial bio-agents as elicitors in plant defense mechanism under biotic stress: A review. Current Research in Microbial Sciences, 2, 100054.
334. Zhang, Y., Li, Y., Liang, S., Zheng, W., Chen, X., Liu, J., & Wang, A. (2022). Study on the Preparation and Effect of Tomato Seedling Disease Biocontrol Compound Seed-Coating Agent. Life, 12(6), 849.
335. Zheng, Z., & Shetty, K. (2000). Enhancement of pea (*Pisum sativum*) seedling vigour and associated phenolic content by extracts of apple pomace fermented with *Trichoderma* spp. Process Biochemistry, 36(1-2), 79-84.
336. Zhou, L., Song, C., Li, Z., & Kuipers, O. P. (2021). Antimicrobial activity screening of rhizosphere soil bacteria from tomato and genome-based analysis of their antimicrobial biosynthetic potential. BMC Genomics, 22(1), 1-14.
337. Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & Van't Riet, K. (1990). Modeling of the bacterial growth curve. Applied and Environmental Microbiology, 56(6), 1875-1881.

Appendix

APPENDIX I

MEDIA COMPOSITION

1. **Nutrient Agar (NA) HiMedia M001**

Ingredients	**g/L**
Peptone	5.0
Sodium chloride	5.0
HM Peptone B	1.5
Yeast Extract	1.5
Agar	15.0
Final pH (at 25°C)	7.4±0.2

2. **Zobell Marine Agar 2216 (ZMA) HiMedia M384**

Ingredients	**g/L**
Peptone	5.0000
Yeast extract	1.0000
Ferric citrate	0.1000
Sodium chloride	19.4500
Magnesium chloride	8.8000
Sodium sulphate	3.2400
Calcium chloride	1.8000
Potassium chloride	0.5500
Sodium bicarbonate	0.1600
Potassium bromide	0.0800
Strontium chloride	0.0340
Boric acid	0.0220
Sodium silicate	0.0040
Sodium fluorate	0.0024
Ammonium nitrate	0.0016
Disodium phosphate	0.0080
Agar	15.0000
Final pH (at 25°C)	7.6±0.2

3. Potato Dextrose Agar (PDA) HiMedia M096

Ingredients	g/L
Potatoes, infusion from	200.0
Dextrose	20.0
Agar	15.0
Final pH (at 25°C)	5.6±0.2

4. Soil Extract Agar (SEA) HiMedia M455

Ingredients	g/L
Glucose	1.00
Dipotassium phosphate	0.50
Soil extract	17.75
Agar	15.00
Final pH (at 25°C)	6.8±0.2

5. M9 Minimal Medium Salts (5X) HiMedia G013

Ingredients	g/L
Disodium hydrogen phosphate	33.90
Potassium dihydrogen phosphate	15.00
Sodium chloride	2.50
Ammonium chloride	5.00

6. Colloidal Chitin Agar

Ingredients	g/L
M9 Minimal Medium Salts (5X)	11.28
Colloidal chitin	2.00
Agar	15.00
Final pH (at 25°C)	7.6±0.2

7. Peptone Water Broth

Ingredients	g/L
Peptone	10.00
Sodium Chloride	5.00
Final pH (at 25°C)	7.2±0.2

8. Laminarin Agar

Ingredients	g/L
M9 Minimal Medium Salts (5X)	11.28
Laminarin	2.00
Agar	15.00
Final pH (at 25°C)	7.6±0.2

9. Starch Agar

Ingredients	g/L
M9 Minimal Medium Salts (5X)	11.28
Starch	2.00
Agar	15.00
Final pH (at 25°C)	7.6±0.2

10. Carboxymethyl Cellulose Agar

Ingredients	g/L
M9 Minimal Medium Salts (5X)	11.28
Carboxymethyl Cellulose	10.00
Agar	15.00
Final pH (at 25°C)	7.6±0.2

11. Casein Agar

Ingredients	g/L
M9 Minimal Medium Salts (5X)	11.28
Casein	10.00
Agar	15.00
Final pH (at 25°C)	7.6±0.2

12. Tributyrin Agar

Ingredients	g/L
M9 Minimal Medium Salts (5X)	11.28
Tributyrin	10.00
Agar	15.00
Final pH (at 25°C)	7.6±0.2

13. Pikovskaya’s Agar Himedia M520

Ingredients	g/L
Yeast extract	0.5000
Dextrose	10.0000
Calcium phosphate	5.0000
Ammonium sulphate	0.5000
Potassium chloride	0.2000
Magnesium sulphate	0.1000
Manganese sulphate	0.0001
Ferrous sulphate	0.0001
Agar	15.0000
Final pH (at 25°C)	7.6±0.2

14. Zinc Solubilizing Agar HiMedia M2068

Ingredients	g/L
Dextrose	10.0
Ammonium sulphate	1.0
Potassium chloride	0.2
Dipotassium hydrogen phosphate	0.1
Magnesium sulphate heptahydrate	0.2
Zinc Oxide	1.0
Agar	15.0
Final pH (at 25°C)	7.6±0.2

15. Aleksandrow Agar HiMedia M1996

Ingredients	g/L
Magnesium sulphate	0.500
Calcium carbonate	0.100
Potassium alumino silicate	2.000
Dextrose	5.000
Ferric chloride	0.005
Calcium phosphate	2.0
Agar	20.0
Final pH (at 25°C)	7.2±0.2

16. Modified Bunt and Rovira medium agar

Ingredients	g/L
Peptone	10.00
Glucose	20.00
Magnesium chloride	0.10
Ferric chloride	0.01
Yeast extract	1.00
Ammonium sulphate	0.50
Disodium hydrogen phosphate	0.40
Magnesium trisilicate	0.25
Agar	20.00
Final pH (at 25°C)	7.0±0.2

17. Urea Agar Base (Christensen) HiMedia M112

Ingredients	g/L
Peptone	1.000
Dextrose	1.000
Sodium chloride	5.000
Disodium hydrogen phosphate	1.200
Potassium dihydrogen phosphate	0.800
Phenol red	0.012
Agar	15.000
Final pH (at 25°C)	6.8±0.2

18. Simmon Citrate Agar HiMedia M099

Ingredients	g/L
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08
Agar	15.00
Final pH (at 25°C)	6.8±0.2

19. Dworkin-foster salts

Ingredients	g/L
Potassium dihydrogen phosphate	4.00
Disodium hydrogen phosphate	6.00
Magnesium sulphate heptahydrate	0.20
Glucose	2.00
Gluconic acid	2.00
Citric acid	2.00
Trace elements	**mg/L**
Ferrous sulphate heptahydrate	1.00
Boric acid	10.00
Manganese sulphate hydrate	11.19
Zinc sulphate heptahydrate	124.60
Copper sulphate pentahydrate	78.22
Molybdenum trioxide	10.00
Final pH (at 25°C)	7.2±0.2

20. Jensen's Agar HiMedia M710

Ingredients	g/L
Sucrose	20.00
Dipotassium phosphate	1.000
Magnesium sulphate	0.500
Sodium chloride 0.500	0.500
Ferrous sulphate	0.100
Sodium molybdate	0.005
Calcium carbonate	2.000
Agar	15.00
Final pH (at 25°C)	7.0±0.2

21. SIM Media HiMedia M181

Ingredients	g/L
HM Peptone B #	3.000
Peptone	30.000
Peptonized iron	0.200
Sodium thiosulphate	0.025
Agar	3.000
Final pH (at 25°C)	7.3±0.2

22. Nitrate Broth HiMedia M439S

Ingredients	g/L
Peptic digest of animal tissue	5.0
Meat extract	3.0
Potassium nitrate	1.0
Sodium chloride	30.0
Final pH (at 25°C)	7.0±0.2

23. Gelatin media

Ingredients	g/L
Peptone	5
Beef Extract	3
Gelatin	120
Final pH (at 25°C)	6.8±0.2

24. Methyl Red - Voges Proskauer Broth (Glucose Phosphate Broth) HiMedia M070

Ingredients	g/L
Buffered Peptone	7.0
Dextrose (Glucose)	5.0
Dipotassium phosphate	5.0
Final pH (at 25°C)	6.9±0.2

25. Anerobic Agar HiMedia M228

Ingredients	g/L
Tryptone	20.000
Dextrose (Glucose)	10.000
Sodium chloride	5.000
Sodium thioglycollate	2.000
Sodium formaldehyde Sulfoxylate	1.000
Methylene blue	0.002
Agar	20.000
Final pH (at 25°C)	7.2±0.2

26. Blood Agar Base (Infusion Agar) HiMedia M073

Ingredients	g/L
Tryptose	10.0
Sodium chloride	5.0
HM Peptone B	10.0
Agar	15.0
Final pH (at 25°C)	7.3±0.2

27. Muller Hinton Agar HiMedia M173

Ingredients	g/L
Acicase	17.5
Starch	1.5
HM infusion B	300.0
Agar	17.0
Final pH (at 25°C)	7.3±0.2

APPENDIX II

REAGENTS AND BUFFERS

1. **Lugol's iodine solution**

   To 20 mL DW, 10 g Potassium iodide was added, followed by 5 g of Iodine. Heat the solution gently with constant stirring until it is dissolved completely. Make up the volume to 100 mL and store in amber coloured bottle.

2. **Rochelle's salt solution**

   To 100 mL ammonia free DW, 50 g Sodium potassium tartarate was added. The solution was boiled and filtered through filter paper.

3. **Nessler's reagent HiMedia R010**

Mercuric chloride	10.0 g
Potassium iodide	7.0 g
Sodium hydroxide	16.0 g
Water (ammonia free)	100 mL
Final pH (at 25°C)	13.2±0.05

4. **Salkowski reagent**

   Perchloric acid (70 %) was diluted to half strength with 250 mL DW water. To this, 10 mL of 0.5 M ferric chloride was added and stored in amber coloured bottle.

5. **DiNitro Salicylic Acid (DNSA) solution**

   To 50 mL DW, 1 g of di-Nitro Salicylic Acid was added, followed by 30 g of Sodium potassium tartarate. This was mixed with 20 mL of 2 N Sodium hydroxide. Volume was made up 100 mL with DW and stored in amber coloured bottle.

6. **Coomassie Brilliant Blue G-250 solution**

   To 5mL ethanol, 10 mg Coomassie Brilliant Blue G-250 was added. This was mixed with 10 mL of concentrated phosphoric acid. Volume was made up 100 mL with DW and filtered to remove any precipitate formed.

7. **Acidified ascorbic acid**

Ascorbic acid (10 g) was dissolved in about 40 mL distilled water. To this, 50 mL of 4.5 M Sulphuric acid (125 mL of concentrated H_2SO_4 added carefully to about 300 mL DW. Upon cooling, the volume was made up to 500 mL with DW) was added and transferred to 100 mL volumetric flask. The volume was made up to 100 mL with distilled water.

8. **Mixed reagent (For phosphate estimation)**

Ammonium heptamolybdate tetrahydrate (6.25 g) was dissolved in about 50 mL distilled water. In a separate beaker, 0.25 g potassium antimony tartarate was dissolved in about 10 mL distilled water. The molybdate solution was added to 175 mL 4.5 M Sulphuric acid (125 mL of concentrated H_2SO_4 added carefully to about 300 mL DW. Upon cooling, the volume was made up to 500 mL with DW) with continuous stirring. Subsequently, the tartarate solution was added and mixed well.

9. **Mixed reagent (For silicon estimation)**

Ammonium heptamolybdate tetrahydrate (3.175 g) was dissolved in about 25 mL distilled water. This was added to 25 mL 4.5 M Sulphuric acid (125 mL of concentrated H_2SO_4 added carefully to about 300 mL DW. Upon cooling, the volume was made up to 500 mL with DW) with continuous stirring.

10. **1X Phosphate buffered saline (PBS)**

Ingredients	**g/L**
Sodium chloride	8.00
Potassium chloride	0.20
Disodium hydrogen phosphate	1.44
Potassium dihydrogen phosphate	0.24
Final pH (at 25°C)	7.4±0.2

11. **Kovac's Reagent HiMedia R008**

p-dimethylamino benzaldehyde	5.0 g
Amyl alcohol	75 mL
Hydrochloric acid, concentrated	25 mL

12. Lactophenol cotton blue HiMedia S016

Phenol crystals	20.00
Cotton blue	0.05
Lactic acid	20.00
Glycerol	20.00
DW	20.00

13. 0.8 % Sulphanilic acid HiMedia R015

Sulphanilic acid	8.0 g
30% Acetic acid	1 L
Final pH (at 25°C)	1.6±0.01

14. α-Naphthylamine Reagent HiMedia R009

α-Naphthylamine	5.0 g
5N Acetic acid	1 L

15. Methyl red indicator HiMedia I007

Methyl red	0.2 g
Ethanol	60 mL
DW	40 mL

16. Barrit's Reagent A HiMedia R029

α-Naphthol (1-Naphthol)	5.0 g
Absolute ethanol	100 mL

17. Barrit's Reagent B HiMedia R030

Potassium hydroxide	40.0 g
DW	100

18. 1 M acetate buffer

Sodium acetate	13.6 g
Glacial acetic acid	6 mL
Add the ingredients to 794 mL DW, adjust the pH as desired and then make the volume to 1 L with DW.	

19. Phosphate buffer

Ingredients	0.05 M	0.1 M
Disodium hydrogen phosphate	7.744 g	15.487 g
Sodium dihydrogen phosphate	2.914 g	5.827 g
Add the ingredients to 800 mL DW, adjust the pH as desired and then make the volume to 1 L with DW.		

20. 0.5 M citrate buffer

Sodium citrate	11.975 g
Citric acid	11.385 g
Add the ingredients to 800 mL DW, adjust the pH as desired and then make the volume to 1 L with DW.	

21. 0.05 M Tris-HCl buffer

To 800 mL DW, 7.88 g Tris-HCl was added. The pH of the solution was adjusted as desired and the volume was made up to 1 L with DW.

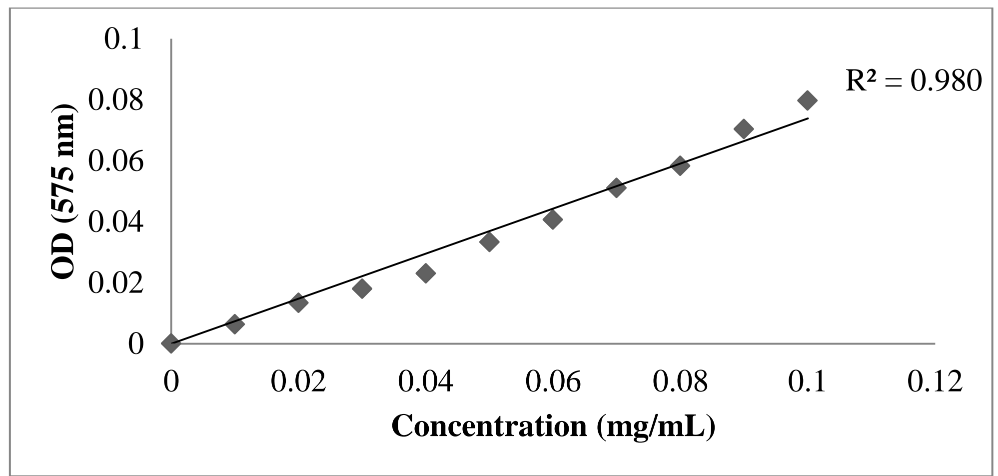
22. Borate buffer

Ingredients	0.1 M	0.2 M
Borax	0.1 g	0.2 g
Boric acid	0.56 g	1.12 g
Add the ingredients to 80 mL DW, adjust the pH as desired and then make the volume to 100 mL with DW.		

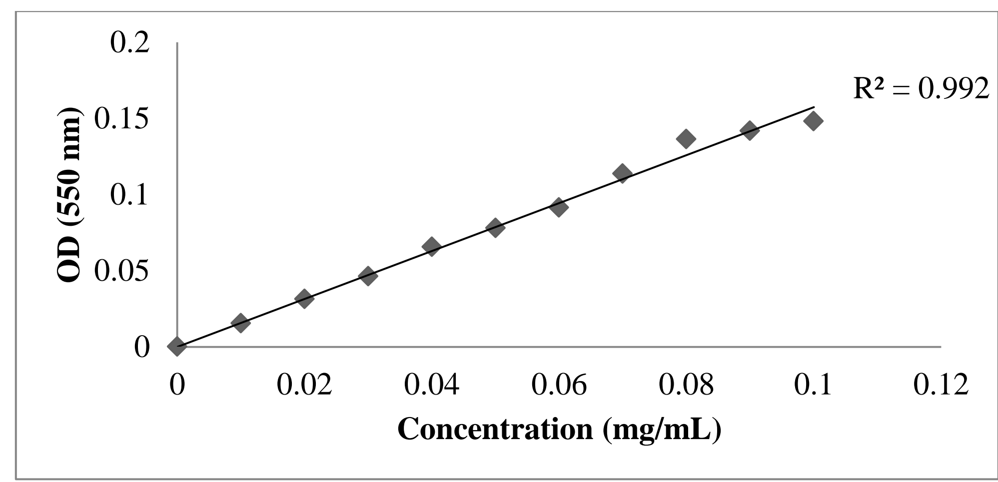
APPENDIX III

STANDARD GRAPHS

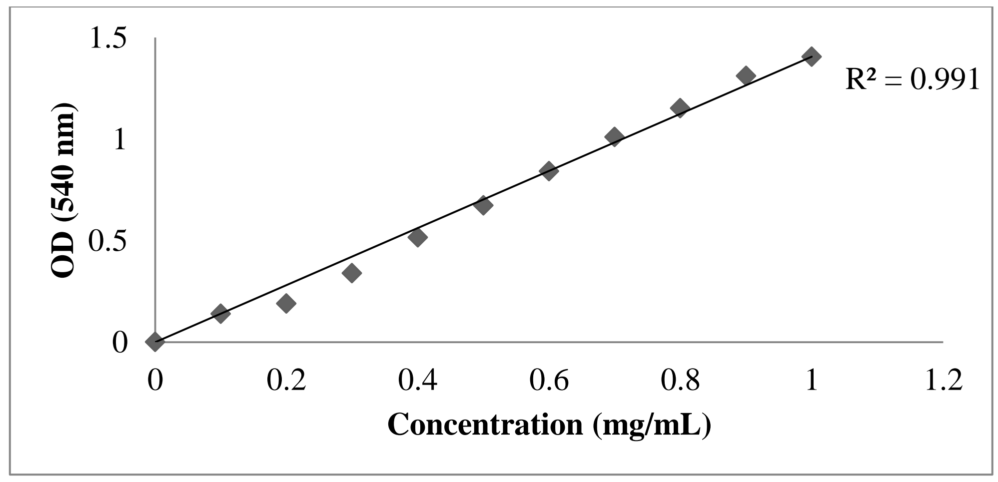
1. **N-Acetylglucosamine (NAGA) standard graph for chitinase estimation**



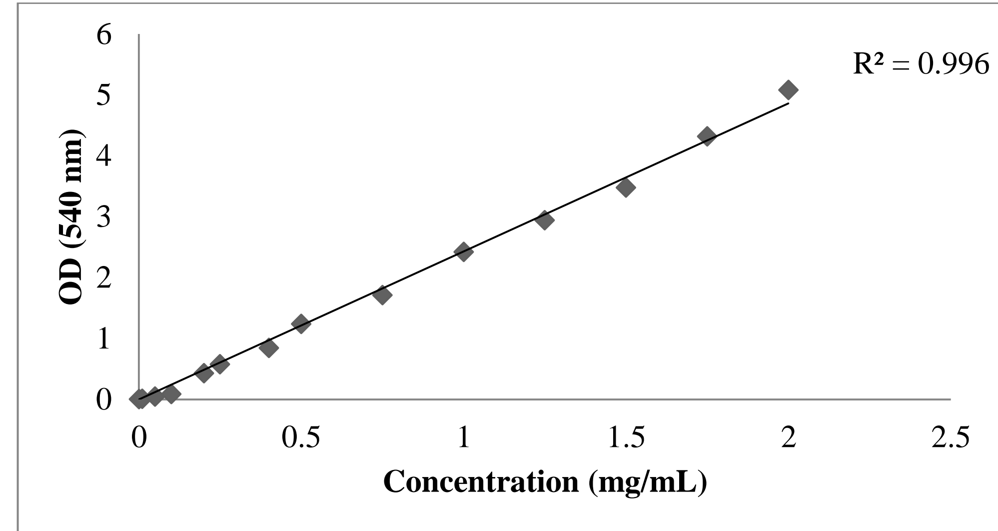
2. **Glucose standard graph for glucanase estimation**



