Poll Res. 16 (4) : 223-227 (1997)

TOXICITY OF INDOLE ACETIC ACID IN TWO RACES OF MULBERRY SILKWORM *BOMBYX MORI* L.

I. K. PAI AND S. N. HEGDE*

Department of Zoology, Goa University, Goa-403 25, India

Key Words : Silkworm *Bombyx mori*, growth regulators, IAA, Toxicity

ABSTRACT

Indole acetic acid, one of the most widely used plant growth promoter to increase yield in plants and animals was tested for its toxicity in one of the best non-mammalian test system viz., mulberry silkworm *Bombyx mori* L. Low concentrations of the chemical was administered to the test system by following egg treatment, oral administration, topical application at III and V instar stage. The toxicity parameters such as fecundity, fertility, hatchability, viability and rate of development were analysed. The results indicate that IAA is toxic to silkworms.

INTRODUCTION

Indole compounds are ubiquitous in the environment including plants. Some of them are also known to be growth hormones in plants (Henbest *et al.*, 1953., Okamoto *et al.*, 1967., Nonoto and Tamura 1970). However there is no evidence for a growth regulating role of auxins, particularly IAA in fungi (Gruen (1959). It is also well known that growth hormones exert biphasic effect; at low concentrations, they are either inactive or stimulate growth, while at high concentrations, they are toxic (Phol and Rother, 1953). Klambt (1961) is of the opinion that, the inhibition of growth caused by excessive concentration of energy of Indole compounds may be caused by exhaustion of energy reserves during detroxification on the blocking of co-enzyme A in the course of detoxification.

There are several reports of using a number of growth regulators to improve yield in plants (Singh *et al.* 1978, Raza 1978, Nelson and Sharples 1980., Agarwal 1984) and animals (Akai 1971., Akai and Kobayashi 1971, Kobari and Akai 1978., 1979., Krishnaswami *et al.* 1979, Pai and Krishnamurthy 1984., Pai *et al.*, 1987). At the same time many of the growth regulators are known to produce Toxicity (Pai *et al.* 1991), dominant lethals and chromosomal aberrations in species of silkworm, Triticum, mouse, rat etc., (Dhingra *et al.*, 1978).

Indole acetic acid (IAA), a plant growth regulator, which is being used in plants and animals to improve yield, is known to do so either by enhancing or inducing the cellular synthesis is being extensively used in increasing the yield of mulberry leaves, which happens to be the sole food of silkworm *Bombyx mori*. However, there are no reports on the toxic effects of IAA, if any, on silkworm B. mori. Hence an attempt has been made in this direction.

MATERIALS AND METHODS

Two races of silkworms Bomyx mori viz., multivoltine Pure Mysore and bivoltine Kalimpong-A maintained for over 25 generations in Sericulature Research Project, University of Mysore, Manasaganotri, Mysore, India, formed the material for the present studies. The chemical was administered by various methods such as (*i*) Oral administration of 5, 10 and 20 ppm of IAA from III instar to spinning stage, (ii) treating the eggs with 5, 10 and 20 ppm of IAA for 30 minutes (iii) topical application of IAA at a concentration of 5,10 and 20 ppm at III instar and V instar separately. The procedure described by Pai *et al.* (1986) was followed for chemical administration. All the experiments were conducted at a standard laboratory conditions following

* Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore-570 006, India.

the silkworm rearing method as described by Krishnaswami (1978). The results obtained were subjected to statistical analysis (ANOVA as described by Snedecor and Cochran 1967) and were presented in Table 1 and 2.

RESULTS

Table 1 shows the effect of IAA on toxicity in multivoltine Pure Mysore race of silkworm *Bombyx mori.* Table reveals that even when IAA was administered orally at low concentrations, induced statistically significant (at 0.05 level by ANOVA) variation with regard to all the eight parameters analysed except for larval duration and hatching percentage.

Administration of IAA by egg treatment method also produces significant changes upto fecundity, while the number of larvae brushed and hatching percentage did not show a significant variation.

