KAVAKA 55 : 71-76 (2020) doi:10.36460/Kavaka/55/2020/71-76

# Isolation and identification of phosphate (P) solubilizing halophytic fungal endophytes

Apurva Sawant and B.F. Rodrigues*
*Department of Botany, Goa University, Taleigao Plateau, Goa 403 206.*
**Corresponding author Email: felinov@gmail.com*
(Submitted on June 20, 2020; Accepted on September 30, 2020)

## ABSTRACT

**Mangrove endophytes are an essential group of halophytic fungi involved in the solubilization of inorganic phosphate (P) into available organic form of P. In the present study, the diversity of potential P solubilizing fungal endophytes was studied by using Pikovskaya's media. The highest colonization of endophytic fungi was recorded in *Avicennia officinalis*. A total of 37 fungal taxa representing 15 genera were isolated along with seven sterile non-sporulating isolates. The relative abundance (RA) and isolation frequency (IF) were recorded highest in *Aspergillus niger* (MEn27) and *Scolecobasidium* sp. (MEn39). It was observed that Shannon-Weiner (H) and Simpson diversity index (D) was 1.5 and 1, respectively. Fungal isolates showed positive P solubilizing activity in solid and liquid media. The highest P solubilization potential was recorded in *Drechslera* sp. A significant decrease in the pH of the liquid media from 7 (un-inoculated control tubes) to 3.11 was observed. The results indicated the potency of endophytic fungi to solubilize P.**

**KEYWORDS:** Mangroves, tropical ecosystem, endophytic fungi, Pikovskaya media, phosphate solubilization.

## INTRODUCTION

Endophytic microorganisms colonizing the internal parts of the plant are known to synthesize diverse groups of secondary metabolites that benefit the host to withstand stress (Wani *et al.*, 2015). Endophytic fungi do not penetrate the cell and grow in-between spaces of a plant cell or within the plant cell wall. These fungi utilize low levels of nutrients from the inter-cellular spaces without causing any damage to the host plant (Tan and Zou, 2001) and have substantial role in the development (Glick, 1995). Literature suggests that such association has a major role in providing host resistance towards pathogens (Azevedo *et al*., 2000) thereby enhancing growth and nutrient uptake (Garg *et al*., 2001). The endophytic species produce several extracellular enzymes that are involved in maintaining a steady flow of carbon and nutrient acquisition in the plant system (White *et al*., 2019). Besides, they are known to be precursor agents that synthesize diverse groups of secondary metabolites having antimicrobial, anticancer and antioxidant properties (Singh *et al*., 2001; Gajalakshmi *et al*., 2013). Therefore, endophytes are designated as a core group among fungal species having biotechnologically important role (Azevedo, 2000).

Endophytes are known symbionts that help host plants in fixing environmental nitrogen (N) and inorganic P (Santoya *et al.*, 2016). Phosphorus is a vital macronutrient involved in various biochemical and physiological processes in the plant system (Sahoo and Gupta, 2018). However, more than 90% of P is available in an insoluble state in the soil (Singh and Sati, 2017) and hence is not available to plants. This situation results in P deficiencies observed in tropical soils. Recent reports suggest that many soil and plant symbionts like fungi, bacteria, and other soil microorganisms play an essential role in P solubilization (Gyaneshwar *et al*., 2002) and mobilization (Scervino *et al*., 2011). In the mangrove ecosystem, the soil pH varies from highly acidic to alkaline (Hossain and Nuruddin, 2016). In mangrove sediments, P gets precipitated due to the abundance of cations (Kpomblekou and Tabatabai, 1994). In acidic soils iron and aluminum binds to P, while in alkaline soils, calcium precipitates P leading to P deficiency (Matos *et al*., 2017). In such cases, the role of P solubilizers is very crucial to help plants overcome P deficiencies. Plant colonizers like endophytic fungi trigger the solubilization and mineralization of inorganic phosphates into soluble forms (Illmer and Schinner, 1995). The conversion of P involves the chelation of cations which are bond to phosphate, at its hydroxyl and carboxyl group. This gets converted into soluble organic form of P by the action of phosphatase enzymes that results in the acidification of the medium in which they are grown (Bashan *et al*., 2013). It involves the release of H+ ions from phosphates that decrease the pH of the medium and the formation of soluble hydrophosphates which is then gets mobilized into the host plant (Matos *et al*., 2017).

