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Improvement in Cocoon Yield Induced by a Juvenile Hormone Analogue, SB-515 in the Bivoltine Silkworm (*Bombyx mori* L.) Hybrid, CSR2 x CSR4 and its Reciprocal Combination

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ABSTRACT

A juvenile hormone analogue JHA, SB-515 (Isopropyl (2E, 4E)-11-methoxy-3,7,11 trimethyl-2-1,4-dodecadienoate) was administered to the popular and high yielding bivoltine silkworm hybrid CSR2×CSR4 and its reciprocal cross for enhancing the cocoon yield. The compound was administered at 24, 48 and 72 h of 5th instar at concentrations such as 1.25, 2.5 and 5 ppm as a single dose. Untreated controls were maintained in parallel to compare the results. Apart from measuring the major commercial traits pertaining to cocoon yield, larval growth pattern was studied. The JHA extended the larval feeding period in the range of 6-48 h depending on the concentration of the compound and the time of administration. SB-515 improved the cocoon traits such as cocoon weight, shell weight and cocoon shell percentage significantly. Maximum increase in the case of CSR2×CSR4 was observed in the larvae treated with 2.5 ppm at 48 h whereas, in CSR4×CSR2 this was in the larvae treated at 72 h with the same concentration. Since the enhancement in the cocoon yield is substantial on JHA administration the compound can be exploited in the commercial sericulture.

Key words: Cocoon traits, juvenile hormone analogue, larval growth, methoprene, commercial sericulture

INTRODUCTION

The subtle interplay of heamolymph hormone titres, principally ecdysteroid and Juvenile Hormone (JH) serves to orchestrate the progression from one developmental stage to the next in insects. In such a process, ecdysteroid regulates the onset and timing of the moult and the JH determines whether the moult would be larval-larval or larval-pupal (Mamatha *et al*., 2006). Corpora allata produce JH with modulations in production occurring during moult cycles. In the last larval instar in silkworm, the JH titre declines to undetectable levels. If this natural decline is delayed or disrupted, the manifestations follow accordingly in the larvae. This delay or disruption can be achieved by administering an exogenous dose of JH analogue by which control of a large number of insect pests can be achieved. But in silkworm, *Bombyx mori* L., exogenous dose of minute quantity of JHA elicits positive response in terms of growth and increased silk production

(Nair *et al.*, 2003). This stimulatory effect was reiterated when commercial traits such as cocoon weight, cocoon shell weight and silk filament length were enhanced through administration of exogenous JH analogues in minute quantities (Mamatha *et al.*, 2008). The enhancement is dependent on the dose of the compound, time of application and number of application (Miranda *et al.*, 2002). This experiment was planned to figure out the dose and critical time of administration of JHA to silkworm which would considerably increase the cocoon yield in the popular high yielding bivoltine silkworm hybrids. This was with an obvious intention of translating this technology to be adopted in the field. SB-515 is a formulation of active RS-methoprene which is widely used for control of mosquitoes and ants in other countries mainly because no health hazard is reported on this third generation insect control agent.

MATERIALS AND METHODS

Silkworm rearing: The study was taken up during the year, 2004-05. Disease free eggs of popular and high yielding bivoltine silkworm hybrids, CSR2×CSR4 and its reciprocal combination, CSR4×CSR2 were used in this study. Silkworms were reared following standard recommended conditions at a higher temperature bracket of 29±1°C (to simulate summer condition) and 75±5% relative humidity under 12:12 (light:dark) photoperiod. Victory-1 mulberry variety leaves, harvested from a frequently irrigated mulberry garden were fed to silkworms three times a day. After the fourth ecdysis, larvae were counted and divided into four groups and continued the rearing in ventilated plastic rearing trays measuring 90×60 cm.

