JASEM ISSN 1119-8362

Full-text Available Online at
http:// www.bioline.org.br/ja

J. Appl. Sci. Environ. Mgt. 2003
Vol. 7 (2) 31 - 38

Influence of Bakuchiol, a JH analogue from Bemchi (*Psoralea corylifolia*) on Silk Production in Silkworm, *Bombyx mori* L. (Bombycidae: Lepidoptera)

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ABSTRACT: The influence of a juvenile hormone analogue (JHA), bakuchiol on the silk yield of silkworm, *Bombyx mori* L. was studied involving two popular commercial hybrids, KA x NB4D2 (bivoltine x bivoltine) and PM x NB4D2 (multivoltine x bivoltine). The compound was administered topically to 5th instars at 24, 48, 72 and 96 h as a single dose. Three-concentration *viz*., 0.625, 1.25 and 2.5 ppm were tested. Economic characters of the larvae and the resultant cocoon traits were measured. The result showed that 1.25 ppm of bakuchiol applied to 48 h old 5th instars was the most favourable treatment for improvement of commercial traits. Cocoon and cocoon shell weight and filament length have increased significantly due to this treatment. The possible role of exogenous JH analogues in eliciting this response in silkworm is discussed @ JASEM

In Insects, the molts and metamorphosis are initiated and coordinated by the circulating hormones (Riddiford, 1994). The interplay of ecdysteroid and juvenile hormone (JH) serves to orchestrate the progression from one developmental stage to the next, with the ecdysteroid regulating the onset and timing of the molt and the JH determining whether the molt would be larval-larval or larval-pupal (Gilbert *et al.*, 1996; Sehnal, 1989; Riddiford, 1994). When the threshold JH level to retain the juvenile or larval features diminishes in the hemolymph below the normal level, the larva prepares to metamorphose into the pupa. When JH is administered to the insect larvae, this exogenous JH disrupts the normal developmental pattern and result in developmental deformity. This has been the basic principle exploited in pest control by administering any of the JH analogues or mimics. (Nair and Trivedy, 1998). But interestingly, the silkworm, *Bombyx mori* L. is known to respond positively to exogenous JH analogues and mimics when administered in minute quantities and enhance commercial traits such as cocoon weight, cocoon shell weight and length of the silk filament (Akai *et al.*, 1985). Such enhancement is dependent on the dose of the compound, time of application and number of application (Chowdhary *et al.*, 1990). The present study, was aimed at examining the suitability of bakuchiol, a JH analogue isolated from the medicinal weed plant Bemchi (*Psoralea corylifolia*) for its suitability for use on silkworm, *B. mori* on larger scale for improvement of commercial traits and to establish the treatment regime eliciting such beneficial response.

MATERIALS AND METHODS

The juvenile hormone analogue (JHA), bakuchiol was procured from Dr. A. Banerji, Bio-organic Division, Bhabha Atomic Research Center, Mumbai, India.. Commercial bivoltine x bivoltine (KA x NB4D2 and multivoltine x bivoltine (PM x NB4D2) silkworm hybrids were reared on fresh mulberry leaves (S36 variety) in the laboratory at 25 ± 1 °C temperature and 75 ± 5 % relative humidity under 12:12 (light :dark) photoperiod following standard procedure. On resumption to 5th instar, 225 larvae of the bivoltine hybrid and 250 larvae of the multi x bivoltine hybrid were counted and reared in ventilated plastic rearing trays measuring 90 x 60 cm in three replicates for the administration of the JHA. Three concentrations *viz*., 0.625, 1.25 and 2.5 ppm of the compound, bakuchiol were prepared in the form of an emulsion using minimum quantity of acetone and tween-20, in water. The concentrations were decided based on the results of an extensive screening process (Nair *et al.*, 1999). The emulsion was administered topically to silkworm in pre-cleaned trays at the rate of 12.5 ml/100 larvae as a single dose by spraying it onto the larvae. After leaving the larvae as such for half an hour, fed with fresh mulberry leaves. Different such batches were treated at 24, 48, 72 and 96 h of 5th instar. Medium and absolute controls were maintained in parallel to compare the results. The medium controls were treated with an equal quantity of emulsion without the JHA while the absolute control was without any treatment. The larval weight was recorded on maximum growth (on the sixth day of 5th instar). Total number of good cocoons, cocoon weight and cocoon shell weight (average of 10 males and 10 females per replication) and filament length were recorded. Further, effective rate of rearing by number (ERR/no.) and weight (ERR/wt.), shell ratio (%) and denier of the filament were calculated as reported earlier (Nair *et al.*, 1997). The experiment was repeated twice. The data of the