Recent studies reveal that endophytic fungi solubilize a higher amount of P compared to bacteria (Vazquez *et al*., 2000). Gyaneshwar *et al*., (2002) revealed that fungal species belonging to *Aspergillus* are known to solubilize a high amount of tri-calcium phosphate under laboratory culture conditions. Therefore, the present study was aimed to document the diversity of endophytic fungi at Chorao Island and to identify the potential P solubilizing endophytes among the fungal population.

## MATERIALS AND METHODS

**Study area:** Goa is a smallest state situated along the southern western coast of Indian Peninsula, commonly known as 'Konkan'. The state is bounded by Terekhol River (North), Karnataka State (South and East) and Arabian Sea (West), forming reservoir of different flora and fauna across the state. Goa is sustained by 11 rivers, *viz*. Terekhol, Mandovi, Baga, Zuari, Colvale, Saleri, Mandre, Harmal, Sal, Talpona and Galjibag flowing across the state. Among the rivers, Mandovi has a great ecological significance for the state as it forms a largest basin in Goa. The present study was undertaken at Chorao (also known as Chodna), which is an island along the Mandovi river. A small part of the Island covering 178 hectares of the area was declared as Reserved Forest under the Indian Forest Act, (1927) to protect and conserve mangrove forests. The reserve area is well known as Dr. Salim Ali Bird Sanctuary. Goa is dominated by 16 mangrove plant species found at the tidal and inter-tidal zones

along the banks of these rivers. These mangroves help in stabilizing the coastal areas and protect land from soil erosion, cyclonic storms, and landslides. Chorao Island is located at a geographic location between 15°54.04" N Latitude and 73°88.47'' E Longitude. The study site is dominated by 12 mangrove plant species. Healthy leaf, stem, and root samples of these mangrove plants species (**Table 1**) were collected and brought to the laboratory in sterile zip-loc bags and processed within 5 hours of collection.

**Isolation of endophytic fungi:** The endophytic fungi were isolated by using the modified method of Arnold *et al*., (2000). Individual plant samples were washed using running tap water, followed by washing with distilled water (DW). Each of the plant samples was further washed using 90% ethanol for 1 minute, followed by rinsing with DW. The samples were subsequently washed using 0.5% sodium hypochlorite for 2 minutes, rinsed with DW and 90% ethanol for 1 minute. Each of the sterilized samples was washed twice with DW and cut into small pieces of approx. 0.5 cm using a sterile blade. The sterilized samples (nine/Petri plate) were placed on Potato Dextrose Agar (PDA) medium, amended with streptomycin sulfate, and penicillin G (150 mg/l). The Petri plates were incubated at 25° C in a tissue culture laboratory.

**Identification of endophytic fungi**: Identification was carried out based on morphological characteristics by using standard methods (Bills, 1996; Tibpromma *et al*., 2018). The colonies were identified based on macroscopic (colony colour, texture) and microscopic characters (hyphae, conidia, and conidiophore structures). The microscopic examination was done by mounting sporulating fungal conidiophore on a clean and dry glass slide amended with lactophenol (for coloured conidia)/lactophenol cotton blue (hyaline conidia) stain. The slides were observed under a bright-field Olympus BX41 microscope. The fungal descriptions were then compared with the available literature. The colonies which failed to sporulate were designated as sterile isolates and were subsequently coded with isolation code.

**Statistical analysis:** The diversity of endophytic fungi at Chorao Island was estimated by using the following statistical tools:

Relative Abundance (RA) = (Number of fungal isolates of a particular species / Total number of fungal isolates) x 100 (Mehrotra and Aneja, 1990; Mehrotra, 1992).

Isolation Frequency (IF) = (Number of plant sample containing particular fungal endophytes / Total number of plants used) x 100 (Nagamani *et al*., 2006)

Shannon and Weiner diversity index (H),

$$H= -\Sigma [(pi) \times \ln (pi)]$$

where, pi = proportion of the particular species (Shannon and Weiner, 1949).

Simpson diversity index (D)

$$Ds= -\Sigma [n (n -1)] / N (N-1)$$

where, n = the number of individuals of each species and N = the total number of all species in a plant host (Simpson, 1949).

Colonization frequency = No. of segments colonized by fungi/Total no. of segments observed x100 (Suryanarayanan *et al*., 2003; Photita *et al*., 2001).

