Journal of Biopesticides 3(1 Special Issue) 212 - 216 (2010) 212

Phyto-Juvenile hormone for augmentation in cocoon yield in silkworm, *Bombyx mori* L.

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ABSTRACT

Juvenile hormone (JH) analogues and mimics regardless of synthetic or of plant/animal origin are potent pest control agents and are popularly referred to as 'third generation pest control agents'. ω-formyl longifolene oxime propargyl ether (NL13), an oxime ether of carbonyl compounds derived from longifolene, a tricyclin sesquiterpene from Indian turpentine oil extracted from the chir pine, *Pinus longifolia* (Roxb., Pinaceae) and bakuchiol, isolated from the medicinal weed, *Psoralea coryllifolia (*Linnaues) (Papilionaceae) are proven biopesticides against *Culex quinquefasciatus* and *Dysdercus* Koenigi, respectively. These two compounds show JH mimicking activities and disrupt the regular developmental pattern and thus identified as potential biopesticides. Since the larvae of silkworm *Bombyx mori* L. are known to respond positively to administration of exogenous JH analogues and mimics in terms of enhanced silk production, minute quantities of these two compounds were administered to fifth instar larvae based on the results of previous broad spectrum dose response studies. Emulsions containing 2.5, 5 and 10 ppm of NL13 and 0.625, 1.25 and 2.5 ppm of bakuchiol were administered to the fifth instar larvae of bivoltine silkworm hybrid, KA x NB_4D_2 at 24, 48, 72 and 96 h at the rate of 12.5 ml/100 larvae, 30 minutes prior to feeding at about 10 am. The compounds elicited notable positive response in silkworm in terms of improved economic traits such as larval and cocoon characters. In the case of NL13, 5 ppm and in the case of bakuchiol, 1.25 ppm of the compounds at 48 h of 5^{th} instar showed the maximum improvement in the cocoon traits in the range of 10~15%. The physiological impact of the compounds on silkworm growth and development and the resultant impact on the commercial traits are discussed.

Key words: Bakuchiol, *Bombyx mori*, JHA, NL13, silkworm.

INTRODUCTION

In insects, the interplay of ecdysteroid and juvenile hormone (JH) serves to orchestrate the progression from one developmental stage to the next, with ecdysteroid regulating the onset and timing of the moult and the JH determining whether the moult would be larval-larval or larval-pupal (Gilbert *et al.,* 1996; Mamatha *et al.*, 2005). Plants are the primary source of juvenoids for phytophagous insecs like silkworm and its role in silkworm development is well established (Khyade *et al.*, 2007). Retention of larval features long enough enabling the silkworm larvae to consume optimum quantity of mulberry leaf is of paramount importance to silk production. When the threshold JH level to retain the juvenile features diminishes in the hemolymph below the normal level, the larva prepares to metamorphose into the pupa. When JH or JH like compounds are administered to the insect larvae, they disrupt the normal developmental pattern leading to developmental deformity. But interestingly, the silkworm, *Bombyx mori* L. is known to have a stimulatory effect on the administration of exogenous JH analogues in minute quantities which lead to enhancement in commercial traits such as cocoon weight, cocoon shell weight and silk filament length (Akai *et al*., 1985; Mamatha *et al*., 2005; 2008). The enhancement is dependent on the dose of the compound, time of application and number of application (Chowdhary *et al*., 1990, Miranda *et al*., 2002; Mamatha *et al*., 2006; 2008). The present study was taken up to examine the effect of two JH mimics *viz*., NL13, isolated from *Pinus lingifolia* and bakuchiol, isolated from the medicinal weed *Psoralea corylifolia* for their suitability to use on silkworm, *B. mori* for improvement of commercial traits and to establish the treatment regime eliciting such beneficial response. If these JH mimics are found effective on silkworm leading to increased silk production, it can replace the imported synthetic JH analogues to a certain extent and save considerable foreign exchange. This study assumes greater significance since JH mimics are now gaining popularity for commercial application at farmers' level.