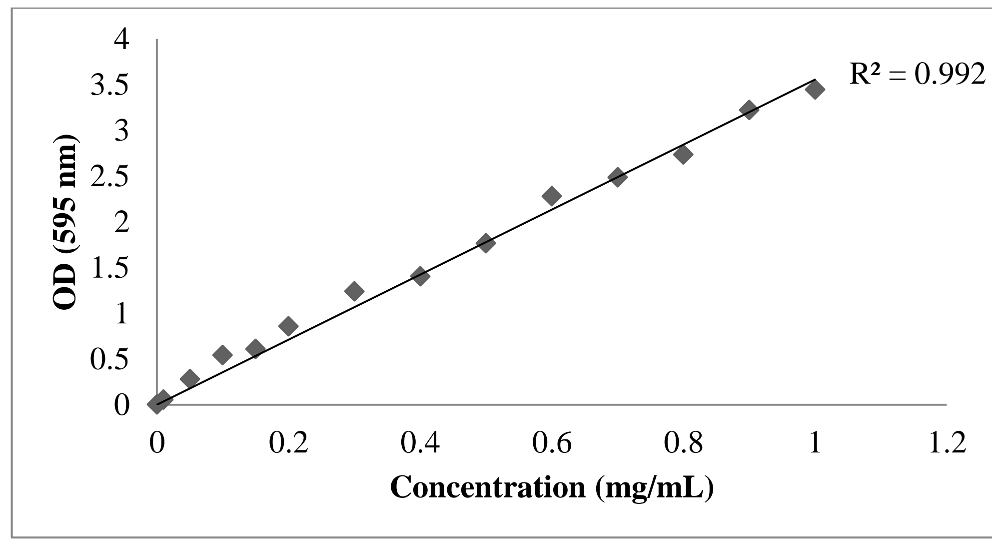
3. **Maltose standard graph for amylase estimation**



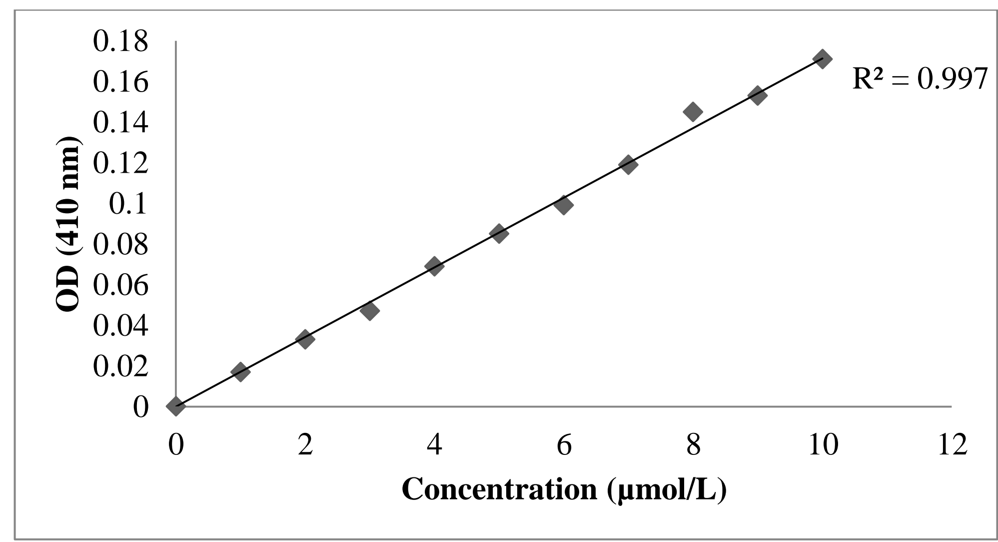
4. Glucose standard graph for cellulase estimation



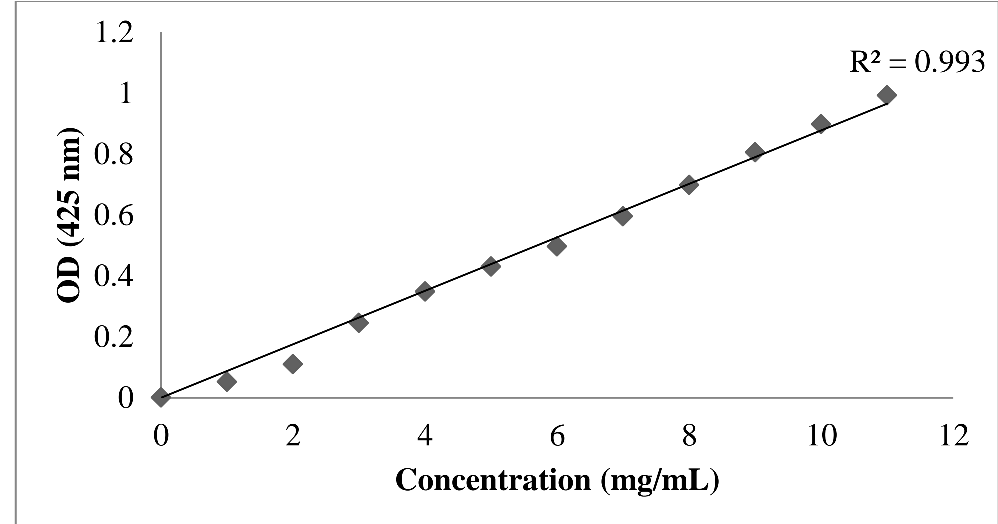
5. Bovine serum albumin standard graph for protease estimation



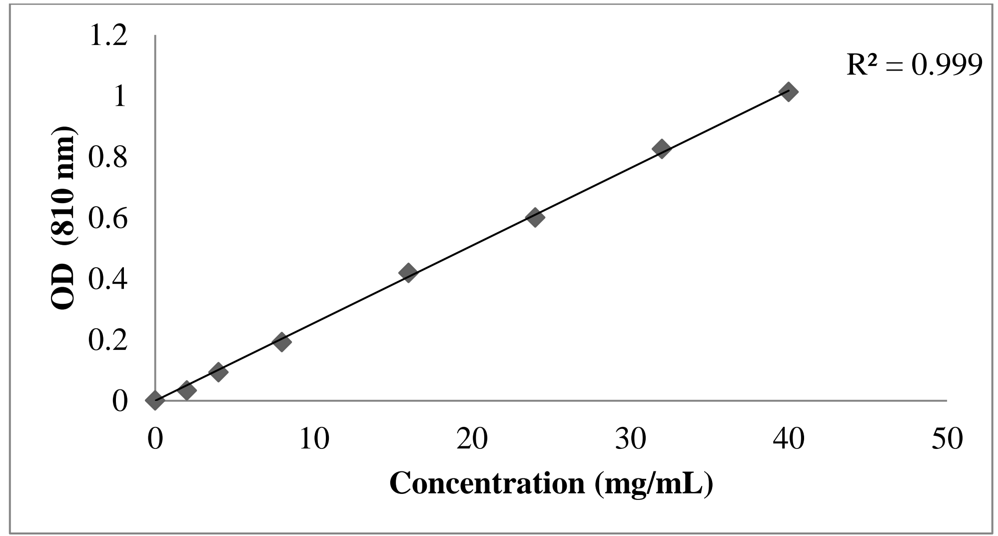
6. p-nitrophenyl standard graph for lipase estimation



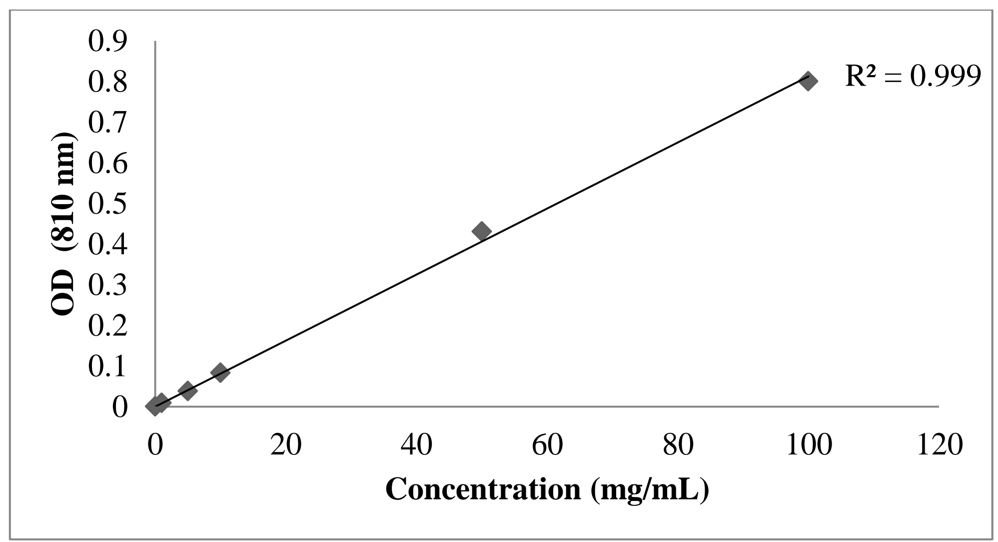
7. Ammonium chloride standard graph for ammonia estimation



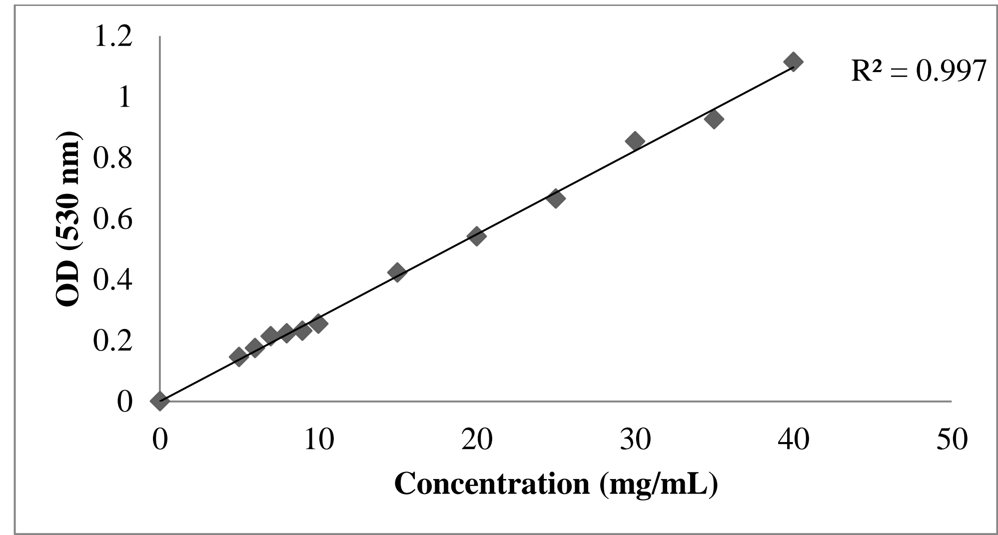
8. Potassium dihydrogen phosphate standard graph for phosphate estimation



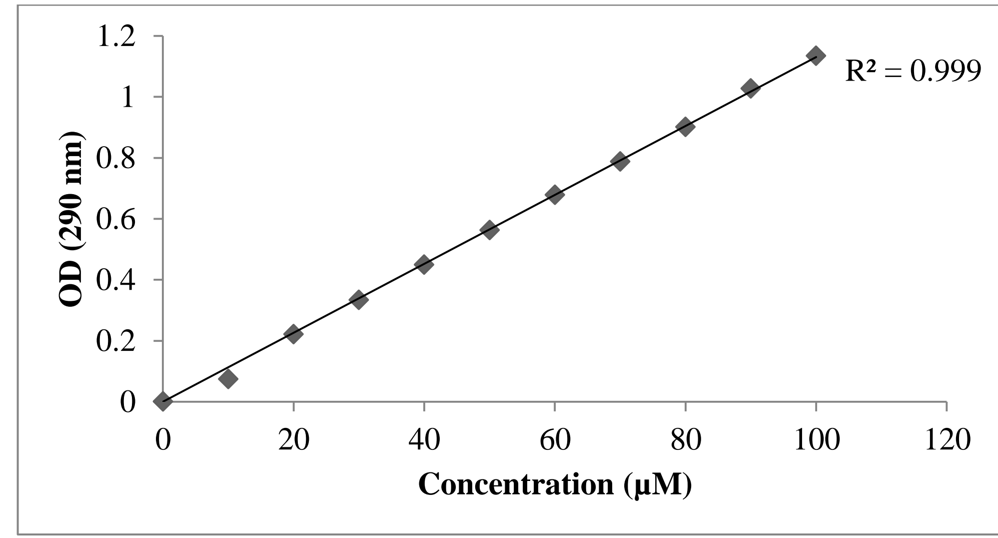
9. Sodium hexafluorosilicate standard graph for silicon estimation



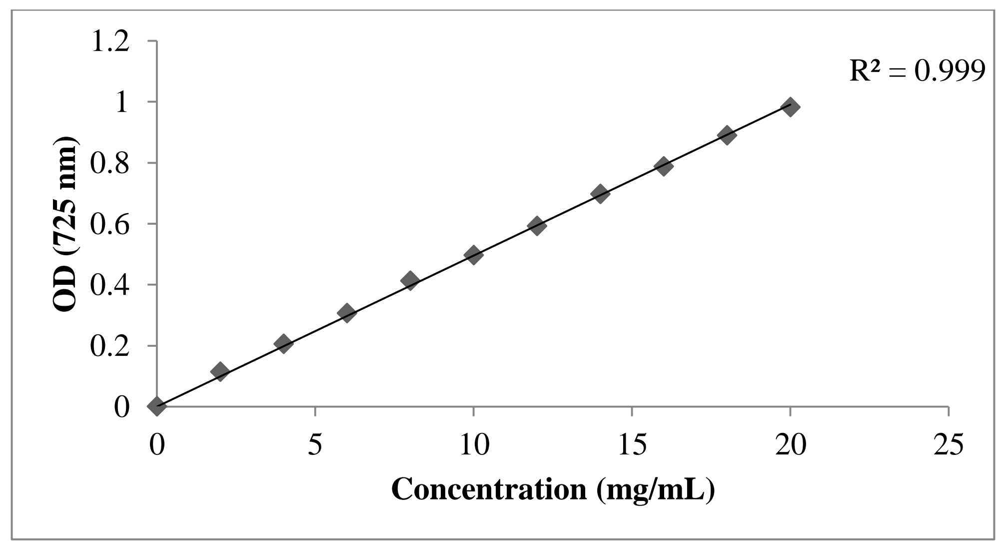
10. Indole Acetic Acid standard graph for IAA estimation



11. Trans-cinnamic acid standard graph for PAL estimation



12. Gallic acid standard graph for TPC estimation



Publications

Manuscripts published:

1. **Pawaskar, M.**, & Kerkar S. (2021). **Microbial biocontrol agents against chilli plant pathogens over synthetic pesticides: a review**. Proceedings of the Indian National Science Academy, 87(4), 578-594.

2. **Pawaskar, M.**, Krishnan, K. P., & Kerkar, S. (2022). **Salt-pan bacteria as potential plant growth promoters and their antagonistic activity to fungal pathogens of *Capsicum annuum* L.** Current Science, 123(9), 1129-1135.

Abstracts published in international and national conferences:

1. "**Statistical optimization of the medium components to enhance production of the antifungal metabolite produced by *Bacillus* sp. strain BGUMS27**" by **Manasi Pawaskar**, Michelle Fernandes, Savita Kerkar at the National Conference of Young Researchers 2017 held at Goa University on 16th -17th March 2017.

2. "**Statistical optimization of the medium components to enhance production of the antibacterial metabolite produced by *Bacillus* sp. strain BGUMS27**" by **Manasi Pawaskar**, Michelle Fernandes, Savita Kerkar at the 4rth Annual International Conference, Prosecution to Litigation: Part III Asserting Exclusive Rights to IP held at Goa College of Pharmacy on 11th-12th November 2019.

3. "**Hypersaline bacteria as potential biocontrol agents against fungal plant pathogens**" by **Manasi Pawaskar** and Savita Kerkar at the National Conference on Frontiers in Biopesticides and Biofertilizers held at P.E.S.'s R.S.N. College of Arts and Science on 6th - 7th December 2019.

4. "***In-vitro* evaluation of *Pseudomonas* spp. from hypersaline environment as potential plant growth promoting bacteria**" by **Manasi Pawaskar** and Savita Kerkar at the International Conference by Biotechnology Society of Nepal (ICBSN-2021), organized by Biotechnology Society of Nepal (BSN) on 12th-14th February 2021.

Manuscripts in preparation:

1. **Production of Surfactin biosurfactant from halotolerant *Bacillus amyloliquefaciens* SK27 and its potential applications** by Ruchira Malik, **Manasi Pawaskar**, Dixita Temkar, Vikash Yadav, Ajeet Kumar Mohanty, Santosh Tilve and Savita Kerkar.

2. **Optimization and characterization of anti-microbial metabolites of a hypersaline *Bacillus* sp. strain BGUMS27 against human and plant pathogens** by Shuvankar Ballav, **Manasi Pawaskar**, Michelle Fernandes and Savita Kerkar.

3. ***In-vivo* application of salt pan strain *Bacillus paralicheniformis* MPSK 23 for controlling *Fusarium* wilt in chilli plants** by **Manasi Pawaskar**, Gourish Karanjalker and Savita Kerkar.

Microbial biocontrol agents against chilli plant pathogens over synthetic pesticides: a review

Manasi Pawaskar & Savita Kerkar

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REVIEW ARTICLE

Microbial biocontrol agents against chilli plant pathogens over synthetic pesticides: a review

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Abstract

India is amongst the top countries consuming, producing and exporting chillies. Chilli (*Capsicum annuum*), known as chilli peppers, belongs to the family Solanaceae. Heavy losses have been observed in the yield due to diseases caused by bacterial, fungal and viral pathogens. Over utilization of synthetic agents to control these diseases causing pathogens have raised serious biological and ecological issues. Along these lines, late endeavours have been centred on utilizing natural ways for controlling plant pathogens. Interest in biological control using microbial agents has increased over the past years as an alternative to chemicals which are toxic to the environment and lead to the development of resistant pathogenic populations. Biological control using microorganisms is a more environmentally friendly alternative and is utilized either on its own or as a part of integrated management strategy to reduce the use of synthetic agents. This research approach reviews various pathogen causing diseases in the *Capsicum annuum*, toxicity of the chemical agents used for controlling the pathogens and the biocontrol agents against chilli phytopathogens along with their suggested mode of action. This article will deepen our knowledge about the consistent beneficial effects of microbial biocontrol agents.

Keywords Biocontrol · Chilli · Disease · Pathogen · Toxicity

Introduction

Chillies (*Capsicum annuum*) also known as 'mirchi', are an indispensable ingredient in Indian cuisine. They were brought in Asia by Portuguese navigators in the 16th Century. Chilli plant belongs to Solanaceae family, are herbaceous annuals, have glabrous or pubescent lanceolate leaves, white flowers and fruit that vary in length, colour and pungency depending on the cultivar (Stommel et al. 2018). They are rich in Vitamin C (ascorbic acid), E (tocopherols and tocotrienols), P (citrin), B1 (thiamine), B2 (riboflavin), B3 (niacin) and provitamin A (β-carotene) (Gopalakrishnan 2007; Bosland et al. 2012). They are also known to be a good source of flavonoids, carotenoids and xanthophylls (Lee et al. 1995). India has been amongst the major countries producing, consuming and exporting chillies. A rising demand for chillies combined with higher value acknowledgment in the domestic market has spurred farmers to expand areas under chilli cultivation for export (Surepeddi and Giridhar 2015). However heavy yield loss of Chilli crop is observed across the country due to diseases caused by both abiotic and biotic factors.

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Abiotic factors affecting chilli plant

Non-living factors responsible for crop damage include extreme levels of temperature, moisture and light, change in nutrients and pH, air pollutants and overdose of pesticides (Peter and Hazra 2012). The major abiotic factor which cause stress conditions in the chilli plant is deficiency or toxicity of the macro and micronutrients. Deficiency of the nutrients may lead to conditions like stunted growth of the plant, chlorosis of leaves, limited foliage and reduced size of fruits. Excessive nutrients are also harmful for the plant and may lead to burning of foliage and root system (Balakrishnan 1999). Due to factors like inconsistent watering, calcium deficiency, increased soil salinity or excessive applications of nitrogen fertilizers; Blossom-End rot is observed in chilli fruit which results in early fruit drop (Taylor and Locascio 2004). This condition manifests in the water soaked lesions on immature fruits, resulting as a prime location for microbial infections by opportunistic

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disease causing pathogens. Exposure to intense sunlight results in 'Sun scalding' where wrinkling of foliage is observed making the plant light-coloured and papery textured. Occasionally fruit skin is affected and cracked open (Rabinowitch et al. 1983). Presence of air pollutants such as peroxyacetyl nitrate (PAN) is reported to cause stunting, chlorosis and even senescence in the chilli plant leaves (Goyal et al. 2020).