**Table 1:** Mangrove diversity at Chorao Island.

| Plant species | True/ associative mangrove | Common Name | Family | Habit |
|---|---|---|---|---|
| *Kandelia candel* Druce | TM | Kandal | *Rhizophoraceae* | Shrub/Small tree |
| *Rhizophora apiculata* Blume | TM | Garjan | *Rhizophoraceae* | Tree |
| *Ceriops tagal* C.B.Rob. | TM | Spurred mangrove | *Rhizophoraceae* | Medium tree |
| *Rhizophora mucronata* Lam. | TM | Red mangrove | *Rhizophoraceae* | Tree |
| *Avicennia marina* (Forssk.) Vierh. | TM | White/Grey mangrove | *Acanthaceae* | Tree |
| *Avicennia officinalis* L. | TM | Indian mangrove | *Acanthaceae* | Tree |
| *Aegiceras corniculatum* (L.) Blanco | TM | Black mangrove | *Primulaceae* | Shrub |
| *Bruguiera cylindrica* (L.) Blume | TM | - | *Rhizophoraceae* | Small tree |
| *Excoecaria agallocha* L. | TM | Milky mangrove | *Euphorbiaceae* | Small tree |
| *Sonneratia alba* Griff. | TM | Apple mangrove | *Lythraceae* | Tree |
| *Sonneratia caseolaris* (L.) Engl. | TM | Apple mangrove | *Lythraceae* | Tree |
| *Clerodendrum inerme* R.Br. | AM | Wild jasmine | *Verbenaceae* | Shrub |
| *Acrosticum aurem* L. | AM | Golden leather fern | *Pteridaceae* | Fern |
| TM=True mangrove; AM=Associate mangrove. | | | | |

**Screening for P solubilization activity:** The emerging fungal culture disc (approx. 3 mm) was inoculated on Pikovskaya's media. The Petri plates were incubated in dark for 7-8 days (depending upon the fungal species) to check the P solubilizing activity of the culture. Halo zone forming fungi were selected to quantify the amount of P solubilization. Further Solubilization Index (S.I.) was calculated by using the formula:

S.I. = Colony diameter + Halo zone diameter/ Colony diameter (Edi–Premono *et al*., 1996)

**Quantitative estimation P solubilization:** Quantitative estimation of P solubilization was carried out using Vanado phosphomolybdate method (Jackson, 1958). The P solubilizing fungal species were separately inoculated into Pikovskaya's broth media in triplicate and incubated at 28ºC for 14 days. 10 mL suspension taken from the flask and centrifuged at 10,000 rpm for 10 min. From this, 5 mL culture filtrate adjusted to 50 mL with distilled water. From the above filtrate, 1 mL was taken, and to this, 2.5 mL of Barton's reagent was added, and the final volume adjusted to 50 mL. After 10 minutes, the resultant, yellow-coloured sample was used to take readings on a spectrophotometer at 420 nm. The mycelia were separated from the culture broth after ten days of incubation. Initial pH and change in pH were noted for all the samples with the help of a digital pH meter.

## RESULTS

**Isolation and identification of endophytic fungi:** From 12 mangrove plant species (378 plant segments), a total of 217 fungal isolates belonging to 37 fungal taxa representing 15 genera along with seven sterile mycelia were obtained. The seven strains remained sterile even after several attempts to

grow them on different media. A similar observation was made by Rajagopal and Suryanarayanan, (2000), which stated that few endophytic strains remain sterile even after prolonged incubation and hence not possible to identify. The highest endophytic isolates were obtained from the leaves of *A. officinalis* followed by *E.agalocha* and *A.corniculatum* (**Fig. 1**). The isolates were purified and later identified by using morphological keys (**Table 2**). Among the fungal isolates, highest RA, and IF (%) was observed for MEn 39 and MEn27 (4.61 and 140, respectively). Lowest RA and IF were observed in MEn10, MEn15, MEn21, MEn26, MEn32, and MEn37 (0.92 and 28, respectively) (**Table 2**). Shannon (H) and Simpson (D) diversity indices for the endophytic fungal population inhabiting the mangrove plant species were 1.5 and 1, respectively.

