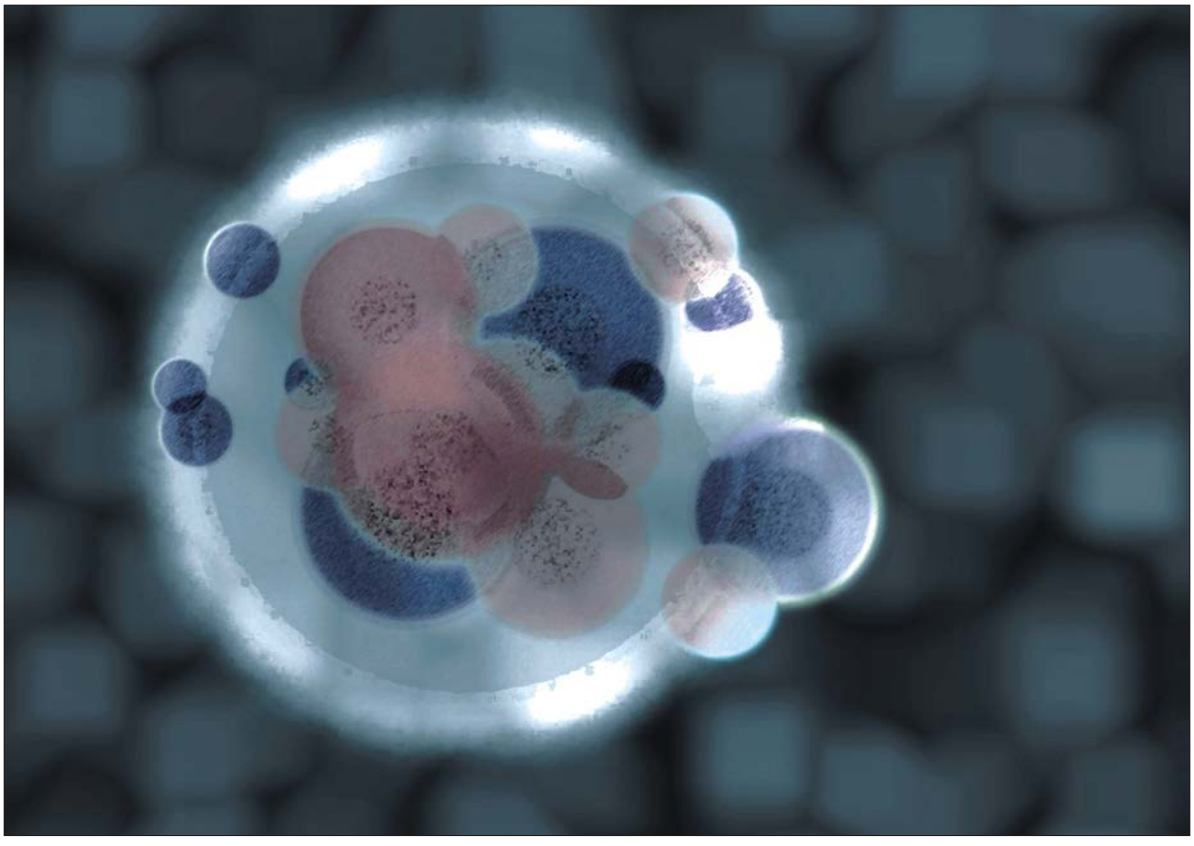

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Impact of Individual and Co-Administrations of Juvenile Hormone Analogue and Phytoecdysteroid on the Crop Management and Performance of Silkworm, *Bombyx mori* L.

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Abstract: Juvenile Hormone Analogues (JHAs) are administered to 5th instar silkworm for improving the cocoon yield and phytoecdysteroid (PE) for hastening the larval maturation events and synchronizing cocoon spinning activities. As individually both these techniques are accepted and quite popular now, doubts persist about their co-administration in the absence of a planned study. This study addresses this concern. JHA and PE were applied to separate and same batches of 5th instar silkworm to ascertain the response in terms of management criteria and crop performance. In three different treatment procedures adopted, T_1 was applied with JHA alone at the 48 h of 5th instar, T_2 was applied with JHA at 48 h and PE at the onset of larval maturation and T_3 with PE alone. T_0 served as untreated control. The popular bivoltine silkworm hybrid, CSR2×CSR4 and multi×bivoltine hybrid, PM×CSR2 were used for this study. The results of two trials showed that JHA treatment alone enhances the cocoon yield quite handsomely with a prolongation in the 5th instar period at least by 24 h. T_2 not only enhances cocoon yield but brings in the advantages of PE administration. The prolongation is limited to 12 h. PE alone hastens and synchronizes larval maturation and reduces the larval period by about 18 h.