Diseases caused by microbial pathogens in chilli plant

Losses incurred in agriculture due to microbial pathogens sums up to around 16% globally (Oerke 2006). The growth and yield of a chilli plant is most affected by bacterial, fungal and viral pathogens (Gachomo et al. 2003). Disease symptoms have been noticed in seedlings, mature plant, fruit or leaves depending on the phytopathogen that attacks the plant. Table 1 enlists the diseases caused by microbial phytopathogens in chilli plants along with the symptoms associated with the disease. Wilting and rotting are manifested in the mature plants by soil borne fungal pathogens belonging to genera *Macrophomina, Phytophthora, Rhizoctonia, Sclerotinia, Fusarium, Sclerotium* and *Verticillium*. Bacterial wilt is particularly caused by a bacterium *Ralstonia solanacearum* leading to the death of the plant (Sanogo 2003). Fungus belonging to genera *Pythium* or *Phomopsis* show damping off symptoms in seeds or young seedlings. Infected seeds fail to germinate and the damaged seedlings generally rot, causing the seedling to wilt and eventually die, or collapse from the ground line (Mishra et al. 2013). Leaves of the plant affected by bacterial pathogens (*Xanthomonas* sp.) and fungal pathogens (*Leveillula* sp., *Alternaria* sp. or *Cercospora* sp.) show powdery mildew and spot symptoms. Formation of mosaic pattern on leaves, curling of leaves, vein bending, mottling of leaves is mostly caused by the viral pathogens affecting the plant (Damiri 2014). Chilli fruits are primarily affected by abiotic factors which can eventually lead to secondary infection by fungal pathogens belonging to the genera *Colletotrichum or Phytophthora*. These pathogens can attack fruit directly or can attack tissue weakened by environmental factors (Reddy 2009; Naik and Savitha 2014).

Use of synthetic pesticides for controlling chilli crop pathogens and the associated problems

The term pesticide in agriculture refers to a component or mixture of components to prevent, control, reduce or inhibit a pest (bacteria, fungi, virus, nematodes, vectors and even unwanted species of plants or animals) interfering with the production, processing, storage or marketing of any agricultural commodity. Synthetic pesticides are those which are formulated or manufactured using chemical processes using chemically synthesized components or by chemically changing any component derived from natural sources (Stoytcheva 2011). For any pest problem, chemical agents are usually preferred over organic ones as they are cheaper, readily available and have longer shelf life than the later. They are mostly broad-spectrum agents thus can be applied against various pests. They are even known to be more persistent in nature thus reducing the frequency of application in the field, which in turn saves time and economics (McCoy and Frank 2020). However due to such properties of persistency and broad spectrum activity, the indiscriminate use of synthetic pesticides has resulted in serious biological and ecological problems (Whipps 2001; Muthukumar et al. 2008). Table 2 summaries the chemical agents used against chilli plant pathogens and the reported toxicity of each agent. As reported by Pimentel (1995), only $< 0.3\%$ of chemical pesticides showed interaction and inhibition only of target pathogens. Most of the chemical pesticides affect the beneficial organisms in the soil which assist natural processes of mycorrhizal colonization, transformation or fixation of nitrogen, improvement of soil porosity and fertility (Aktar et al. 2009). Overuse of pesticides has also resulted in acquirement of resistance by the target pathogens. Some of the chemical agents are seen to be converted to toxic byproducts in the soil thus hampering other plants, animals and even humans (Smith and Perfetti 2020). Studies have revealed many associated adverse effects of the pesticides on human health viz. irritation of eyes and skin, mutations and carcinogenicity, disruption of endocrine functions, impaired reproductive capacity, atherogenicity and interference with neural transmission in the central and peripheral nervous system (Pruett et al. 2001; Atreya and Sitaula 2011; Budzinski and Couderchet 2018). Synthetic pesticides have been detected into aquatic systems due to field runoff or leaching, thus affecting the multiple developmental stages of aquatic life and even wild life (Nabi et al. 2019). Aragaki et al. (1994) has reported the phytotoxicity in a higher plant due to decomposition of a fungicide in water. Disruption of endocrine function, ovarian toxicity, oxidative stress and neuropathological effects has been identified in animals due to toxic chemical agents (Moser et al. 2001; Lu et al. 2004; Tahir and Nour 2009). Considering the risks associated with synthetic pesticides, there is need to shift to biological ways of controlling phytopathogens.

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Table 1 Disease causing phytopathogens in *Capsicum annuum*

Sr. no.	Disease	Causative agent	Symptoms
Fungal diseases			
1	Charcoal Rot (Shahid et al. 2016)	*Macrophomina phaseolina*	Blackening of lower stem and taproot is observed under the epidermis due to the numerous microsclerotia formation. This gives the plant tissue charcoal-sprinkled appearance. As the disease advances, leaves turn yellow, then wilt turning brown and eventually plant dies off
2	Damping off (Majeed et al. 2018)	*Pythium aphanidermatum*	In pre-emergence damping off, the growing points are damaged in the initial stages of seed germination before they come out through the soil. In post emergence damping off, the seedlings topple over the ground due to the collar rotting and rapid shrinkage, followed by spreading to the cortical tissue of the hypocotyls, basal stem and developing taproot resulting in death of plants
3	Fruit rot/Die-back/Anthracnose (Than et al. 2008; Machenhalli 2014)	*Colletotrichum capsici/Colletotrichum gloeosporioides/Colletotrichum truncatum/Colletotrichum acutatum/Colletotrichum scovillei*	Irregular water drenched spots appear on fruits which later become dark depressed lesions with various acervuli in concentric rings. Acervuli formation begins from the focal point of lesion and move towards periphery. They blend and get to be distinctly papery. The infected parts turn black and gets wrinkled and ultimately the fruit shrivels, dries up, rots and fall down. The pathogen may also attack the fruit stalk along the stem causing die-back symptoms. Branches and twigs show necrosis and the entire top of the plant may wither away. Disease spreads quickly under hot and humid conditions
4	Leaf spot/blight (Mishra et al. 2013)	(i) *Alternaria solani/Alternaria alternata*	Circular to oblong spots are observed with light grey to white centres and dark brown margins. Small spots coalesce to each other in extreme diseased condition prompting to defoliation. These leaf spots are covered with a dark greyish brown to black spores which sporulate in moist conditions
		(ii) *Cercospora capsici*	Angular or irregular shaped chlorotic lesions observed which later on turn greyish brown with profuse sporulation at the focal point of the spot. Severely infected leaves drop off prematurely
5	Phomopsis Blight/soft rot (Gopalakrishnan 2007)	*Phomopsis vexans/Diaporthe vexans*	Infected seeds may bring about damping off in the nursery. Lower leaves of seedlings show clearly defined circular brown spots with lighter centre. These spots are papery, often crack and may have short holes. Sometimes infected leaves show big irregular lesions and stems shows lesions with constriction which start from basal part of nodal portion as grey dry rot. This prompts to drying of few twigs or partial wilting of the portion of the plant. Pale to light brown sunken spots develop on the old fruits which expand and coalesce to cover the entire fruit leading to fruit rot

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Table 1 (continued)

Sr. no.	Disease	Causative agent	Symptoms
6	Phytophthora blight/Fruit rot/root rot/leaf blight (Majid et al. 2016)	*Phytophthora capsici*	Occur as crown rot from soil inoculums. At initial stages shrivelling of plants occur due to infection of root and lower portion of the stem. Purplish black lesions are also observed at collar region. Dark green water soaked spots appear later on the fruits which enlarges rapidly to cover the entire surface causing rotting of the fruit. On leaves water soaked bleached spots appear resulting in blight
7	Powdery mildew (Peshama et al. 2017)	*Leveillula taurica*	Whitish spots smaller in size and of circular shape are observed on lower leaf surface. As the disease progresses the entire leaf surface gets covered with dirty white powdery development which are seen in patches. The infection begins from the older leaves but gradually covers the whole plant. Diseased leaves eventually drop off leaving fruits exposed to sunscald
8	Rhizoctonia root rot (Mannai et al. 2018)	*Rhizoctonia solani*	Affects seedling and mature plants and induce dry rot of collar region and root rot which leads to wilting and death of chilli plants. It is easily identified by presence of white mycelia. It also shows symptoms of damping off
9	White/Sclerotia rot (Saharan and Mehta 2008)	*Sclerotinia sclerotiorum*	It is shown up as water soaked rotting of leaves at petiole, stalk and stump region. White mycelium growth is observed all over the infected portion. Seed production of crops is severely affected by this disease collapsing the entire inflorescence. The total rotted portion is converted into compact mat in later stages followed by hard black sclerotial body formation

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Table 1 (continued)

Sr. no.	Disease	Causative agent	Symptoms
10	Wilt (Mullen 2001; Bhat et al. 2003; Suryanto et al. 2010)	(i) *Fusarium oxysporum/Fusarium solani/Fusarium pallidoroseum*	Along with the wilting of the plant, leaf chlorosis and vascular discoloration is observed. Symptoms may appear at 2 stages *viz*. seedling and adult plant stage. Foliage of affected seedling turns yellow and dries there off. On adult plant initially, slight drooping of leaves is seen which led to drying of leaves starting from lower ones extending from root to stem region followed by wilting symptom. Increased temperature and moisture are conducive to symptom development
		(ii) *Sclerotium rolfsii*	The infected plants may show dry root rot, collar rot or stem rot. Rotting of the plant is followed by wilting and eventually plant dies off
		(iii) *Verticillium dahliae/Verticillium albo-atrum*	Slight chlorosis of the lower leaves is observed during initial stages which continue until the leaves become bright yellow, wilt and eventually fall off the plant. During warmer times of the day, plant shoots and overall foliage wilt. Internal vascular tissue of the main stems closer to the crown has a tan to light brown discoloration. Infected plants may exhibit physiological changes such as decreased photosynthesis, increased plant transpiration and respiration. Even if the infected plants do not die completely, plant development and yields can be essentially decreased
Bacterial diseases			
1	Leaf spot (Roach et al. 2018)	*Xanthomonas campesthis* pv. *vesicatoria*	Raised spots arise on young leaves and are yellowish green to dark brown in colour and on older leaves dark, water soaked spots arise which are not raised. Later they appear as straw coloured centre and dark margins. The spots are depressed on the upper leaf surface whereas on the lower leaf surface the spots are raised and slab like. As the bacteria spread along the veins, the spots appear angular. Leaves drop off on turning yellow due to severe spotting. Infected seedlings often lose their foliage except their top leaves. As the disease progress, the spots may enlarge, turn black and become rough giving a scabby appearance. Bacterial spot of pepper is described as blight because of large water-soaked lesions that later become necrotic. On the fruits, smaller blister like spots occur at the early stages and later gain a warty appearance. The disease results in defoliation and severely spotted fruits, both of which cause massive yield losses
2	Soft Rot (Djami-Tchatchou et al. 2019)	*Pectobacterium caratovorum*	Penetrates and infects the chilli fruit through broken peduncles and calyx resulting in soft rot. As the infection progresses, the entire fruit is reduced to a watery mass

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Table 1 (continued)

Sr. no.	Disease	Causative agent	Symptoms
3	Wilt (Sanogo 2003; Tahat and Sijam 2010)	*Ralstonia solanacearum*	The bacterium invades the roots causing wilting of the host plant as a consequence of rapid colonization and multiplication in the vascular tissue, followed by light tan to yellow–brown discoloration of the vascular tissue. Affected leaves turn yellow and remain wilted. The plants attacked by this disease rot, wilt, and eventually die
Viral diseases			
1	Chilli mosaic virus disease (Gopalakrishnan 2007)	*Tobacco mosaic (TMV)/Tomato mosaic (ToMV)/Cucumber mosaic virus*	TMV and ToMV cause light and dark green mosaics and distortion of leaves. Vein clearing, yellowing, mottling are characteristic symptoms. Necrotic flecks and spots can also appear on leaves. Stunted growth of infected plant is observed which does not normally bear fruits. If formed fruits are small, deformed, with chlorosis and necrosis in skin. Ring spot and necrotic spot symptoms are mostly seen in case of cucumber mosaic virus on the leaves or fruits of sweet pepper along with mild to moderate mosaic pattern
2	Leaf curl virus disease (Mishra and Chauvey 2018)	*Pepper leaf curl virus (PepLCV)*	The early symptoms include curling of the leaves toward the midrib at early plant growth stage thus getting deformed. Along with abaxial and adaxial curling; enations, heavy crinkling or puckering, chlorosis or yellowing and size reduction of leaves are observed. This is accompanied by shortening of internodes and petioles, blistering of inter veinal areas and thickening and clearing of the veins. Flower buds abscise before attaining full size and pollen development is hampered, leading to either no fruit set or setting of tiny fruits without any commercial value. In the advanced stages of disease, auxiliary buds are stimulated producing a cluster of small leaves and plant appears bushy with stunted growth producing no fruit
3	Pepper mottle virus disease (Peter and Hazra 2012)	*Pepper Mottle Virus*	Mottling is observed over the entire leaf surface especially in interveinal areas. Fruit becomes distorted and show mosaic pattern
4	Potato virus disease (Peter and Hazra 2012)	*Potato Virus Y*	Vein bending is seen along with the leaf distortion and plant stunting
5	Tomato spotted wilt (Mishra et al. 2013)	*Tomato Spotted Wilt Virus (TSWV)*	Leaves develop black small irregularly shaped to circular spots along with chlorotic and necrotic ring spots. Stems and shoots show black streak or lesions. Fruits develop chlorotic ring, patches and lesions. Severely infected plants are stunted and may wilt

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Table 2 Toxicity of chemical agents used to control plant pathogens

Sr. no.	Chemical agents	Pathogen	Toxicity	References
1	1,3-Dichloropropene	*Ralstonia solanacearum*	Probable human carcinogen Moderate to high aquatic toxicity	Markovitz and Crosby (1984) Krijgsheld and Van der Gen (1986)
2	Azoxystrobin	*Sclerotium rolfsii*	Hazardous to the aquatic environment	van Wijngaarden et al. (2014)
3	Bavistin/Carbendazim	*Alternaria solani* *Cercospora capsici* *Colletotrichum capsici* *Leveillula taurica* *Phomopsis vexans* *Sclerotinia sclerotiorum*	Disturb endocrine function in humans and wildlife, lead to impaired reproductive capacity, and have other toxic effects on sexual differentiation, growth, and development Induce aneuploidy	Lu et al. (2004) Morinaga et al. (2004)
4	Bayleton	*Leveillula taurica*	Toxicological and pathological effects in animals	Bentley et al. (2000) Tahir and Nour (2009)
5	Benlate	*Leveillula taurica*	Phytotoxic	Aragaki et al. (1994)
6	Blitox/copper oxychloride	*Colletotrichum capsici* *Fusarium spp.* *Phytophthora capsici* *Pythium aphanidermatum* *Rhizoctoni solani* *Xanthomonas campesthis*	Hampers growth rate of juveniles and cocoon production in earthworms	Helling et al. (2000)
7	Captan	*Colletotrichum capsici* *Fusarium spp.* *Phytophthora capsici* *Pythium aphanidermatum* *Rhizoctonia solani*	Possible carcinogenic hazard	Bridges et al. (1972)
8	Chloropicrin	*Ralstonia solanacearum* *Verticillium dahlia* *Verticillium alboatrum*	Airborne-irritant	Goldman et al. (1987)
9	Chlorothalonil	*Alternaria solani* *Cercospora capsici* *Colletotrichum capsici*	Airborne-irritant	Lensen et al. (2007)
10	Dazomet	*Sclerotium rolfsii*	Hampers development of mycorrhiza-forming fungi Anti-thyroid activity	Iyer and Wojahn (1976) Buxeraud et al. (1992)
11	Dithane M45	*Colletotrichum capsici* *Colletotrichum gloeosporioides*	Potential mutagenic and carcinogenic agent	Hemavathi and Rahiman (1993)
12	Flutolanil	*Sclerotium rolfsii*	Toxic effects on multiple developmental stages of aquatic life	Yang et al. (2016)
13	Fosetyl-Al	*Fusarium spp.* *Phytophthora capsici* *Pythium aphanidermatum* *Rhizoctoni solani*	Reduced growth of roots and inhibition of mycorrhizal colonization	Sukarno et al. (1998)
14	Hexaconazole	*Colletotrichum capsici*	Shows genotoxic effects Result in thyroid endocrine toxicity and other toxicity in aquatic fishes	Yilmaz et al. (2008) Yu et al. (2013) Wang et al. (2015)
15	Karathane	*Leveillula taurica*	Mutagenic, Clastogenic and Cytotoxic	Çelik et al. (2005)
16	Mancozeb	*Alternaria solani* *Cercospora capsici* *Fusarium spp.* *Phytophthora capsici* *Pythium aphanidermatum* *Rhizoctoni solani* *Sclerotinia sclerotiorum*	It degrades into ethylethiourea (ETU) which shows sperm abnormalities. It affects the central and peripheral nervous systems and causes endocrine disruption. It is carcinogenic – particularly affecting the thyroid teratogenic (interfering with embryonic development), and a general irritant	Atreya and Sitaula (2011)
17	Mercuric chloride	*Xanthomonas campesthis*	Genotoxic potential in a variety of organisms including humans and aquatic species	Betti et al. (1992) Bolognesi et al. (1999)

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Table 2 (continued)

Sr. no.	Chemical agents	Pathogen	Toxicity	References
18	Metam sodium	*Sclerotium rolfsii*	Irritant, adverse effects on both non-target plants and soil microbes, potential for immunological, developmental, carcinogenic, and atherogenic effects	Pruett et al. (2001)
19	Methylbromide	*Sclerotium rolfsii*	Potent ozone depletor Central and peripheral neurotoxic effects	Ristaino and Thomas (1997) DeHaro et al. (1997)
20	Propiconazole	*Leveillula taurica*	Interferes with embryonic development of aquatic animals	Kast-Hutcheson et al. (2001)
21	Propineb	*Phytophthora capsici*	Carcinogenic in animals	Guven et al. (1998)
22	Tebuconazole	*Sclerotium rolfsii*	Neurobehavioral deficits and neuropathology in animals	Moser et al. (2001)
			Disrupts physiological activity in fish	Sancho et al. (2010)
23	Thiophanate-methyl	*Sclerotium rolfsii*	Prolonged exposure is considered as a cytogenetic hazard	Capriglione et al. (2011)
24	Thiram	*Colletotrichum capsici* *Fusarium spp.* *Phytophthora capsici* *Pythium aphanidermatum* *Rhizoctoni solani*	Potential mutagenic and carcinogenic agent	Hemavathi and Rahiman (1993) Perocco et al. (1989)
25	Topsin	*Leveillula taurica*	Ovarian toxicity and oxidative stress in animals	Sakr et al. (2011)

Biocontrol agents against phytopathogens infecting chilli plants

Recent efforts have been focused on developing natural or biological control for the management of plant diseases for increasing the yield. Biocontrol is a safer option as it avoids environmental pollution and is specific to target pathogens. Being from a natural source they don't tend to pose any harm to plants, animals or humans. According to Eilenberg (2006), "biological control or biocontrol is the use of living organisms to suppress the population density or impact a specific pest organism, making it less abundant or less damaging than it would otherwise be". In other words, biological control is a phenomenon related to the antagonism between microorganisms (Cook 1985). Numerous microorganisms have been shown to be capable in suppressing plant pathogens and are therefore considered as biological control agents (BCAs). Table 3 summarizes BCAs against plant pathogens affecting chilli crops along with the suggested mode of action. Single BCA may show different modes of action against a phytopathogen which may be expressed sequentially, concurrently or synergistically. There are three main modes of antagonism exhibited by a candidate BCA against a phytopathogen: direct antagonism, indirect antagonism and mixed path antagonism (Pal and Gardener 2006).