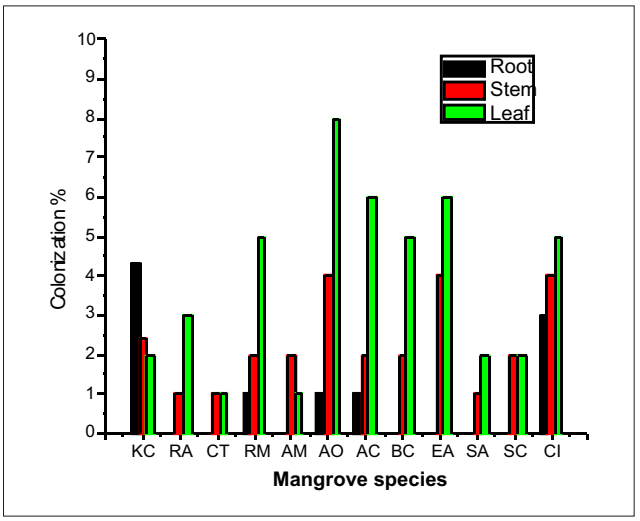


**Fig. 1**: Colonization by fungal endophytes in different plant parts of mangrove plant species. Abbreviations used in histogram refers as KC- *Kandelia candel*; RA- *Rhizophora apiculata*; CT- *Ceriops tagal*; RM- *Rhizophora mucronata*; AM- *Avicennia marina*; AO- *Avicennia officinalis*; AC- *Aegiceras corniculatum*; BC- *Bruguiera cylindrica*; EA- *Excoecaria agallocha*; SA- *Sonneratia alba*; SC- *Sonneratia caseolaris*; CI- *Clerodendrum inerme*.

**Screening for P solubilization activity on solid media:** Mangrove endophytes showed clear halo zones in the culture plates, indicating their potency in P solubilization (**Table 3**). The halo zones were measured to calculate the P solubilizing index (PSI) and P solubilizing efficiency (S.E. %). The results indicated that the P solubilizing index (PSI) ranged from 2 to 3.8 cm. The highest solubilizing index (3.8 cm) was recorded in M.En.19 (*Drechslera* sp.) (**Table 3**).

**Quantitative estimation of P solubilization:** The fungal endophytes recorded a decrease in pH of the culture filtrate, indicating their potency to solubilize P. The total P solubilized by endophytic isolates used in the study ranged from 9.70 to 0.1 µg/mL. The highest P solubilization was recorded in *Drechslera* sp. (9.70 µg/mL). In a liquid medium, a drastic decrease in pH was observed (from 3 to 5.7), indicating their role in P solubilization (**Table 4**).

**Table 2:** Diversity of endophytic fungi along Chorao Island Goa.

| Isolate code | Fungal isolate | Relative Abundance (RA%) | Isolation Frequency (IF %) |
|---|---|---|---|
| M.En.03 | *Scolecobasidium* sp. | 1.84 | 56 |
| M.En.04 | *Drechslera* sp. | 2.30 | 70 |
| M.En.05 | *Fusarium* sp. | 2.76 | 84 |
| M.En.06 | *Pestalotiopsis* sp. | 3.68 | 112 |
| M.En.07 | *Scytalidium lignicola* | 2.76 | 84 |
| M.En.08 | *Penicillium* sp. | 2.30 | 70 |
| M.En.09 | *Fusarium* sp. | 2.31 | 70 |
| M.En.10 | *Aspergillus* sp. | 0.92 | 28 |
| M.En.11 | *Penicillium* sp. | 4.60 | 140 |
| M.En.12 | *Corynesporina elegans* | 2.76 | 84 |
| M.En.13 | *Drechslera* sp. | 2.30 | 70 |
| M.En.14 | *Scytalidium lignicola* | 2.31 | 70 |
| M.En.15 | Sterile mycelia | 0.92 | 28 |
| M.En.16 | *Corynespora* sp. | 4.60 | 140 |
| M.En.17 | *Gilmaniella* sp. | 3.68 | 112 |
| M.En.18 | *Fusarium* sp. | 2.76 | 84 |
| M.En.19 | *Drechslera* sp. | 2.30 | 70 |
| M.En.20 | *Drechslera* sp. | 2.31 | 70 |
| M.En.21 | *Pestalotiopsis* sp. | 0.92 | 28 |
| M.En.22 | No-sporulation | 4.60 | 140 |
| M.En.23 | *Junctospora pulchra* | 2.76 | 84 |
| M.En.24 | *Bipolaris* sp. | 2.30 | 70 |
| M.En.25 | *Scytalidium* sp. | 2.31 | 70 |
| M.En.26 | *Setosphaeria monoceras* | 0.92 | 28 |
| M.En.27 | *Aspergillus niger* | 4.61 | 140 |
| M.En.28 | *Drechslera* sp. | 3.68 | 112 |
| M.En.29 | *Scolecobasidium* sp. | 2.76 | 84 |
| M.En.30 | *Junctospora pulchra* | 2.30 | 70 |
| M.En.31 | *Fusarium* sp. | 2.31 | 70 |
| M.En.32 | *Cladosporium* sp. | 0.92 | 28 |
| M.En.33 | *Myceliophthora* sp. | 4.60 | 140 |
| M.En.34 | Sterile Mycelia | 2.76 | 84 |
| M.En.35 | Sterile Mycelia | 2.30 | 70 |
| M.En.36 | Sterile Mycelia | 2.31 | 70 |
| M.En.37 | Sterile Mycelia | 0.92 | 28 |
| M.En.38 | Sterile Mycelia | 4.60 | 140 |
| M.En.39 | *Scolecobasidium* sp. | 4.61 | 140 |
| MEn- Mangrove Endophyte | | | |