MATERIALS AND METHODS

The oxime ether of longifolene isolated from *Pinus longifolia* coded as NL13 (ω - Formyl longifolene oxime propargyl ether) was obtained from National Chemical Laboratory, Pune and Bakuchiol, from Bioorganic Division, Bhabha Atomic Research Center, Mumbai. Bivoltine x bivoltine (KA x NB_4D_2) silkworm hybrids were reared on fresh mulberry leaves in the laboratory at 25 ± 1° C and 75 ± 5 % RH under 12:12 (L:D) photoperiod following standard procedure (Babu *et al.*, 2010). On resumption to fifth instar, 100 larvae were counted and reared in ventilated plastic trays measuring 56 x 36 cm in three replicates. Three concentrations *viz.*, 2.5, 5 and 10 ppm of NL13 and 0.625, 1.25 and 2.5 ppm of bakuchiol were prepared in the form of an emulsion using 5 ml of acetone and 2 ml of tween-20, in a litre of water. The emulsion was then administered by spraying topically to silkworm in precleaned trays at the rate of 12.5 ml/100 larvae as a single dose. After half an hour, the worms were fed with fresh mulberry leaves. Different batches were treated at 24, 48, 72 and 96 h of fifth instar. Control larvae treated with the medium (acetone and tween-20 in water) were maintained side by side to compare the results. The larval weight was recorded on the sixth day of fifth instar on maximum growth. On maturation of 10-20% of the worms, the larvae were mounted for cocoon formation and on sixth day, the cocoons were harvested. Total number of good cocoons, cocoon weight and cocoon shell weight (average of 10 males and 10 females per replication) and average filament length were recorded as explained in our earlier work (Nair *et al.*, 2001). Further, survival, cocoon yield per 10000 larvae and shell percentage were calculated. The experiment was repeated twice. The data of the two trials were analyzed employing ANOVA to ascertain the statistical significance using 'Analyse-It' statistical package.

RESULTS AND DISCUSSION

The data on the effect of application of NL13 and bakuchiol on the economic traits of silkworm hybrid, KA x NB_4D_2 are presented in Tables 1 and 2. A perusal of Table 1 reveals that larval weight enhanced by a maximum (6.72 %) when NL13 was administered at 48 h with 5 ppm. This was followed by an increase of 5.02 % when treated with 2.5 ppm at 96 h. Significant improvement was also observed on treating the larvae with 10 ppm at 24 and 48 h and also with 5 ppm at 96 h (df:12, $F = 31.4$, $P<0.05$). Table 2 showed substantial positive influence both at 24 and 48 h. The lowest concentration *i.e.*, 0.625 ppm showed notable influence at 72 and 96 h as well. However, 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 %. Similar increase in larval weight on administration of JH compounds was reported earlier (Akai *et al.*, 1985, Trivedy *et al.*, 1997, Miranda *et al.*, 2002, Mamatha *et al.*, 2008).

NL13 did not exert any influence on survival regardless of the concentration and the time of treatment. However, bakuchiol, elicited significant increase in the survival when treated with 1.25 ppm at 24 h and with 2.5 ppm both at 24 and 48 h. There was no decline in this trait in any of the treatment batches. This is a remarkable deviation from the report of Magadum and Magadum (1991) who recorded notable decline in the survival in *Samia cynthia ricini* (Boisdual : Saturniidae) on application of JH compounds but in agreement with the reports of Trivedy *et al.* (1997) who did not observe any substantial change in survival between JH-treated and control silkworms. Cocoon yield showed a fairly better positive change than the survival. In the case of NL13, the improvement was to the tune of 8.24 % when treated at 48 h with 5 ppm. This was followed by an improvement of 5.63 % with 5 ppm application at 72 h. When bakuchiol was administered to the fifth instar silkwom, the highest increase in cocoon yield was observed when treated at 48 h followed by 24 h with an increase of 5.74 and 3.97 %, respectively. These changes were statistically significant (df:12, $F = 26.37$, $P < 0.05$.).