Direct antagonism

Direct antagonism, involves the principle of parasitism or predation. In predation, the predator (in this case a BCA) kills the prey (a phytopathogen) for its survival. Some predatory bacteria use the cytoplasmic constituents of other bacteria as a source of nutrition, thereby killing the later (Köhl et al. 2019). Parasitism is a biological interaction wherein the parasite (in this case a BCA) lives on or inside a host (a phytopathogen), thus harming the later. This type of antagonism is termed as hyperparasitism or mycoparasitism/mycophagy (when target pathogen is a fungus). In mycoparasitism, a BCA secretes lytic enzymes that lyse the fungal cell wall leading to the leakage or disorganisation of cell contents. Hyphal deformation (abnormal swelling, curling and branching of mycelia), vacuolization and disintegration have also been reported. This results in a decrease of pathogen population or even complete inhibition (Heydari and Pessarakli 2010). A study carried out by Sid Ahmed et al. (1999) evaluated *Trichoderma harzianum* as a biocontrol agent for root rot caused by *Phytophthora capsici* in pepper plants. The study revealed how the hypha of *Trichoderma harzianum* coils around those of *Phytophthora capsici* resulting in vacuolization and disintegration of the hyphae of later. Another study exhibited coiling, vacuolation and swelling of the hyphae of *Colletotrichum truncatum* by *Burkholderia rinojensi*, resulting in inhibition of the pathogen, thus suppressing the anthracnose in chillies (Sandani et al. 2019).

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Table 3 BCAs against plant pathogens affecting chilli crops and their mode of action

Sr. no.	Pathogen	Bioagent	Suggested mode of action	References
1	*Alternaria alternata*	*Bacillus spp.*	Hyperparasitism	Sid et al. (2003)
2	*Cercospora capsici*	*Lactobacillus plantarum*	Induced Systemic Resistance	Adedire et al. (2019)
3	*Colletotrichum acutatum*	*Bacillus vallismortis*	Induced Systemic Resistance	Park et al. (2013)
4	*Colletotrichum capsici*	*Pichia guilliermondii* *Trichoderma harzianum* *Pseudomonas fluorescens* *Ophiocordyceps sobolifera*	Induced systemic reistance Hyperparasitism Induced systemic reistance Antibiosis	Chanchaichaovivat et al. (2007) Nantawanit et al. (2010) Ekefan et al. (2009) Anand et al. (2010) Jaihan et al. (2016)
5	*Colletotrichum gloeosporioides*	*Bacillus subtilis* *Streptomyces sp.* *Pseudomonas aeruginosa* *Streptomyces philanthi*	Antibiosis Hyperparasitism Competition Antibiosis	Kim et al. (2010) Ashwini et al. (2014) Kim et al. (2014) Sasirekha and Srividya (2016) Boukaew et al. (2018)
6	*Colletotrichum scovillei*	*Paenibacillus polymyxa*	Antibiosis	Suprapta et al. (2020)
7	*Colletotrichum truncatum*	*Burkholderia rinojensis* *Trichoderma harzianum, Trichoderma asperellum, Paenibacillus dendritiformis*	Hyperparasitism Induced systemic resistance	Sandani et al. (2019) Yadav et al. (2021)
8	*Cucumber mosaic virus*	*Bacillus amyloliquefaciens*	Induced systemic resistance	Lee and Ryu (2016)
9	*Fusarium oxysporum*	*Pseudomonas aeruginosa* *Bacillus subtilis* *Trichoderma viride,* *Trichoderma harzianum*	Competition/ Antibiosis Competition Hyperparasitism	Perveen et al. (1998) Yu et al. (2011) Dar et al. (2015)
10	*Fusarium pallidoroseum*	*Trichoderma viride* *Trichoderma harzianum*	Hyperparasitism Hyperparasitism	Wani and Najar (2012) Dar et al. (2015)
11	*Fusarium solani*	*Pseudomonas aeruginosa* *Bacillus subtilis* *Pseudomonas fluorescens* *Trichoderma viride* *Trichoderma harzianum*	Competition/antibiosis Induced systemic resistance Induced systemic resistance Hyperparasitism Hyperparasitism	Perveen et al. (1998) Sundaramoorthy et al. (2012) Dar et al. (2015) Bhat et al. (2016)
12	*Leveillula taurica*	*Cephalosporium sp.* *Paecilomyces farinosus* *Ampelomyces quisqualis* *Trichoderma harzianum* *Trichoderma asperellum* *Metarhizium anisopliae* *Pseudomonas fluorescens*	Not known Antibiosis Hyperparasitism Hyperparasitism/Induced systemic resistance Secretion of lytic enzymes Induced systemic resistance/ Secretion of lytic enzymes	Kiss (2003) Brand et al. (2009) López et al. (2019) Anand et al. (2010)
13	*Macrophomina phaseolina*	*Streptomyces sp.*	Secretion of lytic enzymes and waste products/Plant growth promoting activities	Alaa Fathalla and El-Sharkawy (2020)
14	*Pectobacterium carotovorum*	*Bacillus vallismortis*	Induced systemic resistance	Park et al. (2013)
15	*Pepper leaf curl virus*	*Streptomyces sp.*	Plant growth promoting activities	Putri et al. (2019)
16	*Phytophthora capsici*	*Trichoderma harzianum* *Bacillus licheniformis* *Streptomyces halstedii* *Bacillus megaterium* *Bacillus subtilis* *Trichoderma hamatum, Pseudomonas aeruginosa*	Hyperparasitism/induced systemic resistance Secretion of lytic enzymes Hyperparasitism/mycophagy Antibiosis Antibiosis Competition/antibiosis	Sid Ahmed et al. (1999) Ahmed et al. (2000) Sid et al. (2003) Joo (2005) Akgül and Mirik (2008) Lee et al. (2008) Chemeltorit et al. (2017)

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Table 3 (continued)

Sr. no.	Pathogen	Bioagent	Suggested mode of action	References
17	*Pythium aphanidermatum*	*Pseudomonas chlororaphis* *Bacillus subtilis* *Calothrix elenkenii*	Competition/antibiosis Induced systemic resistance Antibiosis	Khan et al. (2003) Nakkeeran et al. (2006) Manjunath et al. (2010)
18	*Ralstonia solanacearum*	*Bacillus megaterium* *Enterobacter cloacae* *Pichia guilliermondii* *Candida ethanolica* *Bacillus amyloliquefaciens*	Induced systemic resistance Competition Not known Not known Antibiosis	Nguyen et al. (2010) Hu et al. (2010)
19	*Rhizoctonia solani*	*Pseudomonas aeruginosa* *Bacillus thuringiensis* *Pseudomonas fluorescens* *Pseudomonas putida* *Bacillus subtillis* *Trichoderma viride* *Trichoderma harzianum*	Competition/antibiosis Antibiosis Competition Antibiosis Antibiosis Hyperparasitism Hyperparasitism	Perveen et al. (1998) Mojica-Marín et al. (2008) Abeysinghe (2009) Dar et al. (2015) Lewis and Lumsden (2001)
20	*Sclerotium rolfsii*	*Gliocladium virens* *Pseudomonas fluorescens* *Pseudomonas putida* *Bacillus subtillis* *Trichoderma viride,* *Trichoderma harzianum*	Antibiosis Competition Antibiosis Antibiosis Hyperparasitism	Ristaino et al. (1996) Abeysinghe (2009) Dar et al. (2015)
21	*Tobacco mosaic virus*	*Bacillus cereus*	Induced systemic resistance and plant growth promoting activities	Damayanti et al. (2007)
22	*Verticillium dahliae*	*Pythium oligandrum*	Induced systemic resistance	Al-Rawahi and Hancock (1998) Rekanovic et al. (2007)
23	*Verticillium albo-atrum*	*Pythium oligandrum*	Induced systemic resistance	Rekanovic et al. (2007)
24	*Xanthomonas campestris pv. vesicatoria*	Lactic acid bacteria	Competition	Shrestha et al. (2014)

Indirect antagonism

Indirect modes of antagonism exhibited by BCAs can be either competition for space and nutrition with the pathogen or induction of resistance in the host against the pathogen. Competing for nutrients is generally observed among the species sharing the same ecological niche and having the same physiological prerequisites when resources are constrained. Such competition may lead to reduced proliferation or inhibition of the pathogen (Pal and Gardener 2006). A BCA may limit the growth of the pathogen by competing for host supplied nutrients (exudates, leachates, or senesced tissue), essential soluble nutrients (iron sequestering by producing siderophore) or for colonising root and plant tissues, thus depriving access of the pathogen at the infection site (Vurukonda et al. 2018). When starved of Iron, a *Bacillus* sp. exhibited inhibition of *Fusarium oxysporum* Schl. f. sp. capsici, the causal agent of wilt of *Capsicum annuum* by producing siderophores. This bacterium also did show antagonism against several other plant fungal pathogens, belonging to genus *Fusarium, Colletotrichum, Pythium, Magnaporthe* and *Phytophthora* (Yu et al. 2011). Another study revealed rapid root colonization of chilli plant by Arbuscular mycorrhizal (AM) fungi *Funneliformis caledonium* thus increasing the nutrient acquisition by plant and suppression of *Phytophthora* blight (Hu et al. 2020). Induced systemic resistance is an indirect type of antagonism wherein inoculation with BCAs triggers the induction or enhancement of defence mechanism of plants. This is achieved via production of defence related metabolites (phenolics, reactive oxygen species, phytoalexins, pathogenesis-related proteins) or via activation of pathways (jasmonic acid, salicylic acid) or via formation of physical barriers (modifications of cell walls and cuticles) (Jayapala et al. 2019; Köhl et al. 2019). Suppression of *Colletotrichum truncatum* Anthracnose in Chilli Pepper was observed due to resistance induced by *Trichoderma harzianum*, *Trichoderma asperellum* and *Paenibacillus dendritiformis* in host. The chilli plant exhibited enhancement of the activity of defence-related and antioxidative enzymes, accumulation of phenolic compounds and reactive oxygen species (Yadav et al. 2021). Another study revealed the suppression of disease symptoms caused by *Pectobacterium carotovorum*, *Phytophthora capsici* and *Colletotrichum acutatum* by a *Bacillus* sp. via induction of systemic resistance via a salicylic acid-dependent mechanism (Park et al. 2013). Jisha et al. (2019) reported

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the induction of defense enzymes peroxidase, polyphenol oxidase and phenylalanine ammonia lyase by *Pseudomonas aeruginosa* in chilli plant. Such induction of host systemic resistance also increased the total phenolic contents in the plant. Thus the strain of *Pseudomonas* proved to be capable of reducing antharcnose disease in chilli.

Mixed path antagonism

In this type of antagonism BCAs produce secondary metabolites toxic to the target pathogens. This not only include antibiotics, but also lytic enzymes, unregulated waste products and volatile compounds. Such metabolites interfere in the pathogenesis of the target pathogen (Nega 2014). A BCA can also impart the host plant with growth promoting activities which boost the plant growth and help in disease suppression (Beneduzi et al. 2012). Plant growth promoting BCA can confer the host with various attributes like atmospheric nitrogen fixation, solubilisation of unavailable nutrients such as phosphate, potassium, zinc and silicon thus making it readily available to the plant. They also accelerate production of hormones viz. auxins (Indole Acetic Acid), cytokinin and gibberellins (Bhattacharyya and Jha 2012) or of enzymes such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, thus helping the plant tolerate various stress conditions (Pourbabaee et al. 2016). A BCA can alternatively secret lytic enzymes like proteases, amylases, lipases, chitinases, glucanases and cellulases which target and degrade the cell wall of pathogens (Subrahmanyam et al. 2020). A *Bacillus* sp. isolated from chilli rhizosphere produced appreciable levels of three mycolytic enzymes chitinase, glucanase and cellulase and showed antagonism against *Colletotrichum gloeosporioides* for management of anthracnose disease of chilli (Ashwini and Srividya 2014). Similar studies were undertaken by Nguyen et al. (2012), who reported increased activity of chitinase and β-1,3-glucanase in roots and surrounding rhizosphere soil of pepper plants treated with *Streptomyces griseus*. This resulted in suppression of Root Rot Disease cause *Phytophthora capsici* in the host plant. Microbial unregulated waste products such as Hydrogen cyanide (HCN) may also contribute to pathogen suppression by effectively blocking the cytochrome oxidase pathway. Volatile metabolite such as ammonia at a particular concentration has also been reported to be toxic to various fungal pathogens (Howell et al. 1988). Whereas other volatiles mainly alkenes, alcohols and ketones, are known stimulate plant growth by enhancing mineral uptake, modifying root structure and modulating hormone signalling. Bacterial volatiles also play important role in functions like bacterial motility, biofilm formation and induction of systemic resistance in the host plant (O'Brien 2017). Most of the times a BCA exhibit several of the above mentioned traits to help the host plant in growth and disease suppression. Treatment of chilli plant with a *Streptomyces* sp. positive for production of protease, chitinase, Indole Acetic Acid (IAA), siderophore, ammonia and HCN and phosphate solubilization activity reduced the percentages of damping-off and root rot severity caused by *Rhizoctonia solani* and *Macrophomina phaseolina* (Alaa Fathalla and El-Sharkawy 2020). Shrestha et al. (2014) reported potential of Lactic acid bacteria (LABs) to colonise roots, produce indole-3-acetic acid (IAA) and siderophores and solubilise phosphate. These LABs showed the inhibition of bacterial pathogens *Xanthomonas campestris* pv. vesicatoria and *Ralstonia solanacearum* which are the causative agents of leaf spot disease and wilt in *Capsicum annuum.* Apart from all these secondary metabolites, the major reason for antagonism is the production of antibiotics by BCAs. These are chemically heterogeneous group of organic, low-molecular weight antimicrobial compounds that hamper the growth or metabolism of the target microorganism at a particular concentration (Thomashow et al. 1997). Table 4 enlists some of the antimicrobial compounds produced by BCAs against pathogens affecting chilli plant. Each of these compounds have a different mode of action, thus some organisms are susceptible to certain antibiotics but others are not, depending on the specific moiety of cellular constituent the antibiotic attacks (Ulloa-Ogaz et al. 2015). Vegetative Catalase protein (KatA) produced by a *Bacillus* sp. induced abnormal conidial swelling and elongation and rupture of hyphae of *Colletotrichum capsici* thus suppressing the anthracnose disease of chili pepper (Srikhong et al. 2018). Wu et al. (2019) suggested that the lipopeptides produced by a *Bacillus* sp. inhibited mycelial growth of *Rizoctonia solani*, thus assisting in the suppression of disease symptoms in chilli plant. Another antimicrobial compound Gliotoxin exhibited anti oomycete activity against *Phytophthora capsici* thus suppressing blight in chilli (Tomah et al. 2020). Whereas the study by Ko et al. (2009) suggested that the inhibition of *Phytophthora capsici* by 4-hydroxyphenylacetic acid produced by *Lysobacter antibioticus* could be due to the deformation, lysis, and bending of hyphae of the fungus. Some antibiotics such as fusaricidin have been also known to induce systematic reistance in host (chilli) to suppress *Phytophthora* blight (Lee et al. 2013). Similar results were reported by Sundaramoorthy et al. (2012) wherein the synthesis of phytolaexins by *Bacillus subtilis* induced systemic resistance against wilt disease caused by *Fusarium solani* in chilli. Cell free supernatant of *Paenibacillus polymyxa* containing 3-hydroxy-2-butanone and 2,3-butanediol showed wrinkles on mycelia of *Colletotrichum scovillei* which causes anthracnose in chilli (Suprapta et al. 2020). Antimicrobial compound Phenazine-1-carboxamide produced by *Pseudomonas aeruginosa* showed deformation of mycelia and inhibition of

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Table 4 BCAs producing antimicrobial compounds against phytopathogens infecting chilli crops

Sr no.	BCA	Antimicrobial compound produced	Target pathogen	References
1	*Bacillus subtilis* *Pseudomonas fluorescens*	Phytolaexins	*Fusarium solani*	Sundaramoorthy et al. (2012)
2	*Bacillus subtilis*	Surfactin Iturin Fengycin Bacillomycin	*Rhizoctonia solani*	Wu et al. (2019)
3	*Lysobacter antibioticus*	4-Hydroxyphenylacetic acid	*Phytophthora capsici*	Ko et al. (2009)
4	*Paenibacillus polymyxa*	3-Hydroxy-2-butanone 2,3-Butanediol	*Colletotrichum scovillei*	Suprapta et al. (2020)
5	*Paenibacillus polymyxa*	Fusaricidin	*Phytophthora capsici*	Lee et al. (2013)
6	*Pseudomonas aeruginosa*	Phenazine-1-carboxylicacid	*Leveillula taurica, Colletotrichum capsicum*	Rane et al. (2007)
7	*Pseudomonas aeruginosa*	Phenazine-1-carboxamide	*Colletotrichum capsici*	Kumar et al. (2005)
8	*Pseudomonas fluorescens*	Pyoluteorin Pyrrolnitrin Orfamide A	*Phytophthora capsici*	Kim et al. (2014)
9	*Trichoderma virens*	Gliotoxin	*Phytophthora capsici*	Tomah et al. (2020)
10	*Trichoderma koningiopsis*	Azetidine 2-phenylethanol Ethyl hexadecanoate	*Colletotrichum gloeosporioides*	Ruangwong et al. (2021)

sporulation thus inhibiting the growth of fungal pathogen *Colletotrichum capsici* causative agent of fruit rot in chilli (Kumar et al. 2005).

Conclusion

Biological control agents (BCAs) have generated great enthusiasm as safe and sustainable plant protection tool but still make up only a small percentage of the chilli crop protection market due to lack of availability of formulated products. There is a need for more extended biocontrol research and better understanding of the mechanisms involved in the antagonistic abilities of microbial BCAs so as to improve its efficacy, stability and consistency in fields. The present review identifies potential microbial BCAs with inhibitory activity against various fungal, bacterial and viral pathogens infecting the chilli crop. This review also gives us insight of how BCAs (single or consortium) employ several mechanisms (direct, indirect and mixed path antagonism) to act as an effective biocontrol agent against target pathogens and trigger different promotional effects on chilli plant growth parameters. Being from a natural source these BCAs are also safe to the environment and thus are a great alternative to replace chemical counterparts which are harmful to environment and ecology. Moreover, the field trials of chilli plants inoculated with these microbial BCAs have also proven its effect *in-vivo*. In conclusion, through thorough understanding of biocontrol activities of such microbes, multiple facets of disease suppression and plant growth promotion is revealed. This will thus aid in the achievement of the objective of commercialization of potential strains against the target pathogens and their implementation in the agriculture industry for protection of chilli plants.

Declarations

Conflict of interest The authors declare that they is no conflict of interest.