## DISCUSSION

Mangroves are biodiversity hotspots for marine fungi protecting the coast from high wave action. Recently marine fungi are increasingly gaining attention for their bioactive compounds that help plant in adaptation to changing climatic conditions. The beneficiary aspect of the endophytes and their hosts, particularly in relevance to plant growth promotions, are widely studied (Nautiyal, 1999). The present study revealed the diversity of different endophytic fungal isolates

**Table 3**: Phosphate solubilization activity of fungal endophytes culture in solid media.

| Isolate code | Host plant | Fungal isolate | Solubilization Index (S.I.) cm |
|---|---|---|---|
| M.En.04 | *Avicennia officinalis* L. | *Drechslera* sp. | 2.2 |
| M.En.05 | *Bruguiera cylindrica* (L.) Blume | *Fusarium* sp. | 3.2 |
| M.En.06 | *Avicennia officinalis* L. | *Pestalotiopsis* sp. | 2.1 |
| M.En.08 | *Acrostichum aureum* L. | *Penicillium* sp. | 2.3 |
| M.En.09 | *Acrostichum aureum* L. | *Fusarium* sp. | 2.3 |
| M.En.10 | *Avicennia officinalis* L. | *Aspergillus* sp. | 2.2 |
| M.En.11 | *Sonneratia alba* Griff | *Penicillium* sp. | 2.3 |
| M.En.13 | *Sonneratia alba* Griff | *Drechslera* sp. | 2.2 |
| M.En.15 | *Excoecaria agallocha* L. | Sterile mycelia | 2.9 |
| M.En.18 | *Avicennia marina* L. | *Fusarium* sp. | 2 |
| M.En.19 | *Avicennia marina* L. | *Drechslera* sp. | 4 |
| M.En.20 | *Excoecaria agallocha* L. | *Drechslera* sp. | 3.8 |
| M.En.21 | *Avicennia marina* L. | *Pestalotiopsis* sp. | 2.1 |
| M.En.22 | *Sonneratia alba* Griff | No-sporulation | 2.5 |
| M.En.24 | *Avicennia marina* L. | *Bipolaris* sp. | 3.4 |
| M.En.27 | *Avicennia officinalis* L. | *Aspergillus niger* | 2.8 |
| M.En.28 | *Avicennia marina* L. | *Drechslera* sp. | 2.4 |
| M.En.31 | *Avicennia marina* L. | *Fusarium* sp. | 2.2 |
| M.En.32 | *Avicennia officinalis* L. | *Cladosporium* sp. | 2.1 |
| M.En.34 | *Excoecaria agallocha* L | Sterile mycelia | 2.2 |
| M.En.35 | *Avicennia officinalis* L. | Sterile mycelia | 2.2 |
| M.En.36 | *Avicennia marina* L. | Sterile mycelia | 3.2 |
| M.En.37 | *Aegiceras corniculatum* (L.) Blanco | Sterile mycelia | 3.1 |
| M.En.38 | *Avicennia officinalis* L. | Sterile mycelia | 2.2 |
| M.En- Mangrove Endophyte | | | |

