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EFFECT OF TOPICAL ADMINISTRATION OF JH MIMIC AND PERFORMANCE OF QUALITATIVE AND QUANTITATIVE TRAITS OF CSR_2 AND MG_{408} BIVOLTINE BREEDS OF THE SILKWORM, *BOMBYX MORI L.*

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Abstract: The silk content of the cocoon would be enhanced and achieved the goal by the topical application of juvenile hormonal (JH) mimic namely "samrudhi" on popular bivoltine breeds namely CSR_2 and MG_{408}. During fifth instar at 48h, 72h and 96h of intervals with 15µl, 25µl and 35µl concentrations separately in triplicate to all the three experimental batches were maintained. Each concentration was brought up to 12.50 ml with distilled water then sprayed on 100 silkworm larvae of each batches and an efforts were made to study the degree of improvement in treated batches over control. As per observation made in treated batches exhibited 15-20% improvement in qualitative and quantitative traits with prolongation of fifth instar larval duration.
Keywords: *Bombyx mori* L., economic traits and Samrudhi.

1. INTRODUCTION

The lepidopteran beneficial insect silkworm, *Bombyx mori* L. is monophagous feeds only on *Morus alba*. The silkworm growth, development and metamorphosis are controlled by hormonal regulation. Endocrine organs of any insect system namely brain, suboesophagial ganglion (SG), corpora cardiaca (CC), corpora allata (CA) and prothoracic gland (PG) plays an important role in the development and regulation of the terrestrial insect life. The juvenile hormone controls the silkgland function and prevents their degeneration and can directly cause an increase in silk production (Sehnal and Akai, 1990). With this back ground many workers were used JH mimics in sericulture to enhance the silk yield. Earlier these synthetic juvenoids were used as third generation pesticides. JH analogues are insect specific, it delays the larval maturation and increases the silk production (Akai and Kobayashi, 1971; Akai *et al*., 1973, 1981; Kajiura and Yamashita, 1989) with appropriate dosage but high dosage of JH administration results no sign of cocoon spinning. In insect's prothoracicotrophic hormone (PTTH), Juvenile

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hormone (JH) and ecdysone which plays major role in moulting period and metamorphosis (Wigglesworth, 1985). In India many workers used these JH analogues in sericulture to improve the silk yield (Chowdhary *et al.,* 1990; Trivedy *et al.*, 1993; Nair *et al.,* 2000). With this background an experimental design was made and attempted to improve the qualitative and quantitative traits of oval shaped cocoons of CSR_2 and MG_{408} bivoltine breeds.

2. MATERIALS AND METHODS

JH analogue "SAMRUDHI" a bioactive efficient product developed by Central Sericulture Research & Training Institute, Mysore & Tokyo University of Agriculture & Technology, Tokyo, Japan. This product was purchased from Triumph Pharmaceuticals Private Limited, Industrial area, Metagalli, Mysore, for the research investigation. Two different productive bivoltine breeds were chosen for the study namely CSR_2 and MG_{408} are the oval shaped cocoons were utilized from Silkworm Germplasm Bank, DOS in Sericulture Science, Manasagangothri, Mysore. As per experimental design, disease free layings were selected and rearing was conducted in standard condition at 25±1ºC and 75±5% relative humidity under 12:12 (L: D) photoperiod as per Krishnaswamy (1978) with fresh mulberry leaves. Pre cleaned ventilated plastic trays measuring 90cmx60cm were used for silkworm rearing. JH mimic samrudhi was prepared in three concentrations i.e. 15µl samrudhi added to 12.50ml of distilled water mixed well then used for 100 silkworm larvae. In the same way 25µl & 35µl dosages were prepared. Then selected two robust silkworm breeds were treated at 48h, 72h and 96h intervals and each interval were treated by all the three concentrations in single dosage in fifth instar in three different batches and maintained throughout the completion of life cycle along with respective control. The experiment was repeated twice and recorded all the commercial parameters of treated and control batches. Scientific data were subjected and analyzed by using SPSS 17.00 package.