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two trials were analyzed employing ANOVA to ascertain the statistical significance.

RESULTS AND DISCUSSION

The data on the effect of application of bakuchiol on the economic traits of commercial silkworm hybrids, KA x NB2D2 and PM x NB4D2 are presented in Tables 1 and 2, respectively and in the Figures 1 to 4.

A perusal of the Table 1 reveals that in the bivoltine hybrid, KA x NB4D2, all the three concentrations of the compound used in this study showed substantial positive influence both at 24 and 48 h in the case of larval weight. The lowest concentration, 0.625 ppm has shown notable influence at 72 and 96 h as well. But among all the treatments, 1.25 ppm at 48 h was the best with regard to improvement in the larval weight with a maximum change of 10.68 %. In the case of the multi x bivoltine hybrid, PM x NB4D2, the positive influence in the larval weight has been strictly confined to the treatments at 48 h. Out of the three concentrations, the maximum influence was brought out by 1.25 ppm with a percentage change of 7.80. This was followed by 0.625 ppm with 5.45 % change and 2.5 ppm with 3.66 % change (Table 2). The medium control at any of the treatment hours did not show any remarkable difference compared to control. Similar type of increase in larval weight on administration of JH compounds was reported earlier (Akai *et al.*, 1985; Trivedy *et al., 1997*). In almost such cases, the increase in larval weight was accompanied by a prolongation in the larval period. However, the larvae treated with bakuchiol commenced spinning within 168 h of 5th instar regardless of the concentrations used and the age of treatment although an increase in the larval weight was induced. This result corroborates the results of Muroga *et al.* (1975) who reported an increase in larval weight without an accompanying deviation from the normal developmental duration. Recent studies by Trivedy *et al.* (1996) also found that increase in economic traits of silkworm on JH application need not necessarily be accompanied by an increase in larval period.

From Figures 1 to 4, it is clear that bakuchiol elicited a positive influence on silk production. Though many of the treatments appeared to have exerted substantial influence on silkworm, the maximum improvement in cocoon weight (11.82 %) was observed with 1.25 ppm at 48 h in the case of bivoltine hybrid. This was followed by an enhancement of 8.13 and 7.02 % when treated with 0.625 ppm at 48 and 24 h, respectively (Figure 1A). In multi x bivoltine hybrid, the improvement was maximum (8.46 %) with 1.25 ppm at 48 h followed by 0.625 ppm at the same age (7.23 %) (Figure 1B).

Cocoon shell weight also increased substantially following the administration of bakuchiol. The pattern of improvement in shell weight was also similar to that of cocoon weight. The maximum improvement in cocoon weight was obtained with 1.25 ppm at 48 h (14.55 %) in the bivoltine hybrid. This was followed again by a treatment at same age with 0.625 ppm. The increase was 9.61 % (Figure 2A). In PM x NB4D2, increases in cocoon shell weight of 11.24 % and 8.38 % with 1.25 ppm and 0.625 ppm bakuchiol, respectively were recorded when treated at 48h (Figure 2B). Generally, the silkworms are more sensitive to administration of JH analogues at an age between 24 and 48 h of 5th instar in respect of enhancement in cocoon characters than at a later age (Nair *et al.*, 2000). In this study also the larvae treated at 72 and 96 h showed only meager increase wherever recorded. Shell ratio did not show any marked increase in any of the treatment except the treatment with 1.25 ppm at 72 and 96 h. This could be attributed to the lower pupal weights because a corresponding increase in the cocoon weight was not recorded in these batches. One striking feature in cocoon and cocoon shell weight was that on optimum treatment, bivoltine hybrid showed better response than the multivoltine hybrid. This gives an indication that the bivoltines are far more sensitive to the JH analogues than the multivoltines.