References

Abeysinghe, S.: Efficacy of combine use of biocontrol agents on control of *Sclerotium rolfsii* and *Rhizoctonia solani* of *Capsicum annuum*. Arch Phytopathol Plant Prot **42**(3), 221–227 (2009). https://doi.org/10.1080/03235400600999406

Adedire, O.M., Pitan, A., Farinu, A.O., Ogundipe, W.F.: The biocontrol of soil transmitted *Cercospora capsici* with *Lactobacillus plantarum*. J Adv Appl Microbiol **21**, 1–8 (2019). https://doi.org/10.9734/jamb/2019/v18i330173

Ahmed, A.S., Sánchez, C.P., Candela, M.E.: Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. Eur. J. Plant Pathol. **106**(9), 817–824 (2000). https://doi.org/10.1023/A:1008780022925

Akgül, D.S., Mirik, M.: Biocontrol of *Phytophthora capsici* on pepper plants by *Bacillus megaterium* strains. J. Plant Pathol. **1**, 29–34 (2008)

Aktar, M.W., Sengupta, D., Chowdhury, A.: Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. **2**(1), 1 (2009). https://doi.org/10.2478/v10102-009-0001-7

Alaa Fathalla, A.M., El-Sharkawy, E.E.: Biological control using *Streptomyces* sp. Kp109810 and different genotypes of pepper

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(*Capsicum annuum* L.) on root rot diseases. J. Appl. Res. Plant Prot. **9**(1), 25–37 (2020). https://doi.org/10.21608/japp.2020.130645

Al-Rawahi, A.K., Hancock, J.G.: Parasitism and biological control of *Verticillium dahliae* by *Pythium oligandrum*. Plant Dis **82**(10), 1100–1106 (1998). https://doi.org/10.1094/PDIS.1998.82.10.1100

Anand, T., Chandrasekaran, A., Kuttalam, S., Senthilraja, G., Samiyappan, R.: Integrated control of fruit rot and powdery mildew of chilli using the biocontrol agent *Pseudomonas fluorescens* and a chemical fungicide. Biol. Control **52**(1), 1–7 (2010). https://doi.org/10.1016/j.biocontrol.2009.09.010

Aragaki, M., Uchida, J.Y., Kadooka, C.Y.: Toxicity of Benlate® to cucumber and evidence for a volatile phytotoxic decomposition product. Arch. Environ. Contam. Toxicol. **27**(1), 121–125 (1994). https://doi.org/10.1007/BF00203897

Ashwini, N., Srividya, S.: Potentiality of *Bacillus subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichum gloeosporioides* OGC1. 3 Biotech **4**(2), 127–136 (2014). https://doi.org/10.1007/s13205-013-0134-4

Atreya, K., Sitaula, B.K.: Mancozeb: growing risk for agricultural communities? Himalayan J. Sci **6**(8), 9–10 (2011). https://doi.org/10.3126/hjs.v6i8.1794

Balakrishnan, K.: Studies on nutrients deficiency symptoms in chilli (*Capsicm annum* L.). Indian J. Plant Physiol. **4**(3), 229–231 (1999)

Beneduzi, A., Ambrosini, A., Passaglia, L.M.: Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet. Mol. Biol. **35**, 1044–1051 (2012). https://doi.org/10.1590/S1415-47572012000600020

Bentley, K.S., Kirkland, D., Murphy, M., Marshall, R.: Evaluation of thresholds for benomyl- and carbendazim-induced aneuploidy in cultured human lymphocytes using fluorescence *in situ* hybridization. Mutat. Res. **464**(1), 41–51 (2000). https://doi.org/10.1016/S1383-5718(99)00165-5

Betti, C., Davini, T., Barale, R.: Genotoxic activity of methyl mercury chloride and dimethyl mercury in human lymphocytes. Mutat. Res. Lett. **281**(4), 255–260 (1992). https://doi.org/10.1016/0165-7992(92)90018-D

Bhat, M.N., Mesta, R., Yenjerappa, S.T., Tatagar, M.H., Sardana, H.R., Singh, D., Vennila, S., Sabir, N., Ahmad, M.: Biological control of *Fusarium* wilt of chillies using *Trichoderma* spp. Indian J. Hortic. **73**(1), 74–77 (2016). https://doi.org/10.5958/0974-0112.2016.00021.9

Bhattacharyya, P.N., Jha, D.K.: Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnol. **28**(4), 1327–1350 (2012). https://doi.org/10.1007/s11274-011-0979-9

Bolognesi, C., Landini, E., Roggieri, P., Fabbri, R., Viarengo, A.: Genotoxicity biomarkers in the assessment of heavy metal effects in mussels: experimental studies. Environ. Mol. Mutagen **33**(4), 287–292 (1999). https://doi.org/10.1002/(SICI)1098-2280(1999)33:4%3c287::AID-EM5%3e3.0.CO;2-G

Bosland, P.W., Votava, E.J., Votava, E.M.: Peppers: Vegetable and Spice Capsicums. Cabi, Wallingford (2012)

Boukaew, S., Petlamul, W., Bunkrongcheap, R., Chookaew, T., Kabbua, T., Thippated, A., Prasertsan, P.: Fumigant activity of volatile compounds of *Streptomyces philanthi* RM-1-138 and pure chemicals (acetophenone and phenylethyl alcohol) against anthracnose pathogen in postharvest chili fruit. Crop Prot. **103**, 1–8 (2018). https://doi.org/10.1016/j.cropro.2017.09.002

Brand, M., Messika, Y., Elad, Y., David, D.R., Sztejnberg, A.: Spray treatments combined with climate modification for the management of *Leveillula taurica* in sweet pepper. Eur. J. Plant Pathol. **124**(2), 309–329 (2009). https://doi.org/10.1007/s10658-008-9421-z

Bridges, B.A., Mottershead, R.P., Rothwell, M.A., Green, M.H.: Repair-deficient bacterial strains suitable for mutagenicity screening: tests with the fungicide captan. Chem. Biol.. Interact **5**(2), 77–84 (1972). https://doi.org/10.1016/0009-2797(72)90034-8

Budzinski, H., Couderchet, M.: Environmental and human health issues related to pesticides: from usage and environmental fate to impact. Environ. Sci. Pollut. Res. **25**, 14277–14279 (2018). https://doi.org/10.1007/s11356-018-1738-3

Buxeraud, J., Lagorce, J.F., Comby, F., Raby, C.: Interaction between tetrahydro-3, 5 dimethyl-2h-1, 3, 5thiazidine-2-thione (dmtt or dazomet) and molecular iodine: possible role in thyroid toxicity. Int. J. Environ. Sci. **42**(2–3), 177–186 (1992). https://doi.org/10.1080/00207239208710794

Capriglione, T., De Iorio, S., Gay, F., Capaldo, A., Vaccaro, M.C., Morescalchi, M.A., Laforgia, V.: Genotoxic effects of the fungicide thiophanate-methyl on *Podarcis sicula* assessed by micronucleus test, comet assay and chromosome analysis. Ecotoxicology **20**(4), 885–891 (2011). https://doi.org/10.1007/s10646-011-0655-8

Çelik, M., Ünal, F., Yüzbaşıoğlu, D., Ergün, M.A., Arslan, O., Kasap, R.: *In vitro* effect of karathane LC (dinocap) on human lymphocytes. Mutagenesis **20**(2), 101–104 (2005). https://doi.org/10.1093/mutage/gei013

Chanchaichaovivat, A., Ruenwongsa, P., Panijpan, B.: Screening and identification of yeast strains from fruits and vegetables: Potential for biological control of postharvest chilli anthracnose (*Colletotrichum capsici*). Biol. Control **42**(3), 326–335 (2007). https://doi.org/10.1016/j.biocontrol.2007.05.016

Chemeltorit, P.P., Mutaqin, K.H., Widodo, W.: Combining *Trichoderma hamatum* THSW13 and *Pseudomonas aeruginosa* BJ10–86: a synergistic chili pepper seed treatment for *Phytophthora capsici* infested soil. Eur. J. Plant Pathol. **147**(1), 157–166 (2017). https://doi.org/10.1007/s10658-016-0988-5

Cook, R.J.: Biological control of plant pathogens: theory to application. Phytopathology **75**(1), 25–29 (1985)

Damayanti, T.A., Pardede, H., Mubarik, N.R.: Utililization of root-colonizing bacteria to protect hot-pepper againts tobacco mosaic virus. HAYATI J. Biosci. **14**(3), 105–109 (2007). https://doi.org/10.4308/hjb.14.3.105

Damiri, N.: Mixed viral infection and growth stage on Chilli (*Capsicum annuum* L.) production. Pertanika J. Trop. Agric. Sci. **37**(2), 275–283 (2014)

Dar, G.H., Mir, G.H., Rashid, H., Dar, W.A., Majeed, M.: Evaluation of microbial antagonists for the management of wilt/root rot and damping-off diseases in chilli (*Capsicum annuum*). Vegetos **28**(4), 102–110 (2015). https://doi.org/10.5958/2229-4473.2015.00091.9

DeHaro, L., Gastaut, J.L., Jouglard, J., Renacco, E.: Central and peripheral neurotoxic effects of chronic methyl bromide intoxication. J. Toxicol.: Clin. Toxicol. **35**(1), 29–34 (1997). https://doi.org/10.3109/15563659709001162

Djami-Tchatchou, A.T., Matsaunyane, L.B., Kalu, C.M., Ntushelo, K.: Gene expression and evidence of coregulation of the production of some metabolites of chilli pepper inoculated with *Pectobacterium carotovorum ssp. carotovorum*. Funct. Plant Biol. **46**(12), 1114–1122 (2019). https://doi.org/10.1071/FP18244

Eilenberg, J.: Concepts and visions of biological control. In: Eilenberg, J., Hokkanen, H.M.T. (eds.) An Ecological and Societal Approach to Biological Control, pp. 1–11. Springer, Dordrecht (2006)

Ekefan, E.J., Jama, A., Gowen, S.R.: Potential of *Trichoderma harzianum* isolates in biocontrol of *Colletotrichum capsici* causing anthracnose of pepper (*Capsicum* spp.) in Nigeria. J. Appl. Biosci. **20**, 1138–1145 (2009)

Author's personal copy

Gachomo, E.W., Shonukan, O.O., Kotchoni, S.O.: The molecular initiation and subsequent acquisition of disease resistance in plants. Afr. J. Biotechnol. **2**(2), 26–32 (2003). https://doi.org/10.5897/AJB2003.000-1005

Goldman, L.R., Mengle, D.O., Epstein, D.M., Fredson, D., Kelly, K., Jackson, R.J.: Acute symptoms in persons residing near a field treated with the soil fumigants methyl bromide and chloropicrin. West J. Med. **147**(1), 95 (1987)

Gopalakrishnan TR (2007) Vegetable crops (No. 4). New India Publishing, New Delhi.

Goyal, D., Yadav, A., Vats, T.: Air pollution and its role in stress physiology. In: Saxena, P., Srivastava, A. (eds.) Air Pollution and Environmental Health, pp. 115–140. Springer, Singapore (2020)

Guven, K., Deveci, E., Akba, O., Onen, A., de Pomerai, D.: The accumulation and histological effects of organometallic fungicides Propineb and Maneb in the kidneys of fetus and female rats during pregnancy. Toxicol. Lett. **99**(2), 91–98 (1998). https://doi.org/10.1016/S0378-4274(98)00128-3

Helling, B., Reinecke, S.A., Reinecke, A.J.: Effects of the fungicide copper oxychloride on the growth and reproduction of *Eisenia fetida* (Oligochaeta). Ecotoxicol. Environ. Saf. **46**(1), 108–116 (2000). https://doi.org/10.1006/eesa.1999.1880

Hemavathi, E., Rahiman, M.A.: Toxicological effects of ziram, thiram, and dithane M-45 assessed by sperm shape abnormalities in mice. J. Toxicol .Environ. Health A Curr. Issues **38**(4), 393–398 (1993). https://doi.org/10.1080/15287399309531727

Heydari, A., Pessarakli, M.: A review on biological control of fungal plant pathogens using microbial antagonists. J. Biol. Sci. **10**(4), 273–290 (2010). https://doi.org/10.3923/jbs.2010.273.290

Howell, C.R., Beier, R.C., Stipanovic, R.D.: Production of ammonia by *Enterobacter cloacae* and its possible role in the biological control of *Pythium* preemergence damping-off by the bacterium. Phytopathology **78**(8), 1075–1078 (1988)

Hu, H.Q., Li, X.S., He, H.: Characterization of an antimicrobial material from a newly isolated *Bacillus amyloliquefaciens* from mangrove for biocontrol of *Capsicum* bacterial wilt. Biol. Control **54**(3), 359–365 (2010). https://doi.org/10.1016/j.biocontrol.2010.06.015

Hu, J., Hou, S., Li, M., Wang, J., Wu, F., Lin, X.: The better suppression of pepper *Phytophthora* blight by arbuscular mycorrhizal (AM) fungus than *Purpureocillium lilacinum* alone or combined with AM fungus. J. Soil Sediment. **20**(2), 792–800 (2020). https://doi.org/10.1007/s11368-019-02438-9

Iyer, J.G., Wojahn, K.E.: Effect of the fumigant dazomet on the development of mycorrhizae and growth of nursery stock. Plant Soil **45**(1), 263–266 (1976). https://doi.org/10.1007/BF00011148

Jaihan, P., Sangdee, K., Sangdee, A.: Selection of entomopathogenic fungus for biological control of chili anthracnose disease caused by *Colletotrichum* spp. Eur. J. Plant Pathol. **146**(3), 551–564 (2016). https://doi.org/10.1007/s10658-016-0941-7

Jayapala, N., Mallikarjunaiah, N.H., Puttaswamy, H., Gavirangappa, H., Ramachandrappa, N.S.: Rhizobacteria *Bacillus* spp. induce resistance against anthracnose disease in chili (*Capsicum annuum* L.) through activating host defense response. Egypt. J. Biol. Pest Control **29**(1), 1–9 (2019). https://doi.org/10.1186/s41938-019-0148-2

Jisha, M.S., Linu, M.S., Sreekumar, J.: Induction of systemic resistance in chilli (*Capsicum annuum* L.) by Pseudomonas aeruginosa against anthracnose pathogen *Colletotrichum capsici*. J. Trop. Agric. **56**(2), 153–166 (2019)

Joo, G.J.: Production of an anti-fungal substance for biological control of *Phytophthora capsici* causing *Phytophthora* blight in red-peppers by *Streptomyces halstedii*. Biotechnol. Lett. **27**(3), 201–205 (2005). https://doi.org/10.1007/s10529-004-7879-0

Kast-Hutcheson, K., Rider, C.V., LeBlanc, G.A.: The fungicide propiconazole interferes with embryonic development of the crustacean *Daphnia magna*. Environ. Toxicol. Chem. **3**, 502–509 (2001). https://doi.org/10.1002/etc.5620200308

Khan, A., Sutton, J.C., Grodzinski, B.: Effects of *Pseudomonas chlororaphis* on *Pythium aphanidermatum* and root rot in peppers grown in small-scale hydroponic troughs. Biocontrol. Sci. Technol. **13**(6), 615–630 (2003). https://doi.org/10.1080/0958315031000151783

Kim, P.I., Ryu, J.W., Kim, Y.H., Chi, Y.T.: Production of biosurfactant lipopeptides iturin A, fengycin, and surfactin A from *Bacillus subtilis* CMB32 for control of *Colletotrichum gloeosporioides*. J. Microbiol. Biotechnol. **20**(1), 138–145 (2010). https://doi.org/10.4014/jmb.0905.05007

Kim, T.S., Dutta, S., Lee, S.W., Park, K.: Endophytic bacterium *Pseudomonas fluorescens* strain EP103 was effective against *Phytophthora capsici* causing blight in chili pepper. Korean J. Pestic. Sci. **18**(4), 422–428 (2014). https://doi.org/10.7585/kjps.2014.18.4.422

Kiss, L.: A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Manage. Sci. **59**(4), 475–483 (2003). https://doi.org/10.1002/ps.689

Ko, H.S., Jin, R.D., Krishnan, H.B., Lee, S.B., Kim, K.Y.: Biocontrol ability of *Lysobacter antibioticus* HS124 against *Phytophthora* blight is mediated by the production of 4-hydroxyphenylacetic acid and several lytic enzymes. Curr. Microbiol. **59**(6), 608–615 (2009). https://doi.org/10.1007/s00284-009-9481-0

Köhl, J., Kolnaar, R., Ravensberg, W.J.: Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Front. Plant Sci. **10**, 845 (2019). https://doi.org/10.3389/fpls.2019.00845

Krijgsheld, K.R., Van der Gen, A.: Assessment of the impact of the emission of certain organochlorine compounds on the aquatic environment: Part II: Allylchloride, 1, 3-and 2, 3-dichloropropene. Chemosphere **15**(7), 861–880 (1986). https://doi.org/10.1016/0045-6535(86)90052-4

Kumar, R.S., Ayyadurai, N., Pandiaraja, P., Reddy, A.V., Venkateswarlu, Y., Prakash, O., Sakthivel, N.: Characterization of antifungal metabolite produced by a new strain *Pseudomonas aeruginosa* PUPa3 that exhibits broad-spectrum antifungal activity and biofertilizing traits. J. Appl. Microbiol. **98**(1), 145–154 (2005). https://doi.org/10.1111/j.1365-2672.2004.02435.x

Lee, G.H., Ryu, C.M.: Spraying of leaf-colonizing *Bacillus amyloliquefaciens* protects pepper from Cucumber mosaic virus. Plant Dis. **100**(10), 2099–2105 (2016). https://doi.org/10.1094/PDIS-03-16-0314-RE

Lee, K.J., Kamala-Kannan, S., Sub, H.S., Seong, C.K., Lee, G.W.: Biological control of Phytophthora blight in red pepper (*Capsicum annuum* L.) using Bacillus subtilis. World J. Microbiol. Biotehnol. **24**(7), 1139–1145 (2008). https://doi.org/10.1007/s11274-007-9585-2

Lee, S.H., Cho, Y.E., Park, S.H., Balaraju, K., Park, J.W., Lee, S.W., Park, K.: An antibiotic fusaricidin: a cyclic depsipeptide from *Paenibacillus polymyxa* E681 induces systemic resistance against *Phytophthora* blight of red-pepper. Phytoparasitica **41**(1), 49–58 (2013). https://doi.org/10.1007/s12600-012-0263-z

Lee, Y., Howard, L.R., Villalon, B.: Flavonoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. J. Food Sci. **60**(3), 473–476 (1995). https://doi.org/10.1111/j.1365-2621.1995.tb09806.x

Lensen, G., Jungbauer, F., Gonçalo, M., Coenraads, P.J.: Airborne irritant contact dermatitis and conjunctivitis after occupational exposure to chlorothalonil in textiles. Contact Dermatitis **57**(3), 181–186 (2007). https://doi.org/10.1111/j.1600-0536.2007.01196.x

Lewis, J.A., Lumsden, R.D.: Biocontrol of damping-off of greenhouse-grown crops caused by *Rhizoctonia solani* with a formulation of