3. RESULTS AND DISCUSSION

The research data in relation to the influence of JH mimic samrudhi on fifth instar silkworm larvae and economic characters of oval shaped cocoons of bivoltines breeds are presented on table-1 & 2 in three intervals of time on topical administration. The effect of concentration of juvenile hormonal mimic on egg parameters namely fecundity and hatching percentage is negatively correlated and represented at 48h and 96h of intervals (Table 1).

Whereas at 72h of treatment there is an increase in percent change (1.99%) in CSR_2 breed (table 1). In MG_{408} at 72h and 96h of intervals showed a improvement but at 48h of intervals (Table 2) relatively a decrease was observed (-1.61). Though the egg characters are genetically inherited but it is influenced by the environmental temperature during incubation period.

3.1 Larval traits: The effect of the JH hormone on the larval characters namely larval weight, larval duration is significantly contributed a positive response during late larval stage. The maximum contribution of 10% increase is recorded at 35µl concentration under 96h of intervals. The 48 and 72h proportionately lesser in change in CSR_2 breed (Table 1). Similar trend also observed in MG_{408} in three intervals of time duration in all the three different concentrations.

3.2 Cocoon characters: The cocoon yield by number and weight are the two volumetric parameters evaluated and represented. At 25µl among the three concentrations a drastic increase of more than 15% changes in the 72h of intrevals (Table 1). Whereas, in MG_{408} a marginal improvement of 1.8% in yield by number, but yield by weight has a significant boosting of percent change in all the three concentrations (Table 2). The cocoon characters are assessed and evaluated in all the selected silkworm breeds as important quantitative traits namely cocoon weight shell weight and shell ratio. There is a greater implication were recorded in 72h of intervals which is most responded critical period to maximize the manifestation of the cocoon weight and shell weight (Table 1). But the shell ratio in MG_{408} is declined maximum of 3.5% in all the three concentrations (Table 2).

3.3 Filament length and denier: The increased shell weight represents the converted silk content in the form of bave which is indicated as a qualitative parameter. The 15µl and 25µl dosages of topical administration were insisted the magnification of the length and weight (table 1) but at 35µl of administration a decline is observed in CSR_2 breed (table 1). Whereas in MG_{408} breed a consistent level of result obtained and is very effective (Table 2). Even in the denier the exogenous administration of any compound which is like an analogue and crude extract can also elevate the cocoon parameters.

The role of JH mimics for the stimulation and conversion of silk protein synthesis at molecular level and elicits substantially an improvement in the filament characters including the denier. with this above results in the investigation and believed that the synthetic active functional JH mimic is potential for the benefit of sericulture industry and registered a prominent exogenous hormone for enhancing the activity of feeding and silk production and concluded that hormonal influence can alter the synthesis of silk fibroin and boosting of silk content will help the industry. The JH mimic in an insect growth and development regulates the physiological manifestation and influences the synthesis of the fibroin and cocoon production and prevents the larval maturation (Kurata 1981, Akai et al 1985).

The response of the silkworm in relation to topical administration of the JH mimic depends on the dosage and time (Chowdhury *et al.*, 1990). The JH compound was tested and justified in respective to increase of rate of silk protein, cocoon weight, shell weight was reported by Nair *et al.*, (2002) and screened many JH mimic compounds for the purpose of understanding the effect on silkworm growth and silk yield. Many workers reported an improvement in cocoon and cocoon shell weight and administration of JH analogue increased about 20-50% as reported by Murakashi *et al.*, (1972). Akai also (1985) achieved 26% increase in cocoon shell weight in a repeated treatment of silkworm with JH compound. Magdam and Magdam (1991) also worked and reported in *Samia cynthia ricini* which is an indicative of low ERR by number, but other parameters are substantially improved. Trivedi *et al.,* (1993) did not observed any substantial changes in the ERR among juvenoids treated and control batches.

Table 1: Effect of different concentration of JH mimic (Samrudhi) on economic traits of CSR_2 breed of silkworm, *Bombyx mori* L.