Some workers reported improvement in cocoon and cocoon shell weight on administration of JH analogues, earlier. On administration of a JH analogue, Murakoshi *et al.* (1972) reported an enhanced yield of 20-35 %. Chang *et al.* (1972) reported an increase of 20-50 % in cocoon and pupal weights when final instars were treated up to 6th day. However, the increase was always accompanied by a prolonged feeding period. Muroga *et al.* (1975) was able to get only an enhancement of 9-14 % but without any change in the larval period. Trivedy *et al.* (1997) got considerable increase in the cocoon and shell weight on administration of minute quantities of a strong JH mimic. These reports make it clear that the response of silkworm in terms of improvement in economic traits varies with the compounds used, silkworm races and geographical region. In Indian context, inexpensive and easily available compounds, which are indigenous could be cashed on for using on silkworm.

In both KA x NB4D2 and PM x NB4D2 (Figures 3-4) the effective rate of rearing was not largely influenced by the administration of bakuchiol although marginal increase was noticed in the batches treated at 24 and 48h. Interestingly, there was no decline in this trait in any of the treatment batches barring the one treated at 96 h. But here, the decline was extremely negligible. This is a major deviation from the report of Magadum and Magadum (1991) who reported notable decline in the cocooning percentage (indicative of ERR by number) in *Samia* *cynthia ricini* on application of JH compounds, though other parameters improved considerably. But this is in agreement with the reports of Trivedy *et al.* (1993) who did not observe any substantial change in the ERR between JH-treated and control silkworms.

Table 1: Effect of JH analogue, bakuchiol on the economic traits of silkworm, *Bombyx mori* L. (Hybrid: KA x NB4D2)

Treatment hour (5th instar)	Concentration of JHA (ppm)	Larval weight (g) (10 nos.)	5th instar larval period (h)	Shell ratio (%)	Average filament length (m)	Denier
24	0.625	49.12*	168	20.63	941.09*	2.47
	1.25	49.15*	168	20.62	941.17*	2.49
	2.5	48.23*	168	20.71	908.96*	2.46
	Medium control	46.62	168	20.44	863.08	2.51
48	0.625	49.93*	168	20.59	959.13*	2.48
	1.25	51.31*	168	20.81	975.65*	2.44
	2.5	48.60*	168	20.55	949.73	2.47
	Medium control	46.52	168	20.59	896.04	2.49
72	0.625	49.45*	168	20.59	924.49*	2.46
	1.25	47.81	168	20.89*	935.77*	2.51
	2.5	47.84*	168	20.57	885.31	2.49
	Medium control	46.31	168	20.20	880.74	2.51
96	0.625	48.29*	168	20.57	904.7*	2.46
	1.25	47.19	168	21.40*	892.25	2.46
	2.5	47.22	168	20.66	850.34	2.46
	Medium control	46.08	168	20.51	853.73	2.43
Absolute control		46.36	168	20.32	860.92	2.45
Standard error ± Critical difference at 5%		0.51 ± 1.45	NS	0.19 ± 0.53	12.72 ±35.92	NS

* - Significant ($P < 0.05$) NS - Not significant

The commercially more important trait, ERR by weight showed a fairly better positive change than the ERR by number. The highest increase in the bivoltine hybrid was 5.74% (Figure 4A) and that in the multi x bivoltine hybrids was 7% (Figure 4B). These changes were significant ones. Both these values were found in the silkworm treated with 1.25 ppm at 48 h. This increase in ERR by weight could be attributed to the increase in the cocoon weight on bakuchiol administration.