Topical application at III instar stage brought significant change in cocoon characters and hatching percentage, while it did not bring change in larval duration, fecundity and no. of larvae brushed. However, topical application of IAA at V instar stage showed significant variation in all the eight characters under study.

Table 2 exhibits the effect of IAA administration in bivoltine Kalimpong-A race of silkworm *B. mori.* It is evident from Table 2, that oral administration of IAA brings significant change in all the characters analysed except in hatching percentage. While egg treatment method showed that, the chemical brings significant variation in all the characters analysed except larval duration and yield per 10000 larvae brushed (by weight).

topical application at III instar as well at V instar stage brings significant variation in all the characters analysed.

Table 1. Effect of IAA on toxicity in multivoltine pure Mysore race of silkworm *Bombyx mori* L.

Mode of administration	Concentration	Larval duration (Hrs)	Total No. of cocoons harvested	Total wt. of cocoons harvested	Yield/10000 larvae brushed		Fecundity	Number of larvae brushed	Hatching percentage
					Number	Weight			
Oral	Control	682	96	92.1	9600	9.216	396	379	95.70
administration	Conc. 1	680	66	60.6	6600	6.070	369	355	96.90
	Conc. 2	680	66	60.5	6600	6.050	326	317	97.91
	Conc. 3	696	62	62.0	6200	6.204	308	304	98.90
'F' Value		2.95	7.26*	9.66*	4.87*	14.52*	12.41*	9.02*	2.84
Egg	Control	581	360	351.5	8571	8.374	492	460	97.48
Treatment	Conc. 1	650	401	403.7	9224	9.440	452	437	97.18
	Conc. 2	654	412	406.5	9214	9.755	494	479	96.98
	Conc. 3	651	330	336.4	8809	9.065	480	463	96.98
'F' Value		15.62*	6.18*	6.29*	8.45*	24.81*	4.52*	2.87	1.58
Topical	Control	624	90	85.4	9000	8.540	478	408	88.53
application	Conc. 1	624	96	90.6	9600	9.070	440	394	90.02
at III instar	Conc. 2	624	70	72.9	7000	7.290	420	380	91.12
	Conc. 3	629	58	58.3	5800	5.830	402	364	91.46
'F' Value		2.02	12.70*	13.20*	16.24*	6.14*	2.01	1.14	9.69*
Topical	Control	624	90	85.5	9000	8.541	478	408	85.53
application	Conc. 1	648	90	96.0	9000	9.600	498	426	86.14
at V instar	Conc. 2	648	74	76.4	7400	7.640	421	365	87.12
	Conc. 3	648	70	68.6	7000	6.860	516	456	89.14
'F' Value		5.07*	13.51*	13.91*	8.46*	9.62*	5.25*	19.01*	5.40*

*Statistically significant by ANOVA table Value : 4.07 at 0.05 level

DISCUSSION

Fecundity, hatchability, rate of development (in this case larval duration) viability (in this case., total number and weight of cocoons harvested, yield by number and weight/10000 larvae brushed etc., are some of the important parameters to evaluate the toxicity of any chemical or physical agents in animal test system (Luning, 1966; Sankaranarayanan, 1969; Gayathri and Krishnamurthy, 1979). These serve as good indicators of various somatic effects caused by the chemical in the test substrate (Luning, 1966; Sorsa and Pfeifer, 1973). The parameters analysed in the present studies herald the toxicity of the IAA in silk worms.

Many of the growth regulators are though are known to improve economical characters in silkworms (Akai, 1971; Akai and Kobayashi, 197; Kobari and Akai, 1978, 1979; Shimada 1981; Pai *et al.*, 1987), they are known to produce significant toxicity also (Pai *et al.*, 1991).