**Table 4:** Phosphate solubilization activity of fungal endophytes in the liquid medium.

| Isolate code | Host plant | Fungal isolate | PH | µg/mL |
|---|---|---|---|---|
| M.En.04 | *Avicennia officinalis* L. | *Drechslera* sp. | 4.5 | 9.70 |
| M.En.05 | *Bruguiera cylindrica* (L.) Blume | *Fusarium* sp. | 4.5 | 1.0 |
| M.En.06 | *Avicennia officinalis* L. | *Pestalotiopsis* sp. | 4.4 | 1.0 |
| M.En.08 | *Acrostichum aureum* L. | *Penicillium* sp. | 4.8 | 1.17 |
| M.En.09 | *Acrostichum aureum* L. | *Fusarium* sp. | 5.0 | 0.52 |
| M.En.10 | *Avicennia officinalis* L. | *Aspergillus* sp. | 5.0 | 2.25 |
| M.En.11 | *Sonneratia alba* Griff | *Penicillium* sp. | 4.7 | 0.11 |
| M.En.13 | *Sonneratia alba* Griff | *Drechslera* sp. | 4.3 | 1.71 |
| M.En.15 | *Excoecaria agallocha* L. | Sterile Mycelia | 4.0 | 1.46 |
| M.En.18 | *Avicennia marina* L. | *Fusarium* sp. | 4.0 | 1.84 |
| M.En.19 | *Avicennia marina* L. | *Drechslera* sp. | 4.1 | 8.16 |
| M.En.20 | *Excoecaria agallocha* L. | *Drechslera* sp. | 4.9 | 8.52 |
| M.En.21 | *Avicennia marina* L. | *Pestalotiopsis* sp. | 4.6 | 1.23 |
| M.En.22 | *Sonneratia alba* Griff | Sterile mycelia | 4.5 | 1.66 |
| M.En.24 | *Avicennia marina* L. | *Bipolaris* sp. | 4.6 | 1.71 |
| M.En.27 | *Avicennia officinalis* L. | *Aspergillus niger* | 5.0 | 1.89 |
| M.En.28 | *Avicennia marina* L. | *Drechslera* sp. | 3.1 | 8.47 |
| M.En.31 | *Avicennia marina* L. | *Fusarium* sp. | 5.0 | 2.66 |
| M.En.32 | *Avicennia officinalis* L. | *Cladosporium* sp. | 5.0 | 1.82 |
| M.En.34 | *Excoecaria agallocha* L | Sterile Mycelia | 4.7 | 1.56 |
| M.En.35 | *Avicennia officinalis* L. | Sterile Mycelia | 5.4 | 2.72 |
| M.En.36 | *Avicennia marina* L. | Sterile Mycelia | 5.7 | 1.51 |
| M.En.37 | *Aegiceras corniculatum* (L.) Blanco | Sterile Mycelia | 5.1 | 1.66 |
| M.En.38 | *Avicennia officinalis* L. | Sterile Mycelia | 5.1 | 1.0 |
| M.En. Mangrove Endophyte. | | | | |

at the Chorao Island, having P solubilizing potential. Colonization study revealed the affinity of endophytic fungi to colonize almost all parts of the plant tissue. Endophytic fungi belonging to 15 different genera were recorded from different parts of the mangrove plant species indicating moderate diversity at the island. Study revealed no selective host specificity among the fungal endophytes. On the contrary Gu, *et al*., (2012) noted tissue specificity in *Ceriops tagal* from Hainan Province, China.

Out of 37 assessed fungal taxa, 24 fungal species showed positive activity to solubilize P source present in the media. Among these fungal species *Fusarium, Penicillium, Aspergillus* and *Pestalotiopsis* are the frequently reported P solubilizers under laboratory conditions (Mahadevamurthy, *et al*., 2016). Fungal endophytes showed clear halo zones around the fungal colony that might be due to the release of certain chemical compounds by the growing fungal tips. These fungal species upon culturing in liquid Pikovskaya's media for the period of 14 days, showed a drastic decrease in pH. The reason for such activity is due to acidification and mineralization of P by microbial cells that resulted in lowering the pH in culture conditions as suggested by Singh and Sati (2017). Igual *et al.,* (2001), also reported a similar activity of fungi suggesting, higher solubilizing potential of growing fungi under basic to acidic conditions. The authors are of the opinion that the production of acids can be a sole response of fungus to solubilize insoluble phosphates. Gaind and Gaur (1989) explained a similar behaviour of the fungus involved in decreasing the pH of the medium in which they were grown while solubilizing available inorganic phosphates. Whitelaw *et al.,* (1999) reported that microbial P solubilization involves the chelation of iron (Fe) and aluminum (Al) ions, which are involved in the release of phosphate. Some species belonging to *Aspergillus, Penicillium, Fusarium, Mucor* and *Gliocladium* produce gluconic and citric acid to release P (Shindia *et al*., 2006). According to Khan *et al*., (2014), under liquid media P solubilization is due to the excretion of organic acids (oxalic, citric and lactic acid) by microbes.

Endosymbionts are gaining the attention of the scientific community because of their genetic potential to produce metabolites that are beneficial to the growing pharmaceutical, agricultural, and industrial research (Vitorino and Bessa, 2017). Such mycobiota provides wide variety of direct and indirect interactions between plants and herbivores, including increasing resistance to disease, abiotic stress, and the enhancement of plant growth (Rodriguez, 2009). Compounds produced by fungal endophytes (alkaloids, terpenoids, flavonoids, and steroids) can be used for plant growth promotion, with the synthesis of secondary metabolites and in defense against pathogens, therefore deserve exploration.