The increased cocoon shell weight is understood to have been converted to the end product, the reelable silk filament. A maximum increase of 13.33 and 17.08 % in filament length was observed in KA x NB4D2 (Table 1) and PM x NB4D2 (Table 2), respectively in the batches treated with 1.25 ppm bakuchiol at 48 h. This was followed by notable increase in other treatment batches at 24 and 48 h as well. At the same time, there was no significant change in denier. This is to be appreciated because, if the thicker cocoon shell is unwound to shorter filament of thicker denier, the industry may not be keen to accept it as widely known.

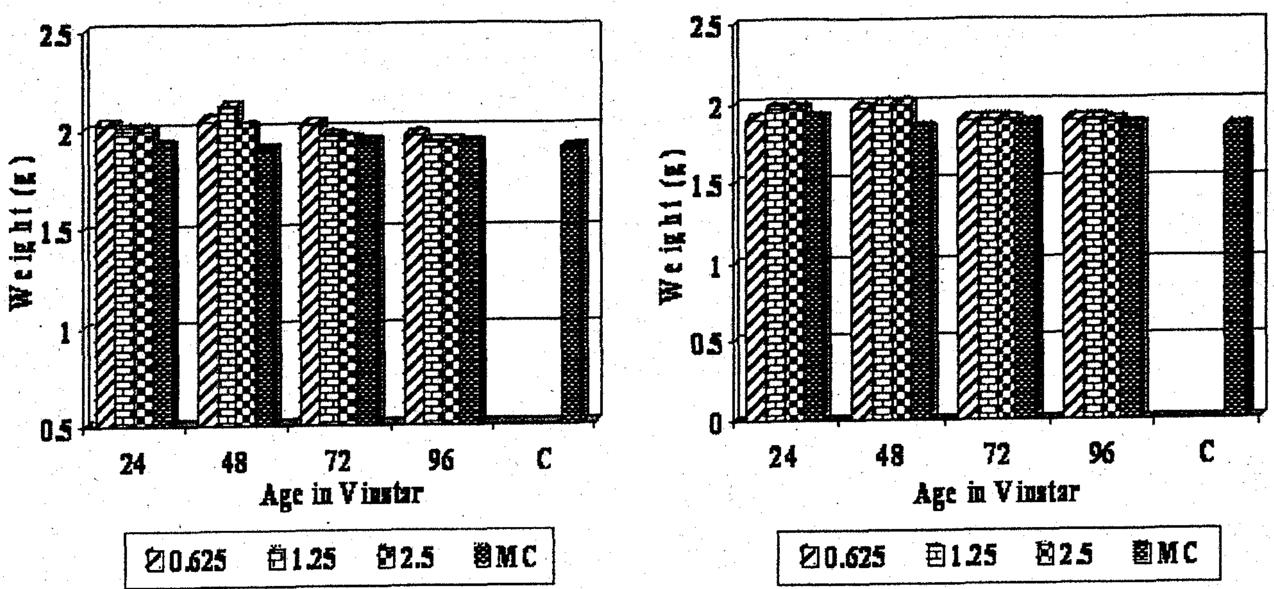

Fig 1. Effect of bakuchiol, on Cocoon weight of silkworm, *Bombyx mori* L. 1A: KA x NB4D2; 1B: PM x NB4D2