Administration of SB-515: The JHA, SB-515 (Isopropyl (2E, 4E)-11-methoxy-3,7,11 trimethyl-2-1,4-dodecadienoate) used in this study was the generous supply from SDS Biotech, KK, Japan as a part of a research understanding. SB-515 emulsion (0.125%) was diluted with water to make concentrations *viz.*, 1.25, 2.5 and 5 ppm. The mixture was then topically applied to different batches of 5th instar silkworm at 24, 48 and 72 h at the rate of 12.5 mL/100 larvae. The larvae were left for 30 min and then fed with fresh mulberry leaves. Untreated control was maintained in parallel to compare the results. Each treatment was replicated 3 times with 250 larvae per replication.

Data collection: Weight of larvae (10 No.) was recorded daily at 10 a.m. and the same was plotted in line graph to monitor the growth of the larvae under the influence of JHA. The duration of the 5th instar was calculated in the treated larvae and control. On maturation, the larvae were transferred onto cocoon building devices and the cocoons were harvested on 6th day. Total number of good cocoons, cocoon weight and cocoon shell weight (average of 10 males and 10 females per replication) were recorded. Further, survival, yield per 10000 larvae and the shell percentage were calculated using standard formulae. The experiment was repeated twice.

Statistical analysis: The data were subjected to statistical analysis employing one way ANOVA to ascertain the significance of the result at 5 and 1% level using 'Analyse-It' statistical package.

RESULTS AND DISCUSSION

Larval growth trend, weight and duration: The growth trend in the silkworm treated with different concentrations is represented graphically (Fig. 1a-c) for the hybrid, CSR2×CSR4. For this, the larval weight was recorded at an interval of 24 h to capture the trend of progressive larval growth on JHA administration. As the growth trend was similar in both the races, the one with CSR2×CSR4 alone is presented.

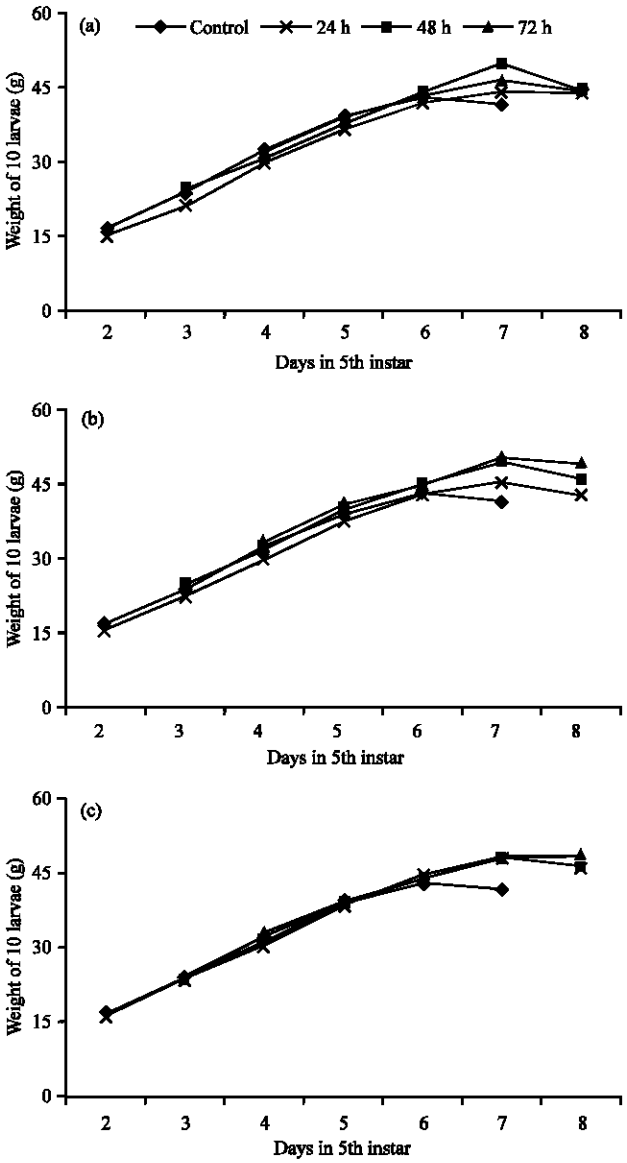

Fig. 1(a-c): Effect of SB-515 on larval weight and growth pattern of bivoltine silkworm hybrid, CSR2×CSR4. (a) At concentration 1.25 ppm, (b) 2.5 ppm and (c) 5 ppm