Parameters	Control Mean±SD	Conc. of JH	Treatment Mean±SD (48 h)		Treatment Mean±SD (72h)		Treatment Mean±SD (96 h)	
Eggs per laying	525.00±9.16	15 µl	516.00±6.56	(-1.744)	524.67±9.07	(-0.064)	524.00±13.527	(-0.191)
		25 µl	528.67±11.50	(+0.694)	533.67±10.97	(+1.624)	522.33±7.023	(-0.511)
		35 µl	516.33±7.02	(-1.679)	535.67±10.07	(+1.991)	514.00±8.000	(-1.697)
Egg Hatching (%)	98.03±0.64	15 µl	98.32±0.76	(+0.288)	98.47±0.54	(+0.443)	98.16±0.90	(+0.132)
		25 µl	97.98±0.75	(-0.058)	97.69±0.55	(-0.355)	97.89±0.16	(-0.150)
		35 µl	97.99±0.44	(-0.044)	97.39±1.26	(-0.661)	98.44±0.37	(+0.410)
Weight. of 5th age larva (g)	4.11±0.07	15 µl	4.30±0.05	(+4.437)	4.40±0.05*	(+6.578)	4.30±0.03	(+4.370)
		25 µl	4.30±0.00*	(+5.505)	4.47±0.26*	(+7.964)	4.40±0.07*	(+6.677)
		35 µl	4.35±0.06*	(+5.505)	4.47±0.05*	(+8.067)	4.50±0.06*	(+8.720)
5th age larval Duration (h)	168.00±0.00	15 µl	178.00±0.00	(+5.618)	178.00±0.00	(+5.618)	178.00±0.00	(+5.618)
		25 µl	178.00±0.00	(+5.618)	178.00±0.00	(+5.618)	178.00±0.00	(+5.618)
		35 µl	183.00±1.00	(+8.197)	183.00±0.00	(+8.197)	188.00±0.00	(+10.638)

Yield/number	8766.67±251.66	15 µl	9066.67±57.3	(+3.309)	9133.33±152.5	(+4.015)	9100.00±100.00	(+3.663)
		25 µl	873.33±152.5	(-0.382)	9133.33±57.73	(+4.015)	9033.33±57.73	(+2.952)
		35 µl	8133.33±152.7	(-7.787)	9066.67±115.7	(+3.309)	9000.00±100.00	(+2.593)
Yield/weight (kgs)	15.77±0.45	15 µl	16.73±0.11*	(+5.777)	18.13±0.50*	(+13.051)	16.60±0.2000*	(+5.020)
		25 µl	16.37±0.30	(+3.666)	18.73±0.15*	(+15.836)	17.20±0.100*	(+8.333)
		35 µl	15.17±0.30	(-3.956)	18.56±0.28*	(+15.081)	17.10±0.200*	(+7.797)
Cocoon weight (g).	1.801±0.00	15 µl	1.850±0.00	(+2.648)	2.00±0.004	(+10.191)	1.832±0.002	(+1.692)
		25 µl	1.882±0.00	(+4.273)	2.05±0.005*	(+12.388)	1.909±0.003*	(+5.676)
		35 µl	1.872±0.00	(+3.761)	2.05±0.002*	(+12.217)	1.905±0.005*	(+5.458)
Shell weight (g).	0.422±0.00	15 µl	0441±0.00	(+4.311)	0.475±0.001*	(+11.221)	0.431±0.001	(+2.316)
		25 µl	0.442±0.00	(+4.657)	0.491±0.001*	(+14.166)	0.431±0.002	(+2.316)
		35 µl	0.442±0.00	(+4.528)	0.491±0.001	(+14.114)	0.446±0.002	(+5.448)
Shell ratio (%)	23.40±0.08	15 µl	23.80±0.02	(+1.680)	23.67±0.055	(+1.155)	23.55±0.055	(+0.637)
		25 µl	23.50±0.11	(+0.425)	23.89±0.060	(+2.051)	24.17±0.069*	(+3.172)
		35 µl	23.59±0.06	(+0.805)	23.92±0.080	(+2.174)	23.40±0.045	(-0.014)
Filament length (m)	1048.66±3.21	15 µl	1071.00±1.00	(+2.086)	1072.66±2.516	(+2.237)	1062.00±2.645	(+1.256)
		25 µl	1070.66±2.08	(+2.055)	1103.00±3.000*	(+4.927)	1092.66±2.516	(+4.027)
		35 µl	1059.66±1.52	(+1.038)	1090.33±1.527	(+3.822)	1068.00±2.081	(+1.841)
Filament weight (g)	0.36±0.00	15 µl	0.37±0.00	(+2.176)	0.36±0.00	(+0.443)	0.361±0.001	(+0.443)
		25 µl	0.36±0.00	(+1.263)	0.38±0.00*	(+7.461)	0.382±0.002*	(+5.912)
		35 µl	0.36±0.00	(+1.452)	0.38±0.00*	(+6.741)	0.362±0.002	(+0.718)
Denier	3.08±0.00	15 µl	3.08±0.01	(0.000)	3.02±0.00	(-1.987)	3.050±0.005	(-0.984)
		25 µl	3.05±0.00	(-0.984)	3.16±0.01	(+2.532)	3.140±0.015	(+1.911)
		35 µl	3.09±0.01	(+0.324)	3.18±0.00	(+3.145)	3.040±0.015	(-1.316)