It is obvious from the results that the JH mimics are capable of eliciting positive response in silk production. Though many of the treatments appeared to have exerted substantial influence on silkworm in terms of improvement in cocoon weight, the maximum improvement (10.83 %) was observed with 5 ppm at 48 h in the case of NL13 ($P<0.05$). This was followed by an enhancement of 7.75 % when treated at 24 h with 10 ppm. When treated with bakuchiol, the high improvement (11.82 %) was observed with 1.25 ppm at 48 h followed by an enhancement of 8.13 and 7.02 % when treated with 0.625 ppm at 48 and at 24 h, respectively. Similarly, cocoon shell weight also improved. The improvement pattern was also similar. The maximum improvement when treated with NL13 was with 5 ppm at 48 h (14.46 %) followed by 10 ppm at 24 h (8.71 %) (df:12, f:22.17, $P < 0.05$). In the case of bakuchiol, the highest improvement was obtained with 1.25 ppm at 48 h (14.55 %) followed by a an improvement of 9.61 % when treated at 48 h with 0.625 ppm. Shell percentage did not change notably except in the larvae treated with 1.25 ppm bakuchiol at 72 and 96 h. This could be attributed to the lower pupal weights of 1.559 and 1.516 g respectively. When the cocoon weight remains almost same, even a marginal reduction in the pupal weight can translate into a relatively higher shell percentage.

Earlier workers reported improvement in cocoon and cocoon shell weight on administration of JHA. Trivedy

Table 1. Effect of the JHA, NL13 on the economic traits of silkworm, *B. mori* L.

Treatment hour (V instar)	Concent -ration(ppm)	Larval weight (g)	Survival (%)	Cocoon yield/ 10000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell percentage	Filament length (m)
24	2.5	4.546	80.78	16.13	1.898	0.386*	20.34	893.44*
	5	4.532	80.33	16.41*	1.957*	0.394*	20.10	899.50*
	10	4.633*	82.33	16.29*	1.999*	0.403*	20.14	875.63*
48	2.5	4.522	79.22	15.77	1.862	0.379*	20.33	915.11*
	5	4.746*	80.44	17.08*	2.060*	0.424*	20.57*	940.09*
	10	4.641*	82.78	16.24	1.944*	0.392*	20.14	909.80*
72	2.5	4.507	79.67	15.71	1.891	0.378*	20.00	892.82*
	5	4.559	81.89	16.67*	1.921*	0.388*	20.19	885.90*
	10	4.444	79.78	15.93	1.897	0.379*	19.95	885.31*
96	2.5	4.670*	80.00	16.22	1.913*	0.381*	19.89	887.72*
	5	4.614*	80.78	15.78	1.881	0.378	20.08	875.58*
	10	4.530	81.33	16.09	1.883	0.373	19.82	833.68
Control		4.447	79.89	15.78	1.859	0.370	19.92	827.56
SE ±		0.043	NS	0.181	0.017	0.003	0.214	16.76
CD at 5%		0.122		0.511	0.049	0.007	0.605	47.34

Post Anova test: Turkey's HSD, *Significant ($P < 0.05$), NS Non-significant.

et al. (1997) got considerable increase in the cocoon and shell weight on administration of minute quantities of a JH mimic, R394. These reports suggest that the response of silkworm in terms of improvement in economic traits varies with the compounds used, silkworm races and geographical region. In the Indian context, inexpensive and easily available compounds could be exploited for using on silkworm for augmentation in the cocoon yield. The increased cocoon shell weight is understood to have been converted to the end product, the reelable silk filament. A maximum increase of 13.60 % in filament length was observed when 5 ppm NL13 was administered at 48 h. In the case of bakuchiol, the maximum increase was 13.33 % when treated with a concentration of 1.25 ppm at 48 h. This was followed by notable increase in other treatment batches at 24 and 48 h as well.