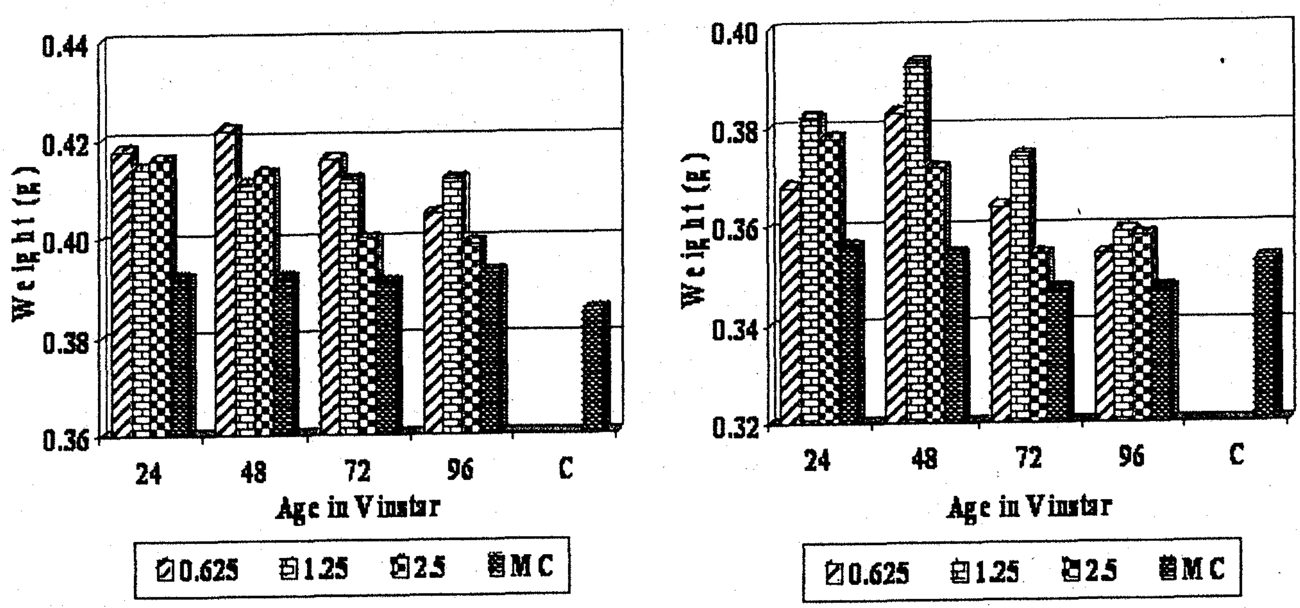

Fig 2: Effect of bakuchiol, on Cocoon shell weight of silkworm, *Bombyx mori* L. 2A: KA x NB4D2; 2B: PM x NB4D2

0.625, 1.25 and 2.5 are the concentrations of Bakuchiol in ppm; MC- medium control; C- absolute control.