## CONCLUSION

From the present study, it can be concluded that endophytic fungi from the mangrove ecosystem could effectively solubilize available P from the Pikovskaya's media. This study would help researchers to use such endophytes to minimize P deficiencies in tropical soil and can probably minimize soil fertility-related problems. The present study was directed to identify the potential marine endophytic fungi, which has a key role in P solubilization in mangrove ecosystem. Further isolation and purification of these isolates would assist in the preparation of biofertilizers.

## ACKNOWLEDGMENT

Assistance in the identification of fungal isolates by Dr. Puja Gawas is gratefully acknowledged.

## REFERENCES

Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. and Kursar, T.A. 2000. Are tropical fungal endophytes hyperdiverse? *Ecology Letters* **3**: 267-274.

Azevedo, J.L., Junior, W.M., Pereira, J.O. and Araujo, W.L. 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electronic Journal of Biotechnology* **3**:40-65.

Bashan, Y., Kamnev, A.A. and Bashan, L.E. 2013. A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. *Biology and Fertility of Soils* **49**: 1-2.

Bills, G.F. 1996. Isolation and analysis of endophytic fungal communities from woody plants. In: *Endophytic fungi in grasses and woody plants: Systematics, ecology, and evolution* (Eds.: Redlin, S.C. and Carris, L.M.). St. Paul, MN: APS Press, pp. 31-65.

Edi–Premono, Moawad, M.A. and Vleck, P.L.G. 1996. Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian Journal of Crop Science* **11**: 13-23.

Gaind, S. and Gaur, A.C. 1989. Effect of pH on phosphate solubilization by microbes. *Current Science* **59** (21):1209-1211.

Gajalakshmi, S., Vijayalakshmi, S. and Devi, R.V. 2013. Pharmacological activities of *Catharanthus roseus*: a perspective review. *International Journal of*

*Pharmacy and Biological Sciences* **4**: 431-9.

Garg, S.K., Bhatnagar, A., Kalla, A. and Narula, N. 2001. *In vitro* fixation, phosphate solubilization, survival and nutrient release by *Azotobacter* strains in aquatic system. *Bioresource Technology* **80**: 101-109.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* **41**: 109–17.

Gu, H.G., Bai, H.J., Zeng, Y.B., Chang, D.D. and Mei, W.L. 2012. Isolation & primary identification of endophytic fungi from mangrove plant *Ceriops tagal*. *Journal of Microbiology* **32**:1-6.

Gyaneshwar, P., Kumar, N.J., Pareka, L.J. and Podle, P.S. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* **245** (1): 83-93.

Hossain, M.D. and Nuruddin, A.A. 2016. Review article soil and mangroves: A review. *Journal of environmental science and technology* **9** (2):198-207.

Igual, J.M., Valverde, A., Cervantes, E. and Velazquez, E. 2001. Phosphate solubilizing bacteria as inoculants for agriculture: use of update molecular techniques in their study. *Agronomy Sustainable Development* **21**: 561-568.

Illmer, P. and Schinner, F. 1995. Solubilization of inorganic calcium phosphates-solubilization mechanisms. *Soil Biology and Biochemistry* **27**: 257-263.

Jackson, M.L. 1958. *Soil Chemical Analysis*. Prentice Hall Inc. Englowood cliff, New Jersey, USA.

Khan, M.S., Zaidi, A. and Ahmad, E. 2014. Mechanism of Phosphate Solubilization and Physiological Functions of Phosphate-Solubilizing Microorganisms In: *Phosphate Solubilizing Microbes for Crop Improvement*. Springer International Publishing, Berlin Heidelberg, p. 31-62.

Kpomblekou, K. and Tabatabai, M.A. 1994. Effect of organic acids on release of phosphorus from phosphate rocks. *Soil Science*. **158**: 442-453.

Matos, A.D.M., Gomes, I.C.P., Nietsche, S., Xavier, A.A., Gomes, W.S., Dos Santos Neto, J. A. and Pereira, M.C.T. 2017. Phosphate solubilization by endophytic bacteria isolated from banana trees. *Anais da Academia Brasileira de Ciências* 1-10.

Mahadevamurthy, M., Channappa T.M., Sidappa M., Raghupathi M.S. and Nagaraj A.K. 2016. Isolation of phosphate solubilizing fungi from rhizosphere soil and its effect on seed growth parameters of different crop plants. *Journal of applied biology and biotechnology* **4** (06): 022-026.