The authors have observed a significant variation (p value < 0.05) with regard to reduction in fecundity in all the modes of administration tested in both the races under investigation, except for topical application at III instar larval stage in Pure Mysore race. The reduced fecundity of an organism is not favoured by the Natural selection, which results in the elimination of the genotype in due course. Fecundity depends on the number of eggs produced, which in turn reflects the number of ovarioles present in the organism. According to Gruwez *et al* (1971) the fecundity and number of ovarioles in *Drosophila melanogaster* are dependent on the condition in which the worms are grown. The fecundity of an organism also depends on its hormonal system. The reduction in fecundity in silkworms in the present studies might be due to the effect of IAA in their hormonal system, which inturn might have affected the development of the ovarioles.

Table 2 shows the group of Pure Mysore and Kalimpong-A races obtained by administering of IAA,

Table 2. Effect of IAA on toxicity in bivoltine kalimpong-A of silkworm *Bombyx mori* L

Mode of administration	Concentration	Larval duration (Hrs)	Total No. of cocoons harvested	Total wt. of cocoons harvested (Gm)	Yield/10000 larvae brushed		Fecundity	Number of larvae brushed	Hatching percentage
					Number	Weight			
Oral administration	Control	624	88	157.5	8800	15.676	416	400	96.63
	Conc. 1	624	84	140.3	8400	14.030	380	363	96.12
	Conc. 2	633	92	175.0	9200	17.500	374	357	95.98
	Conc. 3	633	86	162.3	8600	16.230	361	340	95.13
'F' Value		23.36*	25.96*	22.05*	13.16*	5.11*	8.36*	29.78*	1.12
Egg Treatment	Control	600	373	578.5	8550	13.278	506	491	97.03
	Conc. 1	619	290	526.0	8529	14.469	520	496	98.13
	Conc. 2	619	301	479.0	8390	13.351	514	491	96.12
	Conc. 3	619	304	519.0	8379	14.304	590	540	94.41
'F' Value		1.44	13.20*	8.33*	6.99*	1.77	9.39*	12.39*	5.99*
Topical application at III instar	Control	576	93	180.4	9300	18.240	537	510	95.50
	Conc. 1	605	76	137.8	7600	13.779	515	423	88.14
	Conc. 2	605	68	142.4	6800	14.229	485	445	91.79
	Cone. 3	605	66	120.4	6000	12.041	451	415	94.15
'F' Value		5.79*	15.86*	4.70*	16.78*	7.94*	8.51*	7.33*	4.76*
Topical application at V instar	Control	576	93	153.0	9300	15.221	537	510	95.50
	Conc. 1	600	61	106.0	6100	10.700	520	310	60.10
	Conc. 2	605	58	101.0	5800	10.300	505	420	84.11
	Conc. 3	605	68	98.9	6800	9.910	495	475	96.66
'F' Value		28.30*	39.39*	48.21*	6.61*	6.66*	19.91*	18.19*	12.13*

*Statistically significant by ANOVA table value : 4.07 at 0.05 level exhibiting a reduced hatchability, is attributed to the effect of IAA on growing embryos. This conclusion is in conformity with the earlier studies on *Drosophila* (Luning, 1966., Sankaranarayanan, 1969).

It has also been observed that larval duration prolongs in many instances of administration viz., egg treatment and topical application at V instar to Pure Mysore race., oral administration, topical application at III and V instar stage to Kalimpong-A race. Similar observations were also made by earlier workers (Akai *et al.*, 1978., Krishnaswami *et al.*, 1979., Kuwana *et al.*, 1984., Pai *et al.*, 1986a) when they used several growth regulators and juvenile hormones to improve silk yield. The prolongation of larval duration was attributed to the physiological changes in silkworms due to chemical administration. But Bonneir (1960), is of the opinion that rate of development depends on both genetic and environmental factors. Authors feel that in the present studies the prolongation of larval duration is due to the effect of IAA altering the cellular environment and thus increase larval duration.