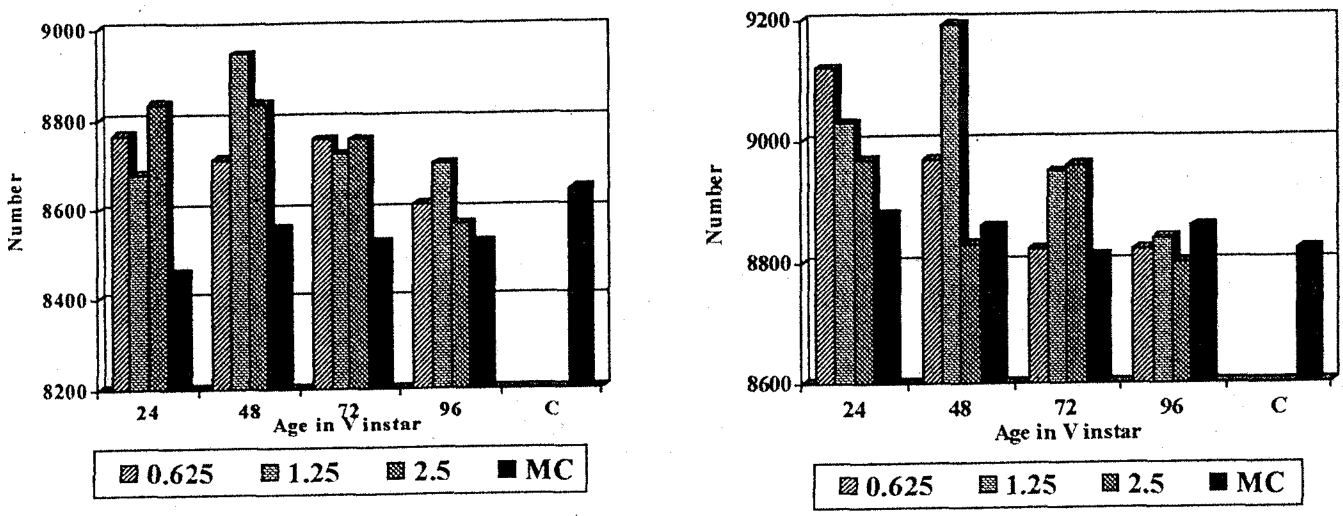

Fig 3. Effect of bakuchiol, on ERR by number of silkworm, *Bombyx mori* L. 3A: KA x NB4D2; 3B: PM x NB4D2

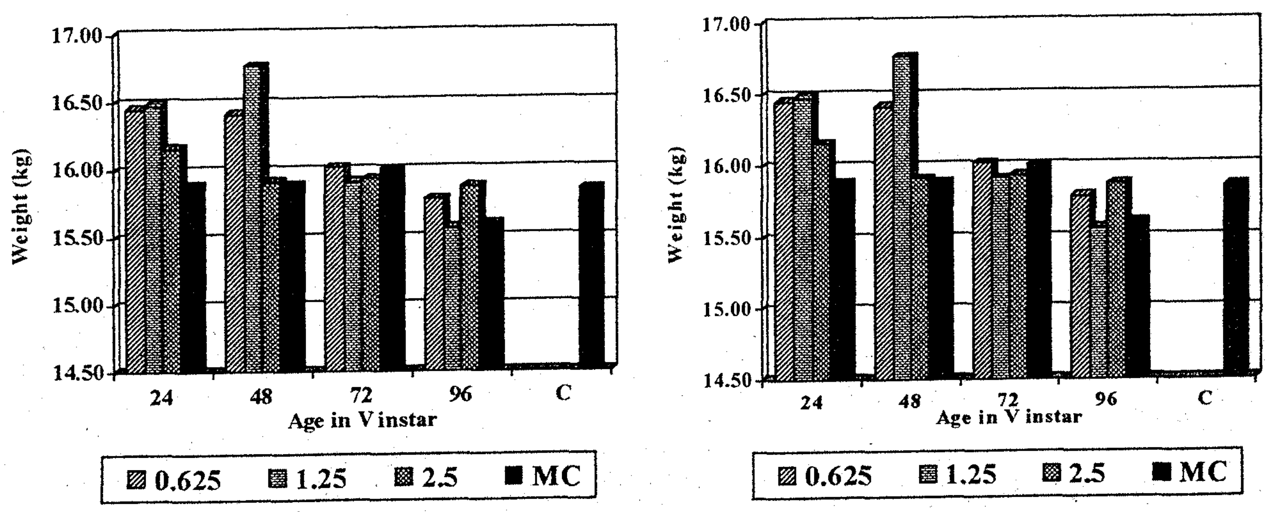

Fig 4: Effect of bakuchiol, on ERR by weight of silkworm, *Bombyx mori* L. 4A: KA x NB4D2; 4B: PM x NB4D2

0.625, 1.25 and 2.5 are the concentrations of Bakuchiol in ppm; MC- medium control; C- absolute control.

Table 2: Effect of JH analogue, bakuchiol on the economic traits of silkworm, *Bombyx mori* L. (Hybrid: PM x NB4D2)