The results of the present investigation indicate that the natural biopesticides which are biologically active in other insects can be judiciously employed in sericulture for the

Table 2. Effect of JHA, bakuchiol on the economic traits of silkworm, *B. mori* L.

Treatment hour (V instar)	Concent -ration(ppm)	Larval weight (g)	Survival (%)	Cocoon yield/ 10000 larvae (kg)	Cocoon weight (g)	Shell weight (g)	Shell percentage	Filament length (m)
24	0.625	4.912*	87.66	16.45*	2.028*	0.418*	20.63	941.09*
	1.25	4.915*	86.77	16.48*	2.007*	0.414*	20.62	941.17*
	2.5	4.823*	88.33*	16.16	2.008*	0.416*	20.71	908.96*
48	0.625	4.993*	87.11	16.41*	2.049*	0.422*	20.59	959.13*
	1.25	5.131*	89.44*	16.76*	2.119*	0.441*	20.81	975.65*
	2.5	4.860*	88.33*	15.91	2.012*	0.413*	20.55	949.73
72	0.625	4.945*	87.55	16.01	2.021*	0.416*	20.59	924.49*
	1.25	4.781	87.22	15.91	1.971*	0.412*	20.89*	935.77*
	2.5	4.784*	87.55	15.93	1.947	0.400*	20.57	885.31
96	0.625	4.829*	86.11	15.78	1.973*	0.405*	20.57	904.7*
	1.25	4.719	87.00	15.56	1.928	0.412*	21.40*	892.25
	2.5	4.722	85.66	15.87	1.933	0.399*	20.66	850.34
Control		4.64	86.44	15.85	1.895	0.385	20.32	860.92
SE ±		0.051	0.49	0.134	0.023	0.004	0.187	12.72
CD at 5%		0.145	1.39	0.379	0.065	0.011	0.527	35.92

Post ANOVA test: Turkey's HSD, *Significant ($P < 0.05$).

benefit of the industry. Some potentially toxic JH mimics at sub-inhibitory concentrations can have stimulatory effects on silkworm. R394, a strong synthetic juvenoid is a classic example which on application to the larvae of *Galleria mellolella* (L., Pyralidae) at the concentration of 10-100 μg 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). NL13 and bakuchiol used in the present study are originally third generation pest control agents as any other JH analogue/ mimic. NL13 has been proved effective in controlling mosquito, *Culex quinquefasciatus* (Culicidae) the vector of filariasis (Sawaikar *et al*., 1995). Bakuchiol was found active against the nymphs of Red Cotton bug, *Dysdercus koenigii* (F.) (Pyrrhocoridae) (Bhan *et al*., 1980).

This investigation gives a clear indication that though JH mimics influence the silk production positively, it is largely dependent on the dose and time of application as stated earlier (Akai *et al.*, 1985, Nair *et al*., 2001). The increase in larval weight of about 7 % in the case of NL13 and about 11 % in the case of bakuchiol was a clear case of manifestation of JH effect. The increase in the body weight naturally got translated into an increased cocoon weight by 11~12 % and cocoon shell weight by about 15 %. This enhancement is the result of the certain physiological phenomenon in which JH plays a critical role. The endogenous JH levels of the last instar larvae deplete within the first one third of the last larval period and hence treatment with JH mimics before this critical stage enhances the feeding activity and silk production. The exogenous JH mimics exert a direct stimulatory effect 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 hormones leading to the changed feeding behaviour and enhanced economic traits.

It is concluded that the JH mimics, NL13 and bakuchiol can be used in sericulture for yield improvement as it can induce a stimulatory effect in silkworm. On elaborate bioassay it has become clear that 5 ppm NL13 and 1.25 ppm bakuchiol administered at 48 h of 5^{th} instar, elicited favourable response in silkworm in significantly improving the cocoon weight up to 11~12 % and cocoon shell weight up to 15 %. This could be due to a direct stimulatory effect of JH mimics on protein synthesis in silk gland.

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