*Significant ($p < 0.05$) values in parentheses are the percentage difference from the control

Table 2: Effect of different concentration of JH mimic (Samrudhi) on economic traits of MG_{408} breed of silkworm, *Bombyx mori* L.

Parameters	**Control Mean±SD**	**Conc. of JH**	**Treatment Mean±SD (48 h)**		**Treatment Mean±SD (72h)**		**Treatment Mean±SD (96 h)**	
Eggs per layings	523.00±10.816	15 µl	516.33±11.060	(-1.291)	532.67±7.505	(+1.815)	527.33±9.018	(+0.822
		25 µl	522.33±7.505	(-0.128)	542.67±15.011	(+3.624)	531.00±13.114	(+1.507)
		35 µl	514.67±5.507	(-1.619)	522.00±9.165	(-0.192)	540.33±9.504	(+3.208)

Egg Hatching (%)	98.22±0.898	15 µl	98.00±0.704	(-0.224)	97.68±0.753	(-0.553)	98.17±0.368	(-0.054)
		25 µl	98.41±0.919	(+0.190)	97.79±0.270	(-0.440)	97.99±0.170	(-0.238)
		35 µl	97.34±0.409	(-0.904)	97.90±0.653	(-0.330)	97.84±0.550	(-0.388)
Weight of 5th age larva (g)	4.227±0.251	15 µl	4.370±0.264	(+3.280)	4.520±0.200*	(+6.490)	4.477±0.251*	(+5.585)
		25 µl	4.400±0.100	(+3.939)	4.627±0.261*	(+8.646)	4.580±0.200*	(+7.715)
		35 µl	4.400±0.100	(+3.939)	4.673±0.261*	(+9.558)	4.610±0.173*	(+8.315)
5th age larval Duration (h)	168.00±0.000	15 µl	178.00±0.000	(+5.618)	178.00±0.000	(+5.618)	178.00±0.000	(+5.618)
		25 µl	178.00±0.000	(+5.618)	178.00±0.000	(+5.618)	178.00±1.000	(+5.618)
		35 µl	183.00±0.000	(+8.197)	183.00±0.000	(+8.197)	188.00±0.000	(+10.638)
Yield/number	8933.33±57.735	15 µl	8966.67±57.73	(+0.372)	9066.67±57.73	(+1.471)	9100.00±0.000	(+1.832)
		25 µl	9033.33±57.73	(+1.107)	9066.67±57.73	(+1.471)	9033.33±115.470	(+1.107)
		35 µl	8966.67±57.73	(+0.372)	9033.33±57.73	(+1.107)	9000.00±100.000	(+0.741)
Yield/weight (kgs)	17.51±0.973	15 µl	17.07±0.152*	(+6.641)	18.13±0.115*	(+12.132)	18.03±0.115*	(+11.645)
		25 µl	17.67±0.115*	(+9.812)	18.67±0.152*	(+14.643)	18.07±0.230*	(+11.808)
		35 µl	17.50±0.100*	(+8.953)	18.60±0.100*	(+14.337)	18.00±0.200*	(+11.482)
cocoon weight (g)	1.808±0.004	15 µl	1.909±0.003*	(+5.290)	2.004±0.004*	(+9.765)	1.994±0.004*	(+9.313)
		25 µl	1.962±0.002*	(+7.848)	2.065±0.005*	(+12.444)	2.005±0.004*	(+9.842)
		35 µl	1.958±0.003*	(+7.679)	2.065±0.005*	(+12.431)	2.039±0.531*	(+11.345)
Shell weight (g)	0.384±0.002	15 µl	0.392±0.002	(+2.139)	0.412±0.002*	(+6.723)	0.408±0.002*	(+5.809)
		25 µl	0.412±0.002	(+6.882)	0.427±0.002*	(+10.147)	0.413±0.000*	(+7.017)
		35 µl	0.407±0.002	(+5.740)	0.428±0.000*	(+10.273)	0.418±0.001*	(+8.216)