Total number and weight of cocoons harvested, yield by number and weight/10000 larvae brushed are the characters which portray the productivity. Further, productivity depends on Darwinian fitness and viability, which is an adaptive trait of an individual, the increase of which qualifies itself to survive and perpetuate in a given environment. It is also known that these characters are vulnerable to the changes in the internal and external environment. The present studies reveals that, there is a significant change with regard to these characters in all the modes of administration in both the races except at a single incidence of yield by weight/1000 larvae brushed for egg treatment method in Kalimpong-A race. Further, it also revealed that most of the times, there was a significant reduction in the characters analysed.

Thus, out of eight parameters analysed in Pure Mysore race, the authors have observed that, there is a significant variation in six characters in oral administration and egg treatment methods; five characters in topical application at III instar stage and all eight characters in topical application at V instar stage.

Similarly in Kalimpong-A race, when IAA was administered orally or at egg stage, there was significant variation in seven out of eight characters analysed. Further, when the mode of administration was topical application at III instar stage or at V instar stage, all the eight characters showed significant variation.

The reason for differences observed between Pure Mysore and Kalimpong-a races is because of the fact that the Pure Mysore race is indegenous, multivoltine race; while Kalimpon-A is a evolved, hybrid, bivoltine race. The variation with regard to the results obtained amongst the modes of administration in the same races is because of the time of the developmental stage at which the chemical was administration, quantity of the chemical entered into the test system., quantity of the chemical assimilated/discarded by the test system., physiological status of the test system for degrading the test chemical etc.,

Therefore on the basis of these results, it can be concluded that IAA is toxic to the silkworms at the concentrations tested.

REFERENCES

Agarwal, S.C. 1984. Effect of IAA and GA on akinite formation of *Pithophora oedogonium* (Mont) Wittrock, *Cur. Sci.*, **53** (4) : 217-218.

Akai, H. 1971. Ultrastructure of fibroin in silkgland of larval *Bombyx mori. Expti. Cell. Res.* **69** : 219-223.

Akai, H. and M. Kobayashi 1971. Induction of prolonged larval instars by JH in *Bombyx mori* L. (Lepidoptera : Bombycidae). *Appl. Ent. Zool.* **6** : 138-139.

Akai, H., Kiguchi, K., Kobari, Y. and A. Shibukawa, 1978 Practical utilization of juvenoids for increasing silk production. Scientific paper of the Institute or Organic and Physical chemistry of Wrociew Tech. University, No. 22, Conf. **7** : 781-792.

Dhingra, G., Sareen, P.K. and J.B. Chowdhury 1987. Pesticide mutagenesis-a tabulated review. Environmental Mutagen Society of India, Bombay, India.

Gayathri, M.V and N.B. Krishnamurthy 1979. differential sensitivity of different species of *Drosophila* to Ceresan - a mercurial fungicide. *Ind. J. Expt. Biol.* **17** (4) : 433-435.

Gruen, H.E. 1959. Auxins and fungi. *Ann. Rev. Plat. Physiol* **10** : 405-439.

Gruwez, G., Hoste, C., Lints, C.V., and F.A. Lints 1971. Oviposition rhythms in *Drosophila melanogaster* and its alteration by a change in photoperidicity. Experintia, **27** : 1414-1416.

Henbest, H.B., Jones E.R.H. and G.F. Smith 1953. Isolation of a new plant growth hormone. 3-indoleacetonitrite. *J. Chem. Soc.* 3796-3801.

Klambt, H.D. 1961. Wachstuminduktion and wwchsstoffmetabolismus in weizenkoleoptizylinder. Reacktion produkte der IES in Abhangigkeit von der inkubation sknonzentration. Plants, **56** : 309-321.

Kobari, Y. and H. Akai 1978. Utilization of Manta (Synthetic compound with Juvenile hormone activity) for silkworm rearing. *J. Seric. Sci.* Japan. **47** : 315-319.

Kobari, Y. and H. Akai 1979. On quantitative increase of silk production by the administration of juvenile hormone (Manta). *J. Seric. Sci.* Japan. **48** (1) : 37-42.