Author's personal copy

Trichoderma spp. Crop Prot. **20**(1), 49–56 (2001). https://doi.org/10.1016/S0261-2194(00)00052-1
López, C.G., Castellanos, L.N., Ortiz, N.A., González, J.A.: Control of powdery mildew (*Leveillula taurica*) using *Trichoderma asperellum* and *Metarhizium anisopliae* in different pepper types. Biocontrol **64**(1), 77–89 (2019). https://doi.org/10.1007/s10526-018-09916-y
Lu, S.Y., Liao, J.W., Kuo, M.L., Wang, S.C., Hwang, J.S., Ueng, T.H.: Endocrine-disrupting activity in carbendazim-induced reproductive and developmental toxicity in rats. J. Toxicol. Environ. Health A **67**(19), 1501–1515 (2004). https://doi.org/10.1080/15287390490486833
Machenhalli, S.: Fruit Rot of Chilli: It's Diversity, Characterization, Epidemology and Integrated Management. Dissertation, University of Agricultural Sciences, Dharwad (2014)
Majeed, M., Mir, G.H., Hassan, M., Mohuiddin, F.A., Paswal, S., Farooq, S.: Damping off in chilli and its biological management–A Review. Int. J. Curr. Microbiol. Appl. Sci. **7**(04), 2175–2185 (2018)
Majid, M.U., Awan, M.F., Fatima, K., Tahir, M.S., Ali, Q., Rashid, B., Rao, A.Q., Nasir, I.A., Husnain, T.: Phytophthora capsici on chilli pepper (*Capsicum annuum* .) and its management through genetic and bio-control: a review. Zemdirbyste-Agric. **103**(4), 419–430 (2016)
Manjunath, M., Prasanna, R., Nain, L., Dureja, P., Singh, R., Kumar, A., Jaggi, S., Kaushik, B.D.: Biocontrol potential of cyanobacterial metabolites against damping off disease caused by *Pythium aphanidermatum* in solanaceous vegetables. Arch. Phytopatol. Plant Prot. **43**(7), 666–677 (2010). https://doi.org/10.1080/03235400802075815
Mannai, S., Jabnoun-Khiareddine, H., Nasraoui, B., Daami-Remadi, M.: *Rhizoctonia* root rot of pepper (*Capsicum annuum*): comparative pathogenicity of causal agent and biocontrol attempt using fungal and bacterial agents. J .Plant Pathol. Microbiol. **9**(2), 431–439 (2018). https://doi.org/10.4172/2157-7471.1000431
Markovitz, A., Crosby, W.H.: Chemical carcinogenesis: a soil fumigant, 1, 3-dichloropropene, as possible cause of hematologic malignancies. Arch. Intern. Med. **144**(7), 1409–1411 (1984). https://doi.org/10.1001/archinte.1984.00350190097018
McCoy, T., Frank, D.: Organic vs. Conventional (Synthetic) Pesticides: Advantages and Disadvantages. Virginia Cooperative Extension (2020). https://vtechworks.lib.vt.edu/handle/10919/99507 Accessed on 8th May 2020
Mishra, R.K., Singh, A.K., Sharma, A.K.: Diseases of Vegetable Crops and their Integrated Management (A Colour Handbook). New Indian Publishing Agency, New Delhi (2013)
Mishra, R.S., Chauvey, A.N.: Chilli leaf curl virus and its management. Acta Sci. Agric. **2**(3), 24–28 (2018)
Mojica-Marín, V., Luna-Olvera, H.A., Sandoval-Coronado, C.F., Pereyra-Alférez, B., Morales-Ramos, L.H., Hernández-Luna, C.E., Alvarado-Gomez, O.G.: Antagonistic activity of selected strains of *Bacillus thuringiensis* against *Rhizoctonia solani* of chili pepper. Afr. J. Biotechnol. **7**(9), 1271–1276 (2008)
Morinaga, H., Yanase, T., Nomura, M., Okabe, T., Goto, K., Harada, N., Nawata, H.: A benzimidazole fungicide, benomyl, and its metabolite, carbendazim, induce aromatase activity in a human ovarian granulose-like tumor cell line (KGN). Endocrinology **145**(4), 1860–1869 (2004). https://doi.org/10.1210/en.2003-1182
Moser, V.C., Barone, S., Jr., Smialowicz, R.J., Harris, M.W., Davis, B.J., Overstreet, D., Mauney, M., Chapin, R.E.: The effects of perinatal tebuconazole exposure on adult neurological, immunological, and reproductive function in rats. Toxicol. Sci. **62**(2), 339–352 (2001). https://doi.org/10.1093/toxsci/62.2.339
Mullen, J.: Southern blight, southern stem blight, white mold. Plant Health Instructor **10**(1), 104 (2001)
Muthukumar, A., Eswaran, A., Sanjeevkumar, K.: Biological control of Pythium aphanidermatum (Edson.) Fitz. Mysore J. Agric. Sci. **42**, 20–25 (2008)
Nabi, Z., Youssouf, M., Manzoor, J.: Impact of pesticides on aquatic life. In: Wani, K.A., Mamta (eds.) Handbook of Research on the Adverse Effects of Pesticide Pollution in Aquatic Ecosystem, pp. 170–181. IGI Global, Pennsylvania (2019)
Naik, M.K., Savitha, A.S.: Diseases of chilli. In: Singh, D., Chowdappa, P., Sharma, P. (eds.) Diseases of Vegetable Crops: Diagnosis and Management, pp. 143–159. Today and Tomorrow's Printers and Publishers, New Delhi (2014)
Nakkeeran, S., Kavitha, K., Chandrasekar, G., Renukadevi, P., Fernando, W.G.: Induction of plant defence compounds by *Pseudomonas chlororaphis* PA23 and *Bacillus subtilis* BSCBE4 in controlling damping-off of hot pepper caused by *Pythium aphanidermatum*. Biocontrol Sci. Technol. **16**(4), 403–416 (2006). https://doi.org/10.1080/09583150500532196
Nantawanit, N., Chanchaichaovivat, A., Panijpan, B., Ruenwongsa, P.: Induction of defense response against *Colletotrichum capsici* in chili fruit by the yeast *Pichia guilliermondii* strain R13. Biol. Control **52**(2), 145–152 (2010). https://doi.org/10.1016/j.biocontrol.2009.10.011
Nega, A.: Review on concepts in biological control of plant pathogens. J. Biol. Agric. Healthcare **4**(27), 33–54 (2014)
Nguyen, X.H., Naing, K.W., Lee, Y.S., Tindwa, H., Lee, G.H., Jeong, B.K., Ro, H.M., Kim, S.J., Jung, W.J., Kim, K.Y.: Biocontrol potential of *Streptomyces griseus* H7602 against root rot disease (*Phytophthora capsici*) in pepper. Plant Pathol. J. **28**(3), 282–289 (2012). https://doi.org/10.5423/PPJ.OA.03.2012.0040
O'Brien, P.A.: Biological control of plant diseases. Australas. Plant Pathol. **46**(4), 293–304 (2017). https://doi.org/10.1007/s13313-017-0481-4
Oerke, E.: Crop losses to pests. J. Agric. Sci. **144**(1), 31–43 (2006). https://doi.org/10.1017/S0021859605005708
Pal, K.K., Gardener, B.M.: Biological control of plant pathogens. Plant Health Instructor (2006). https://doi.org/10.1094/PHI-A-2006-1117-02Accessedon5thOctober2020
Park, J.W., Balaraju, K., Kim, J.W., Lee, S.W., Park, K.: Systemic resistance and growth promotion of chili pepper induced by an antibiotic producing *Bacillus vallismortis* strain BS07. Biol. Control **65**(2), 246–257 (2013). https://doi.org/10.1016/j.biocontrol.2013.02.002
Perocco, P., Alessandra Santucci, M., Campani, A.G., Forti, G.C.: Toxic and DNA-damaging activities of the fungicides mancozeb and thiram (TMTD) on human lymphocytes *in vitro*. Teratog. Carcinog. Mutagen **9**(2), 75–81 (1989). https://doi.org/10.1002/tcm.1770090203
Perveen, S., Ehteshamul-Haque, S., Ghaffar, A.: Efficacy of *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* in the control of root rot-root knot disease complex on some vegetables. Nematol. Mediterr. **26**, 209–212 (1998)
Peshama, M.H., Dadke, M.S., Dandnaik, B.P., Ahamed, B.Z.: Proving pathogencity of *Leveillula taurica* causing powdery mildew of Chilli. Int. J. Curr. Microbiol. Appl. Sci **6**(12), 1855–1858 (2017)
Peter, K.V., Hazra, P.: Handbook of Vegetables. Studium Press LLC, Texas (2012)
Pimentel, D.: Amounts of pesticides reaching target pests: environmental impacts and ethics. J. Agric. Environ. Ethics **8**, 17–29 (1995). https://doi.org/10.1007/BF02286399
Pourbabaee, A.A., Bahmani, E., Alikhani, H.A., Emami, S.: Promotion of wheat growth under salt stress by halotolerant bacteria containing ACC deaminase. J. Agric. Sci. Technol. **18**, 855–864 (2016)
Pruett, S.B., Myers, L.P., Keil, D.E.: Toxicology of metam sodium. J. Toxicol. Environ. Health B **4**(2), 207–222 (2001). https://doi.org/10.1080/10937400117071

Author's personal copy

Putri, R., Sri, S., Triwidodo, A.: Effectiveness of rhizobacteria *Streptomyces* sp to suppress Pepper yellow leaf curl virus on chili in the field. J. Fitopatol. Indonesia (JFI) **14**(5), 183–188 (2019)

Rabinowitch, H.D., Friedmann, M., Ben-David, B.: Sunscald damage in attached and detached pepper and cucumber fruits at various stages of maturity. Sci. Hortic. **19**(1–2), 9–18 (1983). https://doi.org/10.1016/0304-4238(83)90038-9

Rane, M.R., Sarode, P.D., Chaudhari, B.L., Chincholkar, S.B.: Foliar application of *Pseudomonas* metabolite protects *Capsicum annum* (chilli) from fungal phytopathogens. Bionano Front. **1**, 46–53 (2007)

Reddy, P.P.: Advances in Integrated Pest and Disease Management in Horticulture Crops Vol 2 Vegetable Crops. Studium Press, New Delhi (2009)

Rekanovic, E., Milijasevic, S., Todorovic, B., Potocnik, I.: Possibilities of biological and chemical control of *Verticillium* wilt in pepper. Phytoparasitica **35**(5), 436 (2007). https://doi.org/10.1007/BF03020601

Ristaino, J.B., Thomas, W.: Agriculture, methyl bromide, and the ozone hole: can we fill the gaps? Plant Dis/ **81**(9), 964–977 (1997). https://doi.org/10.1094/PDIS.1997.81.9.964

Roach, R., Mann, R., Gambley, C.G., Shivas, R.G., Rodoni, B.: Identification of *Xanthomonas* species associated with bacterial leaf spot of tomato, capsicum and chilli crops in eastern Australia. Eur. J. Plant Pathol. **150**(3), 595–608 (2018). https://doi.org/10.1007/s10658-017-1303-9

Ruangwong, O.U., Pornsuriya, C., Pitija, K., Sunpapao, A.: Biocontrol mechanisms of *Trichoderma koningiopsis* PSU3-2 against postharvest anthracnose of chili pepper. J. Fungi **7**(4), 276 (2021). https://doi.org/10.3390/jof7040276

Saharan, G.S., Mehta, N.: Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management. Springer, Berlin/Heidelberg (2008)

Sakr, S.A., Mahran, H.A., Abdel-Maksoud, A.M.: Suppressive effect of *Ginkgo biloba* extract (EGb 761) on topsin induced ovarian toxicity and oxidative stress in albino rats. J. Appl. Pharm. Sci. **1**(4), 46 (2011)

Sancho, E., Villarroel, M.J., Fernández, C., Andreu, E., Ferrando, M.D.: Short-term exposure to sublethal tebuconazole induces physiological impairment in male zebrafish (*Danio rerio*). Ecotoxicol. Environ. Saf. **73**(3), 370–376 (2010). https://doi.org/10.1016/j.ecoenv.2009.09.020

Sandani, H.B., Ranathunge, N.P., Lakshman, P.L., Weerakoon, W.M.: Biocontrol potential of five *Burkholderia* and *Pseudomonas* strains against *Colletotrichum truncatum* infecting chilli pepper. Biocontrol. Sci. Technol. **29**(8), 727–745 (2019). https://doi.org/10.1080/09583157.2019.1597331

Sanogo, S.: Chile pepper and the threat of wilt diseases. Plant Health Prog. **4**(1), 23 (2003). https://doi.org/10.1094/PHP-2003-0430-01-RV

Sasirekha, B., Srividya, S.: Siderophore production by *Pseudomonas aeruginosa* FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporioides* causing diseases in chilli. Agric. Nat. Resour. **50**(4), 250–256 (2016). https://doi.org/10.1016/j.anres.2016.02.003

Shahid, A.A., Umer, M., Ali, M., Haq, I.M.: Screening and application of rhizobacterial isolates as biological control agent against charcoal rot of chillies. Sci. Int. (Lahore) **28**(2), 1243–1251 (2016)

Shrestha, A., Kim, B.S., Park, D.H.: Biological control of bacterial spot disease and plant growth-promoting effects of lactic acid bacteria on pepper. Biocontrol Sci. Technol. **24**(7), 763–779 (2014). https://doi.org/10.1080/09583157.2014.894495

Sid, A., Ezziyyani, M., Egea-Gilabert, C., Candela, M.E.: Selecting bacterial strains for use in the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternata* in sweet pepper plants. Biol. Plant **47**(4), 569–574 (2003). https://doi.org/10.1023/B:BIOP.0000041063.38176.4a

Sid Ahmed, A., Pérez-Sánchez, C., Egea, C., Candela, M.E.: Evaluation of Trichoderma harzianum for controlling root rot caused by *Phytophthora capsici* in pepper plants. Plant Pathol. **48**(1), 58–65 (1999). https://doi.org/10.1046/j.1365-3059.1999.00317.x

Smith, C.J., Perfetti, T.A.: A comparison of the persistence, toxicity, and exposure to high-volume natural plant-derived and synthetic pesticides. Toxicol. Res. Appl. (2020). https://doi.org/10.1177/2397847320940561

Srikhong, P., Lertmongkonthum, K., Sowanpreecha, R., Rerngsamran, P.: *Bacillus* sp. strain M10 as a potential biocontrol agent protecting chili pepper and tomato fruits from anthracnose disease caused by *Colletotrichum capsici*. BioControl **63**(6), 833–842 (2018). https://doi.org/10.1007/s10526-018-9902-8

Stommel, J.R., Kozlov, M., Griesbach, R.J.: Ornamental pepper (*Capsicum annuum* L.) cultivars comprising the christmas lights cultivar series. HortScience **53**(3), 391–394 (2018). https://doi.org/10.21273/HORTSCI12574-17

Stoytcheva, M.: Pesticides in the Modern World: Trends in Pesticides Analysis . BoD–Books on Demand, Norderstedt (2011)

Subrahmanyam, G., Kumar, A., Sandilya, S.P., Chutia, M., Yadav, A.N.: Diversity, plant growth promoting attributes, and agricultural applications. In: Yadav, A., Singh, J., Rastegari, A., Yadav, N. (eds.) Plant Microbiomes for Sustainable Agriculture, pp. 1–51. Springer, Basingstoke (2020)

Sukarno, N., Smith, F.A., Scott, E.S., Jones, G.P., Smith, S.E.: The effect of fungicides on vesicular–arbuscular mycorrhizal symbiosis. III. The influence of VA mycorrhiza on phytotoxic effects following application of fosetyl-Al and phosphonate. New Phytol. **139**(2), 321–330 (1998). https://doi.org/10.1046/j.1469-8137.1998.00204.x

Sundaramoorthy, S., Raguchander, T., Ragupathi, N., Samiyappan, R.: Combinatorial effect of endophytic and plant growth promoting rhizobacteria against wilt disease of *Capsicum annum* L. caused by *Fusarium solani*. Biol. Control **60**(1), 59–67 (2012). https://doi.org/10.1016/j.biocontrol.2011.10.002

Suprapta, D.N., Darmadi, A.A., Khalimi, K.: Potential antagonistic rhizobacteria to control *Colletotrichum scovillei*, the cause of anthracnose disease in chili pepper. Biodivers. J. Biol. Divers. **4**, 6 (2020). https://doi.org/10.13057/biodiv/d210648

Surepeddi, S.K., Giridhar, K.: Chilli. In: Chadha, K.L., Pal, R.K. (eds.) Vegetables, flowers and plantation crops. Vol. 3. Managing Post-harvest Quality and Losses in Horticulture Crops, pp. 597–631. Daya Publishing House, New Delhi (2015)

Suryanto, D., Patonah, S., Munir, E.: Control of *Fusarium* wilt of chili with chitinolytic bacteria. HAYATI J. Biosci. **17**(1), 5–8 (2010). https://doi.org/10.4308/hjb.17.1.5

Tahat, M.M., Sijam, K.: *Ralstoina solanacearum*: The bacterial wilt causal agent. Asian J. Plant Sci. **9**(7), 385 (2010)

Tahir, Y.F., Nour, S.M.: Oral toxicity of agro-fungicides: Tilt (propiconazole), bayleton (triadimefon) and their mixture to Nubian goats. Sudan J. Med. Sci. **4**(3), 213–220 (2009). https://doi.org/10.4314/sjms.v4i3.48311

Taylor, M.D., Locascio, S.J.: Blossom-end rot: a calcium deficiency. J. Plant Nutr. **27**(1), 123–139 (2004). https://doi.org/10.1081/PLN-120027551

Than, P.P., Prihastuti, H., Phoulivong, S., Taylor, P.W., Hyde, K.D.: Chilli anthracnose disease caused by *Colletotrichum* species. J. Zhejiang Univ. Sci. B **9**(10), 764–778 (2008). https://doi.org/10.1631/jzus.B0860007

Thomashow, L.S., Bonsall, R.F., Weller, D.M.: Antibiotic production by soil and rhizosphere microbes in situ. In: Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D., Walter, M.V. (eds.) Manual of Environmental Microbiology, pp. 493–499. ASM Press, Washington (1997)

Author's personal copy

Tomah, A.A., Abd Alamer, I.S., Li, B., Zhang, J.Z.: A new species of Trichoderma and gliotoxin role: a new observation in enhancing biocontrol potential of *T. virens* against *Phytophthora capsici* on chili pepper. Biol. Control (2020). https://doi.org/10.1016/j.biocontrol.2020.104261

Ulloa-Ogaz, A.L., Muñoz-Castellanos, L.N., Nevárez-Moorillón, G.V.: Biocontrol of phytopathogens: antibiotic production as mechanism of control. In: Méndez-Vilas, A. (ed.) The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs, pp. 305–309. Formatex, Spain (2015)

van Wijngaarden, R.P., Belgers, D.J., Zafar, M.I., Matser, A.M., Boerwinkel, M.C., Arts, G.H.: Chronic aquatic effect assessment for the fungicide azoxystrobin. Environ. Toxicol. Chem. **33**(12), 2775–2785 (2014). https://doi.org/10.1002/etc.2739

Vurukonda, S.S., Giovanardi, D., Stefni, E.: Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. Int. J. Mol. Sci. **19**(4), 952 (2018). https://doi.org/10.3390/ijms19040952

Wang, Y., Xu, L., Li, D., Teng, M., Zhang, R., Zhou, Z., Zhu, W.: Enantioselective bioaccumulation of hexaconazole and its toxic effects in adult zebrafish (*Danio rerio*). Chemosphere **138**, 798–805 (2015). https://doi.org/10.1016/j.chemosphere.2015.08.015

Wani, F.A., Najar, A.G.: Integrated Management of Fusarium Wilt [*Fusarium pallidoroseum* (Cooke) Sacc.] in Chilli (*Capsicum annum* L.). SKUAST J. Res. **14**(1–2), 40–45 (2012)

Whipps, J.M.: Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. **52**(1), 487–511 (2001). https://doi.org/10.1093/jexbot/52.suppl_1.487

Wu, Z., Huang, Y., Li, Y., Dong, J., Liu, X., Li, C.: Biocontrol of Rhizoctonia solani via induction of the defense mechanism and antimicrobial compounds produced by *Bacillus subtilis* SL-44 on Pepper (*Capsicum annuum* L). Front. Microbiol. (2019). https://doi.org/10.3389/fmicb.2019.02676

Yadav, M., Dubey, M.K., Upadhyay, R.S.: Systemic Resistance in Chilli Pepper against Anthracnose (Caused by *Colletotrichum truncatum*) induced by *Trichoderma harzianum*, *Trichoderma asperellum* and *Paenibacillus dendritiformis*. J. Fungi **7**(4), 307 (2021). https://doi.org/10.3390/jof7040307

Yang, Y., Qi, S., Chen, J., Liu, Y., Teng, M., Wang, C.: Toxic effects of bromothalonil and flutolanil on multiple developmental stages in zebrafish. Bull. Environ. Contam. Toxicol. **97**(1), 91–97 (2016). https://doi.org/10.1007/s00128-016-1833-4

Yilmaz, S., Aksoy, H., Ünal, F.A., Celik, M., Yüzbaşıoğlu, D.: Genotoxic action of fungicide Conan 5FL (hexaconazole) on mammalian cells *in vivo* and *in vitro*. Russ. J. Genet. **44**(3), 273–278 (2008). https://doi.org/10.1134/S1022795408030058

Yu, L., Chen, M., Liu, Y., Gui, W., Zhu, G.: Thyroid endocrine disruption in zebrafish larvae following exposure to hexaconazole and tebuconazole. Aquat. Toxicol. **138**, 35–42 (2013). https://doi.org/10.1016/j.aquatox.2013.04.001

Yu, X., Ai, C., Xin, L., Zhou, G.: The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. Eur. J. Soil Biol. **47**(2), 138–145 (2011). https://doi.org/10.1016/j.ejsobi.2010.11.001

Salt-pan bacteria as potential plant growth promoters and their antagonistic activity to fungal pathogens of *Capsicum annuum* L.

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Chilli, an essential condiment worldwide, is generally cultivated in paddy fields and can be infected by fungal pathogens, thus hampering its yield. Due to increasing soil salinization, the efficacy of many biocontrol agents is poor in the fields. In this study, bacteria (about 196) isolated from the Goan salt pans in India were screened for their antifungal activity against *Fusarium oxysporum*, *Fusarium pallidoroseum*, *Rhizoctonia solani* and *Pythium aphanidermatum*. Halotolerant isolates of *Bacillus tequilensis*, *Bacillus subtilis* subsp. *inaquosorum*, *Bacillus cabrialesii*, *Bacillus licheniformis*, *Bacillus paralicheniformis* and *Brevibacterium antiquum* could grow under a wide range of pH, temperature and NaCl concentrations, and also displayed plant growth-promoting attributes.

Keywords: Antagonistic activity, chilli, fungal pathogens, plant growth promotion, salt-pan bacteria.

INDIA is the largest producer of chilli (*Capsicum annuum*; about 1.7 million tonnes) in the world, followed by Thailand and mainland China[1]. Because of its colour, flavour and pungency, chilli, also known as pepper, has become an essential component of Indian cuisine. The phytochemicals of pepper have a remarkable potential in the history of new bioactive compounds and natural ingredients for agro-food, cosmetic and pharma industry[2]. However, every year, a considerable loss in the yield of chilli is observed due to various factors[3].

The major disease is damping off, caused by the genus *Pythium*, affecting up to 90% of the chilli crop[4]. Likewise, the wilt causing *Fusarium* species not only reduces the growth but also affects the fruit quality and causes about 10–80% yield loss in the total global production of chilli[5]. Another root rot-causing saprophytic pathogen, *Rhizoctonia solani* causes up to 33.2% yield loss due to disease incidence at the seedling stage under greenhouse conditions and about 40.2% in the main field[6]. Biocontrol measures are desirable to control these phytopathogens to boost the economy of chilli cultivation.