Shell ratio (%)	21.24±0.070	15 µl	20.56±0.110	(-3.307)	20.55±0.062	(-3.358)	20.46±0.055	(-3.812)
		25 µl	21.02±0.101	(-1.047)	20.70±0.070	(-2.609)	20.60±0.026	(-3.107)
		35 µl	20.80±0.128	(-2.115)	20.73±0.057	(-2.460)	20.53±0.467	(-3.458)
Filament Length (m)	916.66±4.163	15 µl	955.00±5.000	(+4.015)	64.66±4.509	(+4.976)	968.66±3.214*	(+5.368)
		25 µl	990.00±1.000*	(+7.408)	1011.33±12.055*	(+9.361)	996.66±5.859*	(+8.027)
		35 µl	972.33±2.516*	(+5.725)	992.66±5.507*	(+7.656)	1012.33±3.214*	(+9.450)
Filament weight (g)	0.310±0.002	15 µl	0.3200.002	(+3.125)	0.330±0.003*	(+6.061)	0.330±0.003*	(+6.061)
		25 µl	0.3400.000*	(+8.824)	0.350±0.002*	(+11.429)	0.340±0.001*	(+8.824)
		35 µl	0.3300.000*	(+6.061)	0.340±0.002*	(+8.824)	0.350±0.001*	(+11.429)
Denier	3.07±0.011	15 µl	3.0300.005	(-1.320)	3.100±0.015	(+0.968)	3.090±0.023	(+0.647)
		25 µl	3.0800.005	(+0.325)	3.180±0.208	(+3.459)	3.080±0.017	(+0.325)
		35 µl	3.1200.005	(+1.603)	3.140±0.015	(+2.229)	3.150±0.011	(+2.540)

*Significant ($p < 0.05$) values in parentheses are the percentage difference from the control

Mukherji (1993) reported tetranortriturbinoid from *Azadirachta indica* serves as harmonic effect on *Trobolium castinum*. It is believed that the conversion of additional quantity of leaf consumed during extended period and stimulatory effect of the compound on protein synthesis and silkgland are suggested by Kajira and Yamashita (1989). These changes at molecular level might be the results of alterations in the ratio of circulating hormone.

The economic characters of the silkworm breeds exhibited a positive response in juvenoid treated breeds without difference the developmental simultaneity as reported by Muroga (1975), Kurata and Daillie (1978) reported that the juvenoid causes retarded effect on silk gland growth with no such effect on general body weight. It is an indicative of juvenoid exerting an influence mainly on silk gland. The growth of silk gland continues as long as silk protein synthesis takes place in the cells and maintains synthesis of activity of silk protein suggested by Garel (1983). Juvenile hormone is one of the major circulation hormone in insects and retention of larval features is the main function of this hormone apart from controlling other developmental and reproductive activities (Nair *et al*., 1998).