Mehrotra, B.S. 1992. *The fungi- An introduction*. (3rd Edition). Today and Tomorrow Printers and publishers. New Delhi.

Mehrotra, R.S. and Aneja, K.R. 1990. *An introduction to mycology.* Wiley eastern limited, New Delhi.

Nagamani, A., Kunwar, I.K. and Manoharachary, C. 2006. *Handbook of Soil Fungi*. IK International Pvt. Ltd. New Delhi.

Nautiyal, C.S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganism. *FEMS Microbiology Letter* **170**: 265-270.

Photita, W., Lumyong, S., Lumyong, P. and Hyde, K.D. 2001b. Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, in Thailand. *Mycological Research* **105**: 1508-1513.

Rajagopal, R. and Suryanarayanan, T.S. 2000. Isolation of endophytic fungi from leaves of neem (*Azadirachta indica*). *Current science* **78**(4): 1375-1378.

Rodriguez, R.J., White, J.F., Arnold, A.E. and Redman, R.S. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* **182**: 314-330.

Sahoo, H.R. and Gupta, N. 2018. Diversity of endophytic phosphate solubilizing fungi associated with *Pomatocalpa decipiens* (Lindl.) J.J. Smith - an endangered orchid in Barbara forest of Odisha, India. *Studies in Fungi* **3** (1): 84-99.

Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M. and Glick, B.R. 2016. Plant growth - promoting bacterial endophytes. *Microbiological Research* **183**: 92- 99.

Scervino, J.M., Papinutti, V.L., Godoy, M.S. and Rodriguez, J.M. 2011. Medium pH, carbon and nitrogen concentrations modulate the phosphate solubilization efficiency of *Penicillium purpurogenum* through organic acid production. *Journal of Applied Microbiology* **110**:1215-1223.

Shannon, C.E. and Wiener, W. 1949. *The Mathematical Theory of Communication*. University of Illinois press, Urbana, USA.

Shindia, A. A., El-Sherbeny, G.A., El-Esawy A.E. and Sheriff, Y.M.M.M. 2006. Production of gluconic acid by some local fungi. *Mycobiology* **34**(1): 22-29.

Simpson, E.H. 1949. Measurement of Diversity. *Nature* **163**: 688

Singh, S.N., Vats, P., Suri, S., Shyam, R., Kumria, M.M., Ranganathan, S. and Sridharan, K. 2001. Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotoci induced diabetic rats. *Journal of Ethnopharmacology* **76**: 269–77.

Singh, L. and Sati, S.C. 2017. Bio-Prospecting of Root Endophytic Freshwater Fungi *Anguillospora longissima* and *Cylindrocarpon aquaticumas* Phosphate Solubilization Potential. *Journal of Pure and Applied Microbiology* **11**(4): 1929-1937.

Suryanarayanan, T.S., Venkatesan, G. and Murali, T.S. 2003.

Endophytic fungal communities in leaves of tropical forest trees: diversity and distribution patterns. *Current Science* **85** (4): 489-493.

Tan, R.X. and Zou, W.X. 2001. Endophytes: a rich source of functional metabolites. *Natural Product Reports* **18**: 448-459.

Tibpromma, S., Hyde, K., Bhat, J., Mortimer, P., Xu, J., Promputtha, I., Doilom, M., Yang, J., Tang, A. and Karunarathna, S. 2018. Identification of endophytic fungi from leaves of *Pandanaceae* based on their morphotypes and DNA sequence data from southern Thailand. *MycoKeys* **33**: 25–67.

Vazquez, P., Holguin, G., Puente, E.M., Lopez-Cortes, A. and Bashan, Y. 2000. Phosphate- solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility in Soils* **30**: 5-6.

Vitorino, L.C. and Bessa, L.A. 2017. Technological microbiology: Development and applications. *Frontiers on Microbiology* **8**: 827.

Wani, Z.A., Ashraf, N., Mohiuddin, T. and Riyaz-Ul-Hassan, S. 2015. Plant-endophyte symbiosis, an ecological perspective. *Applied Microbiology and Biotechnology* **99**: 2955–2965.

White, J.F., Kingsley, K.L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M.T., Verma S.K., Gond S.K. and Kowalski K.P. 2019. Review : Endophytic microbes and their potential applications in crop management. *Pest Mangement Science* **75**: 2558-2565.

Whitelaw, M.A., Harden, T.J. and Helyar, K.R. 1999. Phosphate solubilization in solution culture by the soil fungus *Penicillium radicum. Soil Biology and Biochemistry* **32**: 655–665.