Biocontrol inoculants based on naturally occurring antagonists are environmental-friendly and effective agents against many soil-borne pathogens[7]. Additional properties like salt tolerance and plant growth promotion can improve their potential application even in saline soils. Among the abiotic factors, soil salinization is the most detrimental and is considered one of the significant limiting factors of agricultural productivity and food security[8]. Worldwide, about 20% of the agricultural land is inundated with salt water, and this is continuously increasing[9]. Halophilic and halotolerant microorganisms from solar salt pans are known to produce several secondary metabolites which can be exploited for various applications[10]. The present study aims to evaluate the potential of halotolerant salt-pan bacteria as plant growth promoters and inhibitors of fungal pathogens affecting chilli crops in saline soils.

Materials and methods

Sample collection and isolation of bacteria from salt pans

Isolation of bacteria was carried out on different strengths of Zobell marine (ZM) agar from water, sediment and biofilm samples from three different salt pans (Ribandar, Batim and Agarwado) in Goa, India. Various physico-chemical properties of these samples, like salinity, temperature, pH, dissolved oxygen, conductivity and total dissolved solids, were assessed[11].

In-vitro antifungal test

Fungal phytopathogens *Fusarium oxysporum* (8302), *Fusarium pallidoroseum* (7890), *Rhizoctonia solani* (5338) and *Pythium aphanidermatum* (4746) were procured from the Indian Type Culture Collection (ITCC), New Delhi. The dual culture assay was carried out to estimate the inhibition percentage (L) of fungal mycelia by the bacterial isolates using the formula $L = [(C - T)/C] \times 100$, where C is the mycelial inhibition in control and T is mycelial inhibition in test[12].

In-vitro screening of salt-pan bacterial isolates for plant growth promoting activity

Isolates with significant antifungal activity (>49%) against all four fungi were screened for plant growth-promoting

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(PGP) activity. Antagonistic bacteria (7) were tested for the production of ammonia in peptone water, siderophore on ChromauzoralS agar and hydrogen cyanide (HCN) on ZM agar amended with glycine[13]. To test indole acetic acid (IAA) and exopolysaccharide (EPS) production, the ZM medium was supplemented with tryptophan, sucrose and Congo red respectively[14]. Production of cell wall-degrading enzymes chitinase, glucanase, amylase, protease, cellulase and lipase was determined by plate assay using 0.2% colloidal chitin, 0.2% laminarin, 0.2% starch, 1% skimmed milk, 1% carboxymethylcellulose and 1% tributyrin as substrate respectively[15]. Isolates were spot-inoculated on Jensen's agar plates to determine the nitrogen-fixing ability and on Dorwin–Fostwer medium supplemented with 1-aminocyclopropane-1-carboxylate (ACC) for testing the ACC deaminase activity[14,16]. Solubilization of insoluble phosphate, zinc, potassium and silicate by the bacterial isolates was determined by calculating the solubilization index of each bacterial isolate on Pikovskaya's, zinc solubilizing, Aleksandrow and modified Bunt and Rovira medium agar plates respectively[13,17,18].

Safety evaluation

The safety of the bacterial isolates was assessed by determining their hemolytic activity with plates containing 5% sterile human blood in agar base and checking the sensitivity of the isolates towards various antibiotics[19]. The antibiotics tested were amikacin, amoxyclav, ampicillin : sulbactam, cephalexin, cephalothin, cephotaxime, chloramphenicol, ciprofloxacin, clindamycin, co-trimoxazole, doxycycline hydrochloride, erythromycin, gentamycin, kanamycin, levofloxacin, lincomycin, nalidixic acid, neomycin, nitrofurantoin, ofloxacin, streptomycin, tetracyclin, tobramycin and vancomycin. The isolates were categorized as sensitive (S), intermediate (I) or resistant (R), as recommended by the Clinical and Laboratory Standards Institute, Pennsylvania, USA, depending on the zone of inhibition[20].

Phenotypic and biochemical characterization

For preliminary phenotypic identification, test strains were Gram-stained and checked for sulphur and nitrate reduction, indole and enzyme (oxidase, catalase, gelatinase and urease) production, citrate and *O*-nitrophenylbeta-D-galactopyranoside (ONPG) utilization and methyl red–Voges Proskauer (MR–VP) test. The utilization of various carbohydrates by bacterial isolates was checked using KB009 HiCarbo™ kits and the results were interpreted according to the manufacturer's instructions. The ability of isolates to grow and replicate in the soil was validated by streaking them on soil extract agar; the oxygen requirement was assessed by growing the isolates in an anaerobic agar medium. Tolerance of the isolates to a broad range of temperature (0–65°C), pH (2.0–14.0) and NaCl concentration (0–20% (w/v)) was also assessed.

SEM analysis and molecular characterization

Cells of 24-h-old bacterial isolates were fixed overnight in 1.5% glutaraldehyde onto glass slides, followed by washing in phosphate buffer and dehydration using ethanol (10–100% v/v concentration). Samples were then mounted on SEM stubs, Sparta-coated and photomicrographs were captured at 15K magnification. Further, the genomic DNA was isolated and the 16S rRNA gene of each isolate was amplified using universal bacterial primers 27F: AGAGTTTGATCCTGGCTCCAG and 1492R: TACGGTTACCTTGTTACGACTT. The PCR product was then sequenced and matched with the GenBank database using NCBI-BLAST.

Statistical analysis

Experimental data were analysed using SPSS software (version 2020). Standard errors were calculated for all mean values. Post-hoc analysis was carried out using Duncan's multiple range test (DMRT) and means were considered significant at the $P \leq 0.05$ level.

Results

Isolation of bacteria from salt pans

A total of 196 bacterial isolates were obtained from the three salt pans of Goa. The physico-chemical properties and number of isolates obtained from each sampling site were compiled (Supplementary Table 1 and Figure 1). The bacterial isolates obtained from water samples (55), sediment samples (48) and biofilm samples (93) were stored on ZM agar for further analysis.

In-vitro antifungal assay

Antifungal dual culture assay revealed that 21 out of 196 bacterial isolates had an inhibitory effect on the mycelia of the fungal pathogens tested (Table 1). The isolates MPSK14, MPSK22, MPSK23, MPSK28, MPSK186, MPSK109 and SK473 showed >49% mycelial inhibition, while MPSK109 showed the highest inhibition against *R. solani* (65.9%), *P. aphanidermatum* (56%), *F. oxysporum* (60.5%) and *F. pallidoroseum* (55%) (Figure 1).

In-vitro screening of bacterial isolates for plant growth promoting activity

Table 2 shows the results of the PGP traits of the selected bacterial isolates. All seven strains tested positive for the

Table 1. Antagonistic activity of salt-pan bacteria against chilli plant pathogens

Isolate	Inhibition (%)			
	Rhizoctonia solani	*Pythium aphanidermatum*	*Fusarium oxysporum*	*Fusarium pallidoroseum*
MPSK109	65.9 ± 1.2^{k}	56 ± 1.5^{j}	60.5 ± 1.5^{j}	55 ± 1.6^{fg}
ABSK11	37.4 ± 1.8^{b}	45 ± 1.3^{b}	47.4 ± 1.2^{ef}	40 ± 1.5^{c}
ABSK171	37.6 ± 0.5^{b}	48 ± 1.4^{cde}	47.4 ± 1.2^{ef}	34.5 ± 0.5^{ab}
MPSK186	56.7 ± 1.3^{h}	51 ± 1.8^{fgh}	55.3 ± 2.1^{i}	56 ± 0.6^{g}
ABSK9	50 ± 1.7^{ef}	49 ± 0.7^{def}	47.4 ± 1.8^{ef}	40 ± 0.5^{c}
BGUM136	47.2 ± 2.2^{cd}	46.9 ± 2.3^{bcd}	42.1 ± 0.8^{d}	32 ± 1.6^{a}
MPSK14	55.5 ± 1.9^{h}	53 ± 1.5^{hi}	52.6 ± 1.6^{ghi}	51 ± 2.1^{de}
BGUM14B	54.3 ± 1.6^{gh}	52.2 ± 1.3^{ghi}	47.4 ± 1.8^{ef}	49 ± 0.3^{d}
BGUM256	47.6 ± 1.3^{de}	44.4 ± 2.5^{b}	47.4 ± 2^{ef}	32 ± 2.2^{a}
BGUM359	44.5 ± 0.5^{c}	45 ± 0.5^{b}	42.1 ± 1.3^{d}	32 ± 1.3^{a}
BGUM370	36 ± 0.5^{b}	45.5 ± 1.5^{bc}	47.4 ± 0.9^{ef}	40 ± 0.7^{c}
BGUM440	45 ± 2.2^{cd}	44.4 ± 2^{b}	34.2 ± 1.7^{b}	36 ± 0.6^{b}
SK473	61.6 ± 2.3^{ij}	54 ± 1.5^{ij}	54.2 ± 1.8^{hi}	60 ± 0.5^{h}
BGUM93	47 ± 1.3^{cd}	44 ± 1.9^{b}	42.1 ± 1.3^{d}	40 ± 1.5^{c}
MPSK20	51 ± 1.1^{f}	50 ± 1.7^{efg}	45 ± 1.5^{e}	50 ± 1.3^{de}
MPSK22	51.4 ± 1.8^{ef}	56 ± 1.2^{j}	52 ± 2.1^{gh}	54.2 ± 1.9^{fg}
MPSK23	63.2 ± 0.9^{j}	61 ± 1.1^{k}	54.2 ± 1.7^{hi}	56.3 ± 1.9^{g}
MPSK51	52.5 ± 0.6^{fg}	51 ± 0.5^{fgh}	39 ± 0.5^{c}	34.2 ± 1.1^{ab}
MPSK28	52.4 ± 1.2^{fg}	49.7 ± 1.8^{efg}	50 ± 0.7^{fg}	49.6 ± 2.2^{de}
MPSK6	37 ± 1.1^{b}	40 ± 0.5^{a}	32 ± 1^{b}	34.2 ± 0.9^{ab}
MPSK8	29.5 ± 1.8^{a}	40 ± 1.5^{a}	29 ± 2.2^{a}	35.4 ± 1.6^{b}

Values are mean ± standard deviation of three independent experiments. Means followed by the same letter within columns are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 2. *In-vitro* characterization of salt-pan bacterial isolates for plant growth promotion attributes and hydrolytic enzymes

Isolate	MPSK22	MPSK23	MPSK186	MPSK109	MPSK14	SK473	MPSK28
IAA	Moderate	Moderate	Moderate	Low	High	High	Moderate
Siderophore	No	No	Low	Low	No	No	No
HCN	No	No	No	No	No	No	No
Ammonia	High	Moderate	High	Low	Low	Moderate	Moderate
Amylase	Moderate	Low	Moderate	High	Moderate	High	No
Protease	Moderate	Moderate	High	High	Moderate	Low	No
Lipase	Low	Moderate	High	High	High	High	No
Chitinase	Moderate	Moderate	Moderate	No	No	No	No
β-Glucanase	Moderate	Low	High	No	No	No	No
Cellulase	Low	Low	Moderate	High	High	High	No
N fixation	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Zinc	Low	High	Moderate	Low	Low	Low	No
Silicon	No	No	No	No	No	No	No
Potassium	No	No	No	No	No	No	No
Phosphate	No	No	No	No	No	No	No
EPS	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	No
ACC deaminase	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate

Scale: No activity, Low activity, Moderate activity, High activity

production of IAA, ammonia and ACC deaminase, and were able to grow on nitrogen-free media. Except for MPSK28, all the isolates produced EPS and amylase, lipase, protease and cellulase enzymes. These cultures were also able to solubilize zinc in the media. None of the isolates exhibited the production of HCN or solubilization of silicon, potassium and phosphate. Only two isolates, viz. MPSK186 and MPSK109 produced siderophores and three isolates, viz. MPSK22, MPSK23 and MPSK186 utilized colloidal chitin and laminarin.

Safety evaluation

All seven isolates exhibited γ-hemolysis on human blood agar, indicating negative hemolysin production against human blood cells (Figure 2). The sensitivity of the seven isolates to 24 different antibiotics and the interpretive criteria are given in Supplementary Table 2. The results indicate the susceptibility of the isolates to >50% of the antibiotics tested.

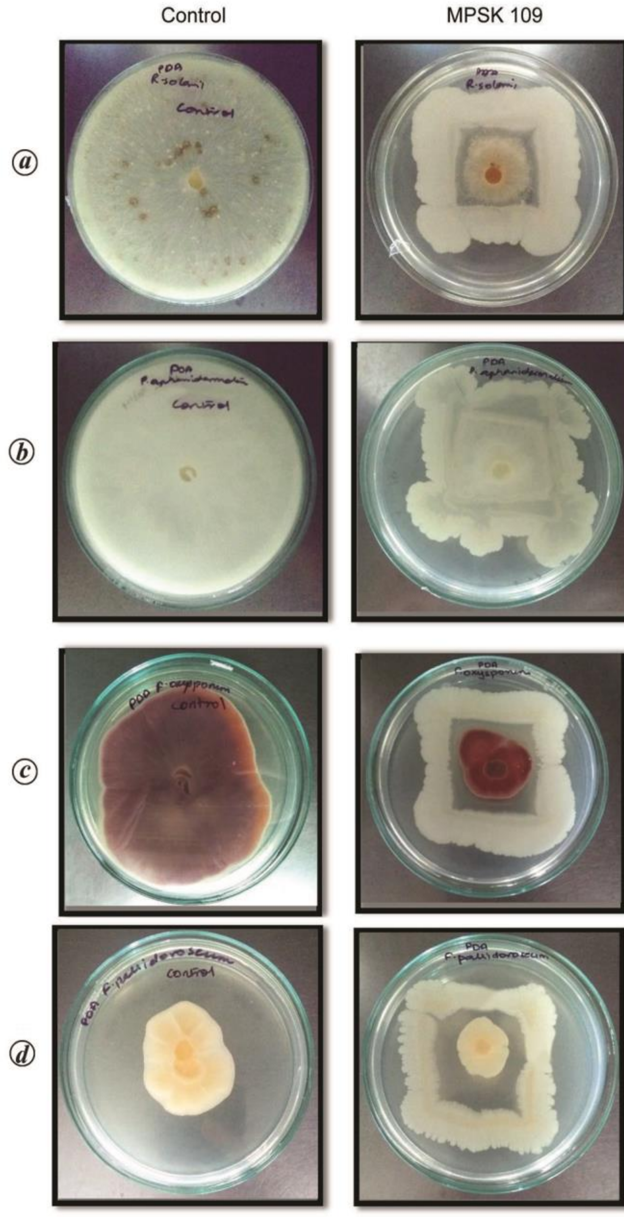

Figure 1. Antagonism exhibited by *Bacillus cabrialesii* strain MPSK 109 against fungal phytopathogens. ***a***, *Rhizoctonia solani*; ***b***, *Pythium aphanidermatum*; ***c***, *Fusarium oxysporum*; ***d***, *Fusarium pallidoroseum*.

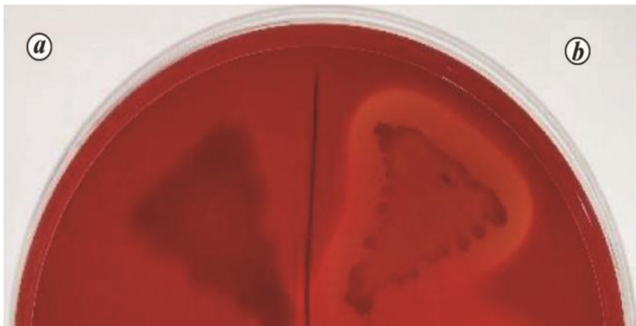

Figure 2. ***a***, γ-Hemolysis of human blood cells by MPSK 28. ***b***, β-Hemolysis of human blood cells by *Vibrio parahaemolyticus*.

Phenotypic and biochemical characterization

In the sulphur, indole, motility (SIM) medium, all the isolates except MPSK28 exhibited diffused growth suggesting motility; however, all isolates tested negative for sulphur reduction. The biochemical tests revealed that all the bacterial isolates were positive for oxidase and catalase production and negative for gelatinase and indole production. Only two isolates, viz. MPSK22 and MPSK23 showed the production of urease enzyme; nitrate reduction was observed in all the isolates except MPSK28. MPSK14, MPSK22, MPSK23 and SK473 utilized ONPG, whereas MPSK14, MPSK22, MPSK23, MPSK109 and MPSK186 utilized citrate. It was also observed that except for MPSK28, all the isolates showed a positive VP reaction and MPSK186 solely showed a positive MR reaction. Based on the Gram-staining and the above results, isolates MPSK14, MPSK22, MPSK23, MPSK109, MPSK186 and SK473 were inferred to belong to the genus *Bacillus*, whereas MPSK28 to the genus *Brevibacterium*. The results showed that all the isolates were halotolerant and could tolerate pH values up to 12. MPSK22, MPSK23 and MPSK28 grew in the media with up to 17% NaCl and MPSK14, MPSK109, MPSK186 and SK473 could tolerate up to 15% of NaCl. The minimum temperature which supported the growth of all the isolates was 15°C; however, the maximum temperature was 30°C for MPSK28, 50°C for MPSK22 and MPSK23, and 60°C for the remaining isolates. Further, MPSK22 and MPSK23 were determined to be facultative aerobes, whereas the remaining isolates were obligate aerobes. The ability of all these isolates to replicate in the soil was confirmed by the growth observed on the soil extract agar. The utilization pattern of 31 different carbohydrates by the seven halotolerant isolates is given in Supplementary Table 3.

SEM analysis and molecular characterization

Scanning electron microscope images confirmed that all the isolates except MPSK28 were long rods, with dimensions ranging from 2.056 μm × 715.3 nm to 2.563 μm × 889.5 nm (Figure 3). Cells of MPSK28 appeared as joint short rods with two cells orienting to give a V-shape, which is typical of the genus *Brevibacterium*. Molecular sequencing of MPSK22 and MPSK23 showed similarity to *Bacillus licheniformis* and *Bacillus paralicheniformis* respectively. Similarly, MPSK186 showed similarity to *Bacillus subtilis* subsp. *inaquosorum* and MPSK109 showed similarity to *Bacillus cabrialessi*. Both MPSK14 and SK473 showed high similarity to *Bacillus tequilensis*, and MPSK28 showed high similarity to *Brevibacterium antiquum*. The partial 16S rRNA gene sequences of the antagonistic strains were deposited at NCBI GenBank and the accession numbers were obtained (Figure 4).