It is fairly clear from Fig. 1a that the larval growth was affected by 1.25 ppm of JHA but depending on the time of application, there was a variation in the weight gain. Although the difference between the 72 h treated silkworm and the control was much higher, that between 24 h treated and the control was negligible. The larvae treated at 48 h were placed between 72 h treatment and 24 h treatment. In the case of 2.5 ppm concentration, the ones treated at 48 and 72 h behaved almost similarly but the larvae treated at 24 h had comparatively low weight right through their growth in the 5th instar. Quite interestingly, in the initial days, the controls were performing at par with the larvae treated at 48 and 72 h, but the difference grew wider towards the end (Fig. 1b). The case was far more striking when treated with 5 ppm concentration. In this case, regardless of the time of the treatment, the growth trend was similar and there was no

Table 1: Effect of administration of a JHA, SB-515 on the economic traits of CSR2×CSR4 hybrid of silkworm, *Bombyx mori* L.

Time of administration in 5th instar	Concentration (ppm)	V instar larval duration (h)	Cocoon weight (g)	Shell weight (g)	Shell (%) percentage	Survival (%)	Yield/10000 larvae (kg)
24 h	1.25	156** (+12)	1.808 (+2.62)	0.413 (+2.38)	22.87 (0.00)	88.10 (+1.85)	15.55 (+2.06)
	2.5	158** (+14)	1.804 (+2.43)	0.411 (+1.84)	22.75(-0.52)	89.28* (+3.21)	15.58 (+2.30)
	5	165** (+21)	1.886** (+7.08)	0.427* (+5.92)	22.64 (-1.00)	88.14 (+1.90)	16.00 (+5.01)
48 h	1.25	170** (+26)	1.917** (+8.83)	0.444** (+10.10)	23.15 (+1.23)	89.42* (+3.38)	16.69** (+9.55)
	2.5	170** (+26)	1.947** (+10.51)	0.452** (+12.00)	23.17 (+1.30)	90.01** (+4.06)	17.57** (+15.32)
	5	178** (+34)	1.932** (+9.68)	0.453** (+12.24)	23.41 (+2.37)	87.81 (+1.51)	17.19** (+12.86)
72 h	1.25	173** (+29)	1.901** (+7.93)	0.457** (+13.26)	23.94* (+4.69)	87.94 (+1.66)	16.65** (+9.29)
	2.5	173** (+29)	1.868** (+6.05)	0.454** (+12.66)	24.32** (+6.34)	88.13 (+1.88)	16.59** (+8.93)
	5	179** (+35)	1.861** (+5.67)	0.446** (+10.62)	23.98* (+4.84)	86.90 (+0.47)	16.11* (+5.79)
Control		144	1.761	0.403	22.87	86.50	15.23
SE±		1.740	0.022	0.007	0.291	0.877	0.293
CD at 5%		1.440	0.063	0.021	0.833	2.501	0.838
CD at 1%		4.117	0.085	0.028	1.115	3.355	1.122

Values in parentheses are percentage difference from the control except in larval duration which shows actual difference in hours. *Significant ($p<0.05$), **Significant ($p<0.01$)

Table 2: Effect of administration of a JHA, SB515 on the economic traits of CSR4×CSR2 hybrid of silkworm, *Bombyx mori* L.