Chemical compounds that are analogues to the structure of function of JH have been identified and synthesized primarily to administered to insect pests and control them (Retnakaran *et al*., 1985). These compounds were aptly christened as third generation pesticides. It was remarkable that these compounds, when administered to silkworm, *Bombyx mori* larvae in minute quantities elicited a hormonetic response and elevated the productivity, but with a prolonged larval feeding period (Akai *et al*., 1971). Future, it was consolidated that the silk output can be improved on JH administration to 5th instar silkworm larvae (Chang *et al*., 1972; Nihmura *et al.,* 1972; Muroga *et al*., 1975).

However, response to such treatment varies depending on the dosage, time and number of applications (Aomori *et al.*, 1977). Among large number of synthetic JH formulations surfaced at different parts of the globe context particularly for pest control, many of were quite effective in improving the economic traits of silkworm, *Bombyx mori* L. (Shimada *et al*., 1979; Akai *et al*., 1985). Subsequently, some of these JH compounds were tried in India. An experimental basis and good results in respect of increased cocoon and shell weight were reported Chowdhary *et al*., 1986; Trivedy *et al*., 1993. In KA x NB_4D_2, a notable change in ERR by number was noticed in the treatments with concentration of 5 and 10 ppm at 24 and 48h. Such increase was also observed with 10 ppm at 72h but in PM x NB_4D_2, the effective rate of rearing which may otherwise be understood as survival in general entomological arena was not largely affected although a marginal change was noticed with 10ppm at 48h. Earlier workers did not notice any remarkable change in ERR (Trivedy *et al*., 1993). ERR by weight, a commercially very important trait, had a substantially positive change in the treated silkworm. In KA X NB_4D_2, the improvement was to the tuned upto 8.73% when treated at 48h with 5ppm. This was followed by an improvement of 5.56% with 5ppm application at 24h. 10ppm at 24h and 48h and 5ppm at 72h also had significant positive changes. In PM X NB_4D_2, the maximum increase was about 8% at 48h with 5ppm. This increase must be attributed mainly towards cocoon weight on JH application along with the increase in the ERR by number. The results of the present investigation clearly pointed out that the natural or synthetic JH mimics tested and justified bioactive compounds in other insects can be judiciously employed in sericulture for the benefit of the industry. The compound used in the present study, ω-formyl longifolene oxime citronellyl ether has been proved to be effective in controlling the mosquito, *Culex quinquefasciatus*, and the vector of filariasis (Sawaikar *et al*., 1995). The present results clearly show that although JH mimicing compounds influence the silk production positively, it is largely dependent on the dose and time of application as stated earlier (Akai *et al*., 1988, Trivedy *et al*., 1993). When cocoon/cocoon shell weight was improved, a corresponding improvement in larval weight was also noticed. It is obvious that the changes in larval weight were not as prominent as that in cocoon shell weight. This probably gives an indication that an elevated larval weight is not a prerequisite for improved cocoon shell weight, but physiologically more sensible to read temporary inhibition on JH administration.

4. CONCLUSION

The findings of the research is that the enhancement and manifestation of the commercial traits are not only accompanied by a prolonged feeding period, but due to a direct stimulatory effect of exogenous administration of JH mimicing compound for the synthesis silk protein in silk gland Another possibility is the increased efficiency of converting the ingested food into digestibility of food. These changes at the physiological or molecular level might be the resulting the alteration of the circulation of neurohormones. It may be summarized that the

JH mimic, is one of the potential hormonal mimic could boost the activity level of the enzymes for the interconversion of ingested food into cellular and sub cellular energy mobilization for the production of the silk protein in turns improvement of the commercial traits of the cocoons though it is originally considered as a third generation pesticide. On elaborate study on topical application of JH mimic. it has become clear that the compound at 5ppm concentration would be most effective at 48h of interval during 5^{th} instar and elicits the most favourable response in silkworm are illustrated in the present study for the improve of economic parameters.

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