Treatment hour (5th instar)	Concentration of JHA (ppm)	Larval weight (g) (10 nos.)	5th instar larval period (h)	Shell ratio (%)	Average filament length (m)	Denier
24	0.625	41.90	168	19.23	844.02*	2.47
	1.25	41.98	168	19.44	871.26*	2.47
	2.5	42.04	168	19.27	832.58*	2.48
	Medium control	41.52	168	18.75	797.52	2.50
48	0.625	43.28*	168	19.45	851.59*	2.47
	1.25	44.25*	168	19.73	902.74*	2.49
	2.5	42.55*	168	18.97	856.05*	2.49
	Medium control	41.29	168	19.30	798.94	2.48
72	0.625	41.24	168	19.27	840.69*	2.49
	1.25	41.58	168	19.79*	845.12*	2.48
	2.5	41.41	168	18.68*	821.70	2.48
	Medium control	41.17	168	18.68	799.15	2.52
96	0.625	42.27	168	18.69*	809.51	2.47
	1.25	41.06	168	19.00	815.99*	2.48
	2.5	40.29	168	18.99	793.11	2.46
	Medium control	40.69	168	18.71*	777.16	2.44
Absolute control		41.04	168	19.23	771.04	2.49
SE ±		0.504	NS	0.180	15.77	NS
Cd at 5%		1.423		0.509	44.55	

SE = standard error * - Significant ($P < 0.05$) NS - Not significant Cd = Critical difference

The result of the present investigation indicate that natural or synthetic bio-active compounds mimicking juvenile hormone activity in other insects can be judiciously employed in sericulture for the benefit of the industry. Some potentially toxic substances at sub-inhibitory concentrations can have stimulatory effects. This phenomenon is termed as hormesis. Even a highly effective pest control agent azadirachtin was reported to have hormetic effect in *Tribolium castaneum* (Mukherjee *et al.*, 1993). A better example is R394, a strong juvenoid which on application to 7th instar larvae of *Galleria mellonella* at the concentration of 10-100 µg on 3rd -6th day led to imperfect super larvae (Sehnal *et al.*, 1986) but on extremely minute concentration enhanced silk production in *Bombyx mori,* considerably (Trivedy *et al.*, 1997). Bakuchiol used in the present study is originally a third generation pest control agent as any other JH analogue/mimic. It is isolated from the medicinal weed plant, *Psoralea corylifolia.* The high activity of the petroleum ether extracts called for further studies, which led to the isolation of this main active principle. Bakuchiol was found highly active against the nymphs of Red Cotton bug, *Dysdercus koenigii* (Bhan *et al.*, 1980).

This investigation gives a clear indication that though JH mimicking compounds influence the silk production positively, it is largely dependent on the dose and time of application as stated earlier (Akai *et al.*, 1985; Trivedy *et al.*, 1993). The endogenous JH levels of the last instar larvae deplete within the first one third of the last larval period and hence juvenoid treatment before this critical stage enhances the feeding activity and silk production. It may be interpreted that the enhanced silk production is a concerted effect of the conversion of additional quantity of leaf consumed during the extended period and a direct stimulatory effect of the compound on protein synthesis in silk gland as suggested by Kajiura and Yamashita (1989). These changes at the physiological or molecular level might be the result of an alteration in the ratio of the circulating hormone.

It is concluded that the JH analogue, bakuchiol can be used in sericulture for yield improvement as it can induce a hormetic effect in silkworm. On elaborate bioassay it has become clear that the compound at 1.25 ppm when treated at 48 h of 5[th] instar, elicits favourable response in silkworm in improving the commercial traits notably.

Acknowledgements: The authors are grateful to Dr. A. Banerji, Bio-organic Division, Bhabha Atomic Research Center, Mumbai, India for his kind supply of the JH analogue. They are also grateful to Dr. R.K.Datta, former director of Central Sericultural Research and Training Institute, Karnataka, India for providing all the facilities for accomplishing the research work.

REFERENCES

Akai, H; Kimura, K; Kiuchi, M; Shibukawa, A (1985). Increase of silk production by repeated treatments with a juvenile hormone analogue. J Seric Sci Jpn 54: 297-299.

Bhan, P; Soman, R; Dev, S (1980). Insect juvenile hormone mimics based on bakuchiol. Agric Biol Chem 44:1483-1487.

Chang, C F.; Murakoshi, S; Tamura, S (1972). Giant cocoon formation in the silkworm, *Bombyx mori* L. topically treated with methylnedioxyphenyl derivatives. Agric Biol Chem 36: 692-694.