Time of administration in 5th instar	Concentration (ppm)	V instar larval duration (h)	Cocoon weight (g)	Shell weight (g)	Shell (%) percentage	Survival (%)	Yield/10000 larvae (kg)
24 h	1.25	146* (+6)	1.771 (-0.77)	0.429 (+5.70)	24.23 (+6.41)	85.51 (-1.18)	13.76 (-7.30)
	2.5	146* (+6)	1.753 (-1.78)	0.393 (-3.00)	22.42 (-1.56)	89.08 (+2.95)	15.54 (+4.66)
	5	146* (+6)	1.754 (-1.72)	0.412 (+1.51)	23.44 (+2.92)	86.53 (0.00)	15.87 (+6.87)
48 h	1.25	164** (+24)	1.768 (-0.91)	0.412 (+1.74)	23.33 (+2.45)	87.83 (+1.50)	15.58 (+4.95)
	2.5	164** (+24)	1.959** (+9.80)	0.462* (+14.07)	23.58 (+3.56)	89.13 (+3.00)	17.40** (+17.20)
	5	164** (+24)	1.987** (+11.38)	0.487** (+20.00)	24.49 (+7.56)	88.06 (+1.76)	17.65** (+18.86)
72 h	1.25	168** (+28)	1.904 (+6.69)	0.482** (+18.94)	25.33* (+11.22)	85.53 (-1.16)	15.99 (+7.71)
	2.5	188** (+48)	2.097** (+17.50)	0.527** (+29.92)	25.13* (+10.36)	84.07 (-2.84)	16.92* (+13.98)
	5	188** (+48)	2.001** (+12.13)	0.497** (+22.47)	24.82 (+8.98)	85.90 (-0.73)	16.68* (+12.31)
Control		140	1.784	0.405	22.77	86.53	14.85
SE±		1.920	0.043	0.015	0.758	2.086	0.594
CD at 5%		5.706	0.126	0.046	2.252	6.200	1.764
CD at 1%		7.817	0.173	0.063	3.085	8.493	2.417

Values in parentheses are percentage difference from the control except in larval duration which shows actual difference in hours. * Significant ($p<0.05$), ** Significant ($p<0.01$)

difference among the three treatment times and they had almost similar difference in the weight gain from the control (Fig. 1c). Ultimately, it was the concentration and time of treatment that decided the total increment in the larval weight and duration. In CSR2×CSR4, the larvae treated at 48 h with 2.5 ppm had the maximum larval weight with an improvement of 15% over the control followed by that treated with the concentrations of 5 and 1.25 ppm at 48 h. Treatment with 2.5 ppm at 72 h also recorded improvement of the same magnitude. These treatments extended the larval feeding period by 26~34 h when compared to the control (Table 1). The extension in larval period in the case of CSR4×CSR2 was 24 h in the larvae treated at 48 h regardless of the concentration used. This was interesting because the gain in larval weight was not uniform in all the treatment. Five ppm resulted in maximum weight gain (11.55%). Although, the weight gain in the larvae treated at 72 h was in the range of 8.5~10.98%, the extension in larval period was a whopping 24~48 h (Table 2).

The extension of larval feeding period and corresponding increase in the larval weight on JHA administration is not uncommon. In our exclusive study to examine this phenomenon on application of a juveiod, R394, it was proved unequivocally that the larval feeding period indeed extends (Nair *et al.*, 2004). But it can be compound, dose and time dependent. This result is also in agreement with that of Akai and Kobayashi (1971) and Sohn (1986), who reported that such increase in larval weight is invariably accompanied by an increase in the larval period. Similar type of increase in larval weight on administration of JH compounds was also reported earlier (Akai *et al.*, 1985). In almost all such cases, the increase in larval weight was accompanied by a prolongation in the larval period. In this study, the larvae treated with SB-515 commenced spinning in 144~188 h as against 140~144 h in the control.