Discussion

Several *Bacillus* spp. have been reported as potential biocontrol agents (BCAs) against phytopathogens affecting

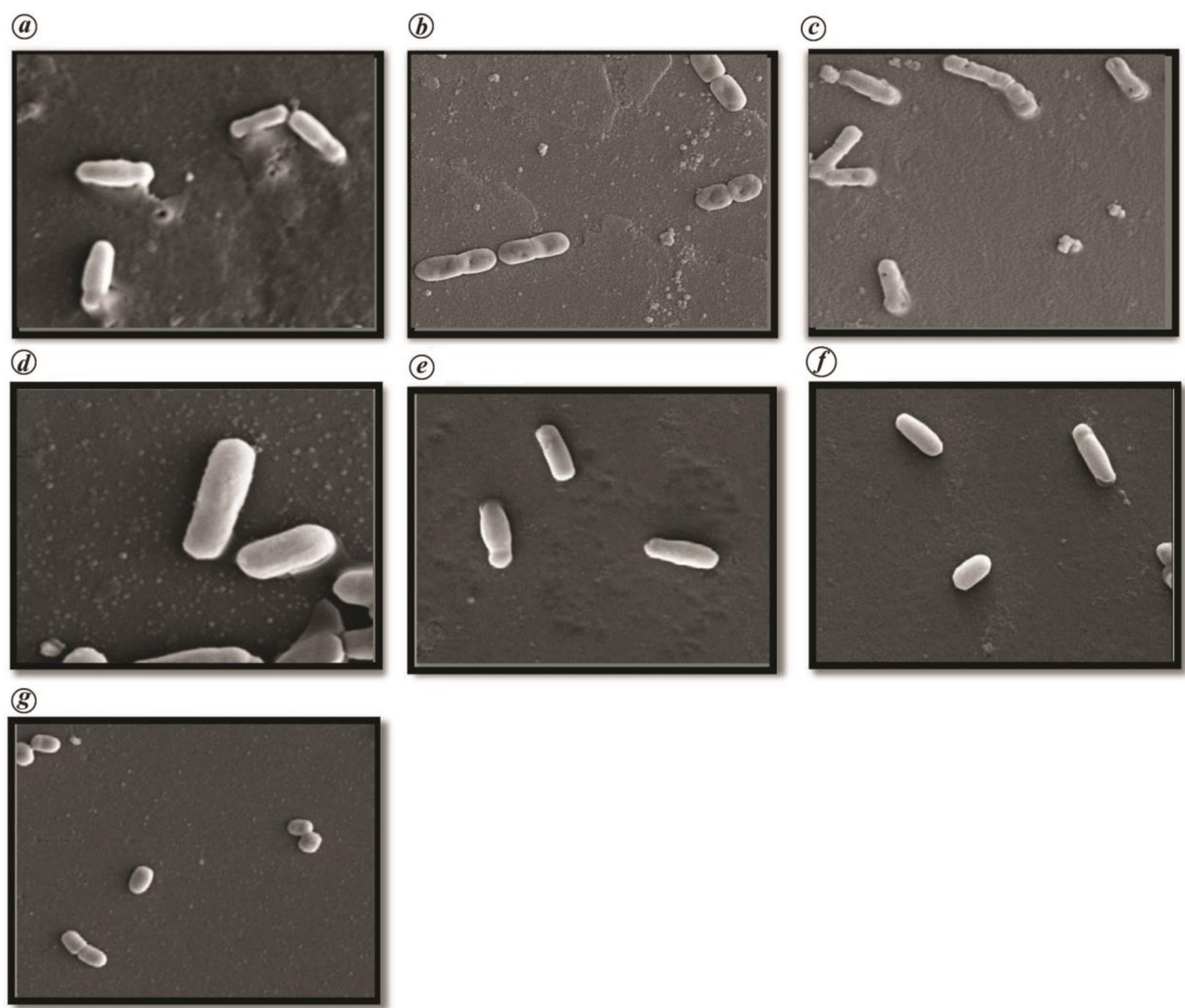

Figure 3. Scanning electron photomicrographs showing individual cells of (***a***) MPSK 22, (***b***) MPSK 23, (***c***) MPSK 186, (***d***) MPSK 109, (***e***) MPSK 14, (***f***) SK 473, (***g***) MPSK 28.

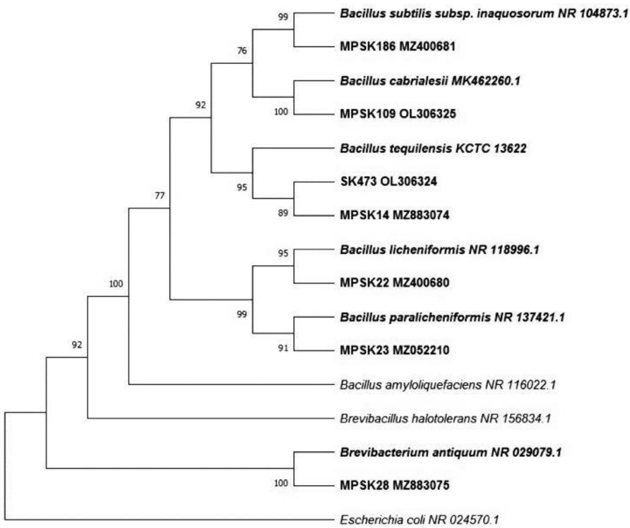

Figure 4. Phylogenetic tree of partial 16S rDNA sequences of halotolerant strains with those of maximum similar entries of type strains from NCBI nucleotide database. The tree was made maximum likelihood method with 1000 bootstrap resamplings.

chilli plants. However, no studies have reported the application of salt-pan bacteria as BCAs against fungal pathogens *R. solani*, *P. aphanidermatum*, *F. oxysporum* and *F. pallidoroseum*[3]. In this study, we explored halotolerant *Brevibacterium* and *Bacillus* strains from different salt pans of Goa (Batim, Ribandar and Agarwada) for their antagonistic activity against fungal pathogens. The isolates of *B. tequilensis* (MPSK14 and SK473), *B. licheniformis* (MPSK22), *B. paralicheniformis* (MPSK23), *B. antiquum* (MPSK28), *B. subtilis* subsp. *inaquosorum* (MPSK186) and *B. cabrialessi* (MPSK109) were all able to grow on soil extract agar and exhibited tolerance towards a wide range of temperature (15–60°C), pH (5–12) and NaCl concentration (0–17%), thus demonstrating their ability to survive and proliferate in the varying dynamics of the soil. These isolates did not haemolyse human erythrocytes *in-vitro*, proving safe for *in-vivo* applications[20]. Further, the results of the antibiotic susceptibility test showed their strong susceptibility to tetracycline, a broad-spectrum antibiotic which could be used in case of an emergency.

Bacillus tequilensis as a BCA has been reported previously[21–23]. In the present study, we show the antagonistic activity of salt-pan *B. tequilensis* (MPSK14 and SK473) against *F. pallidoroseum.* Previous studies have also proved the antifungal activity of *B. paralicheniformis* against *F. oxysporum* and *R. solani*[24,25]. However, to our knowledge, no previous studies on salt-pan *B. paralicheniformis* (MPSK23) were found to be antagonistic to *F. pallidoroseum* and *P. aphanidermatum*. The present study shows the potential of a halotolerant *B. subtilis* subsp. *inaquosorum* (MPSK186) as a BCA against *F. pallidoroseum*, *P. aphanidermatum* and *R. solani*. Another interesting finding of this study is the antifungal activity exhibited by the salt-pan *B. cabrialesii* (MPSK109) against *P. aphanidermatum*, *F. oxysporum* and *F. pallidoroseum.* Although previous studies have reported antifungal activity of *B. cabrialesii* against *Bipolaris sorokiniana* affecting wheat and *Botrytis cinerea*, *Rhizoctonia solani*, *Verticillium dahlia* and *Phytophthora infestans* affecting tomato[26,27], no report is available on its activity against the pathogens tested in the present study. In the case of *B. antiquum*, there is a report on its antagonistic activity against *Macrophomina phaseolina*, the causal agent of charcoal rot[28]. The present study demonstrates the biocontrol ability of *B. antiquum* (MPSK 28) against other disease-causing phytopathogens.

These salt-pan isolates exhibited several PGP activities which have not been reported previously. *B. licheniformis* strain MPSK22, *B. paralicheniformis* strain MPSK23 and *B. cabrialesii* strain MPSK109 showed solubilization of insoluble zinc from the medium. Micronutrients such as Zn, Fe and Mn are deficient in most soils[29]. Zn solubilization by microorganisms in this context proves to be beneficial and economical in agricultural applications. *B. paralicheniformis* strain MPSK23 also exhibited ACC deaminase activity, and production of cell wall-degrading chitinase and glucanase. Various bacterial isolates have been shown to excrete chitinases and glucanases to digest chitin and β-glucans from the fungal hyphae, and the products of digestion are used as energy sources[30]. This enzymatic digestion of the fungal pathogens could present an effective method for biological control by bacteria[31]. ACC deaminase acts by degrading ACC, the precursor of ethylene, resulting in the production of α-ketobutyrate and ammonia, which prevents excessive increase in the synthesis of ethylene under various stress conditions. It is one of the most efficient mechanisms to induce plant tolerance to salt stress[32]. *B. subtilis* subsp. *inaquosorum* strain MPSK186, used in the present study, has shown siderophore production, dinitrogen fixation and ACC deaminase activity. It is known that microbial siderophores provide plants with iron nutrition to enhance their growth when the bioavailability of Fe is low. Siderophores also compete with phytopathogens for iron, thus negatively affecting the growth of several fungal pathogens[33]. Furthermore, due to the ability of the bacteria to fix atmospheric nitrogen, the dinitrogen is converted to ammonia which is assimilated by the plants[34]. The present study has also demonstrated nitrogen fixation, ammonia production and ACC deaminase activity in the salt-pan isolates of *B. cabrialesii* strain MPSK109 and *B. antiquum* strain MPSK28. Ammonia production is known to not only satisfy the nitrogen demand of the host plant but, in excess, can reduce the colonization of plants by pathogens, thus protecting the plant from various diseases. *B. cabrialesii* strain MPSK109 also produced cellulase, lipase and amylase enzymes. Cell wall-degrading enzymes like cellulases help in the penetration of plant tissue by PGP bacteria, and enzymes like lipases, amylases and proteases are known to degrade the cell wall of pathogens to a considerable extent[35,36].

Conclusion

The results of this study indicated the potential of halotolerant bacteria isolated from the salt pans of Goa as promising antifungal agents, with additional PGP activities against pathogens of chilli. Further field trials will confirm their synergistic effect on the environment, ascertaining their use as sustainable BCAs. Overall, the present study showed the potential application of such bacteria as candidates for yield improvement and biological control for integrated use in disease and nutrient management strategies in saline soils.

Conflict of interest: The authors declare that there is no conflict of interest.

1. FAO, FAOSTAT 2020. Food and Agriculture Organization, Rome, Italy, retrieved from https://www.fao.org/faostat/en/#data/QCL/visualize
2. Baenas, N., Belović, M., Ilic, N., Moreno, D. A. and García-Viguera, C., Industrial use of pepper (*Capsicum annum* L.) derived products: technological benefits and biological advantages. *Food Chem.*, 2019, **274**, 872–885; https://doi.org/10.1016/j.foodchem.2018.09.047.
3. Pawaskar, M. and Kerkar, S., Microbial biocontrol agents against chilli plant pathogens over synthetic pesticides: a review. *Proc. Indian Natl. Sci. Acad.*, 2021, **87**, 578–594; https://doi.org/10.1007/s43538-021-00053-2.
4. Arora, H., Sharma, A., Sharma, S., Haron, F. F., Gafur, A., Sayyed, R. Z. and Datta, R., *Pythium* damping-off and root rot of *Capsicum annuum* L.: impacts, diagnosis, and management. *Microorganisms*, 2021, **9**, 823; https://doi.org/10.3390/microorganisms9040823.
5. Bashir, M. R., Atiq, M., Sajid, M., Mohsan, M., Abbas, W., Alam, M. W. and Bashair, M., Antifungal exploitation of fungicides against *Fusarium oxysporum* f. sp. *capsici* causing Fusarium wilt of chilli pepper in Pakistan. *Environ. Sci. Pollut. Res.*, 2018, **25**, 6797–6801; https://doi.org/10.1007/s11356-017-1032-9.
6. Rini, C. R. and Sulochana, K. K., Management of seedling rot of chilli (*Capsicum annuum* L.) using *Trichoderma* spp. and fluorescent pseudomonads (*Pseudomonas fluorescens*). *J. Trop. Agric.*, 2007, **44**, 79–82; http://jtropag.kau.in/index.php/ojs2/article/view/159/159.
7. Lugtenberg, B. and Kamilova, F., Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.*, 2009, **63**, 541–556; https://doi.org/10.1146/annurev.micro.62.081307.162918.
8. Daliakopoulos, I. N., Tsanis, I. K., Koutroulis, A., Kourgialas, N. N., Varouchakis, A. E., Karatzas, G. P. and Ritsema, C. J., The threat of soil salinity: a European scale review. *Sci. Total Environ.*, 2016, **573**, 727–739; https://doi.org/10.1016/j.scitotenv.2016.08.177.

9. Gupta, B. and Huang, B., Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int. J. Genomics*, 2014, **2014**, 1–18; https://doi.org/10.1155/2014/701596.
10. Waditee-Sirisattha, R., Kageyama, H. and Takabe, T., Halophilic microorganism resources and their applications in industrial and environmental biotechnology. *AIMS Microbiol.*, 2016, **2**, 42–54.
11. Kamat, T. and Kerkar, S., Bacteria from salt pans: a potential resource of antibacterial metabolites. *Rec. Res. Sci. Technol.*, 2011, **3**, 46–52; http://irgu.unigoa.ac.in/drs/bitstream/handle/unigoa/2521/RecentRes_Sci_Technol_3_46.pdf?sequence=1&isAllowed=y
12. Karimi, E., Safaie, N., Shams-Baksh, M. and Mahmoudi, B., *Bacillus amyloliquefaciens* SB14 from rhizosphere alleviates *Rhizoctonia* damping-off disease on sugar beet. *Microbiol. Res.*, 2016, **192**, 221–230; https://doi.org/10.1016/j.micres.2016.06.011.
13. Sahu, P. K. *et al.*, Endophytic bacilli from medicinal-aromatic perennial Holy basil (*Ocimum tenuiflorum* L.) modulate plant growth promotion and induced systemic resistance against *Rhizoctonia solani* in rice (*Oryza sativa* L.). *Biol. Control*, 2020, **150**, 104353; https://doi.org/10.1016/j.biocontrol.2020.104353.
14. Shet, S. A. and Garg, S., Plant growth promotion of *Vigna unguiculata* in arid sandy soil using bacterial species from coastal sand dune. *Agric. Res.*, 2022, **11**, 1–12; https://doi.org/10.1007/s40003-022-00613-y.
15. Karimi, E., Sadeghi, A., Abbaszadeh Dahaji, P., Dalvand, Y., Omidvari, M. and Kakuei Nezhad, M., Biocontrol activity of salt tolerant *Streptomyces* isolates against phytopathogens causing root rot of sugar beet. *Biocontrol. Sci. Technol.*, 2012, **22**, 333–349; https://doi.org/10.1080/09583157.2012.658552.
16. Kayasth, M., Gera, R., Dudeja, S. S., Sharma, P. K. and Kumar, V., Studies on salinization in Haryana soils on free-living nitrogen-fixing bacterial populations and their activity. *J. Basic Microbiol.*, 2014, **54**, 170–179; https://doi.org/10.1002/jobm.201200158.
17. Pirhadi, M., Enayatizamir, N., Motamedi, H. and Sorkheh, K., Screening of salt tolerant sugarcane endophytic bacteria with potassium and zinc for their solubilizing and antifungal activity. *Biosci. Biotechnol. Res. Commun.*, 2016, **9**, 530–538; https://www.researchgate.net/profile/Naeimeh-Enayatizamir/publication/333712846_Screening_of_salt_tolerant_sugarcane_endophytic_bacteria_with_potassium_and_zinc_for_their_solubilizing_and_antifungal_activity/links/5d0b9f6ba6fdcc11782233a2/Screening-of-salt-tolerant-sugarcane-endophytic-bacteria-with-potassium-and-zinc-for-their-solubilizing-and-antifungal-activity.pdf
18. Chandrakala, C., Voleti, S. R., Bandeppa, S., Kumar, N. S. and Latha, P. C., Silicate solubilization and plant growth promoting potential of *Rhizobium* sp. isolated from rice rhizosphere. *Silicon*, 2019, **11**, 2895–2906; https://doi.org/10.1007/s12633-019-0079-2.
19. Fernandes, S., Kerkar, S., Leitao, J. and Mishra, A., Probiotic role of salt pan bacteria in enhancing the growth of whiteleg shrimp, *Litopenaeus vannamei*. *Probiot. Antimicrob. Protein.*, 2019, **11**, 1309–1323; https://doi.org/10.1007/s12602-018-9503-y.
20. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Clinical and Laboratory Standards Institute, 2017.
21. Kim, M. J. *et al.*, Safety evaluations of *Bifidobacterium bifidum* BGN4 and *Bifidobacterium longum* BORI. *Int. J. Mol. Sci.*, 2018, **19**, 1422; https://doi.org/10.3390/ijms19051422.
22. Bhattacharya, A. *et al.*, Intervention of bio-protective endophyte *Bacillus tequilensis* enhance physiological strength of tomato during *Fusarium* wilt infection. *Biol. Control*, 2019, **139**, 104074; https://doi.org/10.1016/j.biocontrol.2019.104074.
23. Zhou, H., Zhu, H., Ren, Z., Li, X., Zhong, J. and Liu, E., Efficacy of *Bacillus tequilensis* strain JN-369 to biocontrol of rice blast and enhance rice growth. *Biol. Control*, 2021, **160**, 104652; https://doi.org/10.1016/j.biocontrol.2021.104652.
24. Paul, N. C., Ji, S. H., Deng, J. X. and Yu, S. H., Assemblages of endophytic bacteria in chili4 pepper (*Capsicum annuum* L.) and their antifungal activity against phytopathogens *in vitro*. *Plant Omics*, 2013, **6**, 441–448; https://search.informit.org/doi/abs/10.3316/INFORMIT.844523344855871
25. Ramírez-Cariño, H. F. *et al.*, Biocontrol of *Alternaria alternata* and *Fusarium oxysporum* by *Trichoderma asperelloides* and *Bacillus paralicheniformis* in tomato plants. *Antonie van Leeuwenhoek*, 2020, **113**, 1247–1261; https://doi.org/10.1007/s10482-020-01433-2.
26. Kannan, C., Mishra, D., Rekha, G., Maruthi, P., Shaik, H. and Sundaram, R. M., Diversity analysis of antagonistic microbes against bacterial leaf and fungal sheath blight diseases of rice. *Egypt. J. Biol. Pest Control*, 2021, **31**, 1–16; https://doi.org/10.1186/s41938-021-00462-x.
27. de los Santos Villalobos, S., Robles, R. I., Cota, F. I. P., Larsen, J., Lozano, P. and Tiedje, J. M., *Bacillus cabrialesii* sp. nov., an endophytic plant growth promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley, Mexico. *Int. J. Syst. Evol. Microbiol.*, 2019, **69**, 3939–3945; https://doi.org/10.1099/ijsem.0.003711.
28. Zhou, L., Song, C., Li, Z. and Kuipers, O. P., Antimicrobial activity screening of rhizosphere soil bacteria from tomato and genome-based analysis of their antimicrobial biosynthetic potential. *BMC Genomics*, 2021, **22**, 1–14; https://doi.org/10.1186/s12864-020-07346-8.
29. Gopalakrishnan, S., Humayun, P., Kiran, B. K., Kannan, I. G. K., Vidya, M. S., Deepthi, K. and Rupela, O., Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. *World J. Microbiol. Biotechnol.*, 2011, **27**, 1313–1321; https://doi.org/10.1007/s11274-010-0579-0.
30. Alloway, B. J., *Zinc in Soils and Crop Nutrition*, International Zinc Association Communications, IZA Publications, Brussels, Belgium, 2004.
31. Garbeva, P., Van Veen, J. A. and Van Elsas, J. D., Assessment of the diversity, and antagonism towards *Rhizoctonia solani* AG3, of *Pseudomonas* species in soil from different agricultural regimes. *FEMS Microbiol. Ecol.*, 2004, **47**, 51–64; https://doi.org/10.1016/S0168-6496(03)00234-4.
32. Arora, N. K., Kim, M. J., Kang, S. C. and Maheshwari, D. K., Role of chitinase and β-1,3-glucanase activities produced by a fluorescent pseudomonad and *in vitro* inhibition of *Phytophthora capsici* and *Rhizoctonia solani*. *Can. J. Microbiol.*, 2007, **53**, 207–212; https://doi.org/10.1139/w06-119.
33. del Carmen Orozco-Mosqueda, M., Glick, B. R. and Santoyo, G., ACC deaminase in plant growth-promoting bacteria (PGPB): an efficient mechanism to counter salt stress in crops. *Microbiol. Res.*, 2020, **235**, 126439; https://doi.org/10.1016/j.micres.2020.126439.
34. Ahmed, E. and Holmström, S. J., Siderophores in environmental research: roles and applications. *Microb. Biotechnol.*, 2014, **7**, 196–208; https://doi.org/10.1111/1751-7915.12117.
35. Olanrewaju, O. S., Glick, B. R. and Babalola, O. O., Mechanisms of action of plant growth promoting bacteria. *World J. Microbiol. Biotechnol.*, 2017, **33**, 1–16; https://doi.org/10.1007/s11274-017-2364-9.
36. Rodrigues, A. A., Forzani, M. V., Soares, R. D. S., Sibov, S. T. and Vieira, J. D. G., Isolation and selection of plant growth-promoting bacteria associated with sugarcane. *Pesqui. Agropecu. Trop.*, 2016, **46**, 149–158; https://doi.org/10.1590/1983-40632016v4639526.
37. Friedrich, N., Hagedorn, M., Soldati-Favre, D. and Soldati, T., Prison break: pathogens' strategies to egress from host cells. *Microbiol. Mol. Biol. Rev.*, 2012, **76**, 707–720; https://doi.org/10.1128/MMBR.00024-12.

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