Krishnaswami, S. 1978. New technology of silkworm rearing. Bulletin No. 2 CSR and TI, Mysore, India.

Krishnaswami, S., Singh K., Raghuraman R. and R.G. Geethadevi 1979. To study the effect of chloramphenicol sprayed leaf as growth promoter. Ann. Rep. CSR and TI, Mysore India.

Kuwana, E., Takeya, R., M. Eto 1984. Synthesis and antijuvenile activity of 1 citronelly 1-5 substituted imidazole. *Agric. Biol. Chem.* **48** (12) : 3115-3119.

Luning, K.G. 1966. *Drosophila* tests in Pharmacology. Nature 209, 84-86.

Nomoto, M. and S. Tamura. 1970. Isolation and identification of indole derivatives in club-roots of chinese cabbage. *Agric. Biol. Chem.* **34** : 1590-1592.

Nelson, J.M. and C.C. Sharples. 1980. Effect of growth regulators on germination of cucumber (*Cucumis sativus*) and other cucurbit seeds at sub-optimal temperature. Hortsci. **15** (3) part-I : 253-254.

Okamoto, T., Isogai, Y., Koizumi, T., Fujishiro, H. and Y. Sato 1967. Studies on plant growth regulators, III isolation of indole-3-acetonitrile and methyl indole -3-acetate from the neutral fraction of Moyashi extract. *Chem. Pharm. Bull.* **15** : 163-168.

Pai, I.K. and N.B. Krishnamurthy. 1984. Effect of a growth promoter in multivoltine and bivoltine races of silkworm *Bombyx mori* L. XIV Int. Seri. Cong. Bangalore (Abst : Sericologia : France, **24** (3) : pp 429.

Pai, I.K., Hegde, S.N. and N.B. Krishnamurthy. 1986. Effect of Paraaminobenzoic acid on the hatchability in silkworm *Bombyx mori* (L.) Indian Zoologist, **10** (1 and 2) : 7-13.

Pai, I.K., Hegde S.N. and N.B. Krishnamurthy. 1987. Use of Paraaminobenzoicacid to improve yield in silkworm *Bombyx mori* L. *Ann. Trop. Res.* Philippines **9** : 178-183.

Pai, I.K., Hegde, S.N. and N.B. Krishnamurthy, 1991. Toxicity of Paraaminobenzoic acid in Pure Mysore and Kalimpong-A races of the silkworm *Bombyx mori L.* Sericologia, France, **31** (2) : 311-318.

Pohl, R. and W. Rother. 1953. Sporenkeimungund myzelwachstrum von *Phycomyces blakesleeanus* unter dem einfluss von wuchs and Hemmstoffen der hoheren pflanzen. Biol. Zent. **72** : 364-372.

Raza, S.H. 1978. Effect of some growth regulators on the seed germination of Kalyanasona wheat. *Ind. J. Agric. Res.* **12** (3) : 201-202.

Sankaranarayanan, K. 1969. The effect of nitrogen and oxygen frequency on X-ray induced dominant lethals and the physiology of sperms in *Drosophila melanogaster* Mut. Res. 641-661.

Singh, G., Sekhon, N. and M. Kaur. 1978. Effect of growth regulators on some yield contributing parameters in *Arachis hypogea. J. Res Punjab Agric. Univ.* **15** (1) : 106-111.

Shimada, S., Atsushi, K. and A. Shiji. 1981. The utilization of methoprene, a synthetic compound with JH activity on silkworm rearing. 3. Absorption, distribution with excretion of methoprene by the silkworm *Bombyx mori. J. Sericult.* (Japan), **50** (5) : 391-395.

Snedecor, W.G. and C.W. Cochran 1967. (Eds) Statistical methods. Oxford and IBH Publications. New Delhi, India.

Sorsa, M. and S. Pfeifer 1973. Effect of cadmium on development time and prepupal puffing patterns of *Drosophila melanogaster* Hereditas, **74** : 273-277.