Cocoon traits: The data on cocoon traits indicate that SB-515 elicited a remarkably positive influence on silk production. Though many of the treatments appeared to have exerted substantial influence on silkworm, the maximum improvement in cocoon weight was observed with 2.5 ppm at 48 h in the case of the hybrid, CSR2×CSR4. The improvement was to the tune of 10.51%. This was followed by the treatment with 5% concentration at 48 h with an improvement of 9.68% (Table 1). In CSR4×CSR2, 2.5 ppm when treated at 72 h had the maximum impact with an increase of 17.5%. 5 ppm concentration at 48 and 72 h also had considerable positive impact on cocoon weight with a surge of 11.38 and 12.13%, respectively (Table 2).

Cocoon shell weight also increased substantially following the administration of SB-515. The pattern of improvement in shell weight was similar to that of cocoon weight but with a higher margin. Although, the maximum improvement in cocoon shell weight in CSR2×CSR4 was obtained with 1.25 ppm at 72 h (13.26%), all the treatments at 48 and 72 h registered significant improvement (Table 1). In CSR4×CSR2, increase in cocoon shell weight was prominent with 19~30% surge when treated at 72 h. The treatments at 48 h with 2.5 and 5 ppm concentrations also recorded good improvement in the range of 14-20% (Table 2). Generally, the silkworms are more sensitive to administration of JH analogues at an age between 48 and 72 h of 5th instar in respect of enhancement in cocoon characters (Nair *et al.*, 2000). This study reiterated this theory in respect of cocoon and cocoon shell weight.

Shell percentage did not show any significant increase when treated at 24 and 48 h although marginal increase was registered. But those treated at 72 h had quantum increase in the shell percentage in the case of CSR4×CSR2 (Table 2). This could be attributed to the higher magnitude of increase in the cocoon shell weight which was comparatively more than that in the cocoon weight and the increase in cocoon weight did not commensurate with that in shell weight. One striking feature in cocoon and cocoon shell weight as understandable was that the higher increase was accompanied by increased prolongation in larval duration.

Quite a few 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. 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.

Survival and cocoon yield: In CSR2×CSR4, the survival was marginally but positively influenced by the administration of SB-515 in general and the improvement in the case of

treatment with 2.5 ppm at 24 and 48 h and that with 1.25 ppm at 48 h were statistically significant. But at the same time, the slight improvement in survival found in the reciprocal cross was not significant. This is a major deviation from the results of Magadum and Magadum (1991), who reported notable decline in the cocooning percentage (which can otherwise be referred to as survival) 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 survival between JH-treated and control silkworms.

The commercially most important trait, the cocoon yield showed considerable positive change on SB-515 administration. The highest increase in CSR2×CSR4 hybrid was 15.32% when treated with 2.5 ppm at 48 h. There was also considerable improvement in the treatment with 5 ppm at 48 h and with these concentrations at 72 h (Table 1). In the case of CSR4×CSR2, the maximum increase was noticed with 5 ppm at 48 h (18.86%) followed by 2.5 ppm (17.20%) and 2.5 (13.98%) and 5 ppm at 72 h (12.31%) (Table 2). These significant changes were very important in preparation to the field trials of the formulation.

The results of this 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 especially insect hormone analogues at sub-inhibitory concentrations can have stimulatory effects. A striking example is R394, a strong JHA 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). JH compounds in combination with phytogenous ecdysteroids on administration also proved effective not only in enhancing the yield but in managing the prolonged larval period (Nair *et al.*, 2008). SB-515 used in the present study is originally a third generation pest control agent as any other JH analogue/mimic and used extensively for mosquito and ant control. Although, improvements are prominent in the 72 h treatment, considering the prolongation in the larval duration, 48 h treatment was considered to be a better option for field evaluation.

It is concluded that the JH analogue, SB-515 on administration to silkworm larvae in the 5th instar at 1.25, 2.5 and 5 ppm concentration enhances the yield substantially. On the elaborate laboratory study, it has become clear that the compound at 2.5 ppm when treated at 48 h of 5th instar, was the most beneficial as the extension in larval duration was within the manageable limits.

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