Chowdhary, S K; Raju, P S; Ogra, R K (1990). Effect of JH analogues on silkworm, *Bombyx mori* L. growth and developments of silk gland. Sericologia 30: 155-165.

Gilbert, L I; Rybczynski, R; Tobe, S (1996). Endocrine cascade in insect metamorphosis. In: Gilbert, L I; Tata, J; Atkison, P (Eds). Metamorphosis: Postembryonic reprogramming of gene expression in Amphibian and Insect Cells. Academic Press, San Diego. P. 59.

Kajiura , S; Yamashita, O (1989). Super growth of silk glands in the dauer larvae of silkworm, *Bombyx mori* induced by a juvenile hormone analogue. J Seric Sci Jpn 58: 39-46.

Magadum, V B; Magadum, S B (1991). Influence of manta on some economical characters of Eri silkworm, *Samia cynthia ricini* Biosduval. Korean J Seric Sci 33: 93-96.

Mukherjee, S N; Rawal, S K; Ghumare, S S; Sharma, R N (1993). Hormetic concentrations of azadirachtin and isoesterase profiles in *Tribolium casteneum* (Herbst) (Coleoptera:Tenebrionidae). Experientia 49:557-560.

Murakoshi , S; Chang, C F; Tamura, S (1972). Increase in silk production by the silkworm, *Bombyx mori* L. due to oral administration of a juvenile hormone analog. Agric Biol Chem 36: 695-696.

Muroga, A; Nakajima, M; Aomori, S; Ozawa, Y; Nihmura M (1975). Utilization of the synthetic juvenile hormone analog to the silkworm rearing on mulberry leaves. J Seric Sci Jpn 44:267-273.

Nair, K S; Trivedy, K (1998). Juvenile hormone mimics and silk productivity. Indian Silk 37: 23-25.

Nair, K S; Trivedy, K; Magadum, S B; Datta, R K (1997). Improvement of economic characters of mulberry silkworm, *Bombyx mori* L., by a phytohormone, Abscisic acid. J Enotmol Res 21: 343:349.

Nair, K S; Vijyan, V A; Nair, J S; Trivedy, K (1999). Juvenilomimic compounds for enhanced productivity in silkworm, *Bombyx mori* L. A screening. Indian J Seric 38: 119-124.

Nair, K S; Vijayan, V A; Nair, J S; Trivedy, K; Chinya, P K (2000). Influence of a plant based juvenile hormone mimic. w-formyl longifolene oxime citronellyl ether on silkworm, *Bombyx mori* L. Sericologia 40:551-557.

Riddiford, L M (1994). Cellular and molecular action of juvenile hormone. General consideration and premetamorphic actions. Adv Insect Physiol 24: 213-274.

Sehnal, F (1989). Hormonal role of ecdysteroids in insect larvae and during metamorphosis. In: Koolman, J. (Ed) Ecdysone. Georg Thieme Verlag. Stuttgart. P. 271.

Sehnal, F; Delbecque, J; Maroy, P; Mala, J (1986). Ecdysteroid titres during larval life and metamorphosis of *Galleria mellonella.* Insect Biochem 16: 156-162.

Trivedy, K; Nair, K S; Ahsan, M M; Datta, R K (1997). A juvenile hormone mimic modulated enhancement of silk productivity in silkworm, *Bombyx mori* L. Indian J Seric 36: 35-38.

Trivedy, K; Porcheron, P; Lafont, R; Magadum, S B; Datta, R K (1996). Alteration of ecdysteroid titre and spinning by juvenile hormone analogue, R394 in silkworm, *Bombyx mori.* Indian J Exp Biol 34543-546.

Trivedy, K; Remadevi, O K; Magadum, S B; Datta, R K (1993). Effect of juvenile hormone analogue, labomin on the growth and economic characters of silkworm, *Bombyx mori* L. Indian J Seric 32:162-168.