Key words: *Bombyx mori*, JHA, phytoecdysteroid, silkworm

INTRODUCTION

Feeding of exogenous ecdysteroid to the larvae of silkworm, at certain stages of development has been shown to enhance synchronous development of the larvae. The response of the silkworm to the treatment of ecdysteroid largely depends on the time of application and also the dose of the compound (Chou and Lu, 1980). For the purpose of administration to silkworm, ecdysteroid from plant sources i.e., phytoecdysteroids (PE) are used owing to its effectiveness, economic viability and easy availability from locally available plant materials. Such treatments have considerable potential to improve the efficiency of silkworm rearing especially since the hormonal treatments could be based on extracts of appropriate indigenous plant species (Dinan, 2001). PEs are used on silkworm mainly to combat the non-uniform larval maturation, staggered larval mounting process and the resultant manpower and resources wastage (Nair *et al*., 2002, 2005). The procedure became quite popular due to its visible impact on the sericulture economy. The success of this technology also prompted the introduction of Juvenile Hormone Analogue (JHA) administration to silkworm larvae to increase cocoon production as it ensures an improvement of 10-20% in the cocoon and silk yield (Nair *et al*., 2003). Large scale tests at the field level showed a consistent improvement of 10~20% in the cocoon weight and shell weight with no notable change in the shell percentage. There are no documented results especially in the Indian context to show whether these two technologies can be used together on the same batch of silkworm so that the yield could be enhanced to certain extent and the cocoon spinning process could be hastened and synchronized. The present study examines the impact of individual and co-administration of JHA and PE on the last instars of silkworm.

MATERIALS AND METHODS

Silkworm rearing: Disease free eggs of bivoltine silkworm hybrid, CSR2×CSR4 and multi×bivoltine hybrid, PM×CSR2 were used in this study. Silkworms were reared under standard recommended conditions at 25±1°C temperature, 75±5% relative humidity under 12:12 (light:dark) photoperiod. Mulberry leaves of Victory-1 genotype, harvested from a periodically irrigated mulberry garden were fed to silkworms three times a day.

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Administration of JHA and PE: Just before resumption to 5th instar, batches of 250 larvae were counted and kept in ventilated plastic trays measuring 3 ft×2 ft for different treatments and labeled as T_1, T_2, T_3 and T_0. The following treatment procedure was followed:

T_1 : Administration of JHA at 48 h of 5th instar
T_2 : Administration of JHA at 48 h of 5th instar and PE at the onset of cocoon spinning.
T_3 : Administration of PE at the onset of cocoon spinning.
T_0 : Control

The JHA, RS-methoprene (E, E)-(RS)-11-methoxy, 3, 7, 11-trimethyl dodeca-2, 4-dienoate), was mixed thoroughly in water in the presence of a surfactant to form an emulsion to a concentration of 2.5 ppm. The JHA formulation was administered topically to the larvae of T_1 and T_2 at the rate of 12.5 mL per 100 larvae on completion of 48 h in 5th instar. The larvae were fed after 45 min of JHA treatment. Ecdysteroid was dissolved in clean water at the rate of 25 mg L^{-1}. The mixture was sprayed onto fresh mulberry leaves at the rate of 100 mL to 1 kg of leaf. The leaf sprayed with PE was then fed to the larvae of T_2 and T_3 when a few larvae showed symptoms of maturation and cocoon spinning. The larvae of T_0 were maintained as control. Each treatment was replicated 5 times.

Collection of data and statistical analysis: On attaining maximum growth, the weight of 10 larvae was recorded from each replication. The larval duration in the 5th instar from the time of resumption till completion of mounting also was recorded. Cocoons were harvested on 6th day from completion of mounting. The cocoons were sorted out from each replication and the numbers recorded. After recording the total weight, cocoons were cut open and the weight of cocoon and cocoon shell of 10 males and 10 females were recorded. Finally, traits such as survival, cocoon yield for 10000 larvae and shell percentage were calculated from the observations recorded.

The experiment was tried twice in the year 2006 at Central Sericultural Research and Training Institute, Mysore. Data were compiled and subjected to statistical analysis using ANOVA to find out the significance in the difference found between the treatments and the control.

RESULTS AND DISCUSSION

JHA administration to silkworm larvae is a practice to improve the cocoon yield whereas, PE is usually administered to induce hastened larval maturation and synchronized cocoon spinning. In this study, JHA and PE were administered individually and also in combination to two popular silkworm hybrids. The results of these treatments on various economic traits are presented in Table 1 and 2.

Larval duration and weight: In both the bivoltine and multi×bivoltine hybrids, the larval duration was influenced by the treatment. In the bivoltine hybrid, T_1 and T_2 took 174 and 162 h, respectively to complete the 5th larval stadium whereas, the control took only 150 h. These were 24 and 12 h more than what the control took. At the same time, T_3 took only 132 h in the 5th stadium which was 18 h less than the 5th instar period of the control, 30 h less than that of T_2 and 42 h less than that of T_1. In the multi×bivoltine hybrid as well, a similar pattern of difference in the larval duration was noticed. T_1 and T_2 recorded 24 and 12 h more 5th instar period compared to the control and T_3 recorded 18 h less 5th instar duration compared to the control. On one hand, JHA on application to silkworm prolongs the larval period (Miranda *et al*., 2002) and on the other hand, PE administration shortens it (Chou and Lu, 1980). In the Indian sericultural scenario, a wide deviation in the larval duration in the 5th instar is approached with interest. Usually, the economic viability of silkworm rearing is examined in the light of 5th instar duration. It is generally believed that, a shorter larval duration translates into better economics on account of reduced leaf and labour consumption. But if examined *vis-à-vis* the increased yield and if it commensurate with the prolonged period, the concept of producing more by inducing the silkworm to consume more will have enough takers as epitomized by the popularity of JHA technology in rural India.

In the larval weight also, a pattern of change similar to that of larval duration was observed. In the bivoltine hybrid, T_1 recorded the maximum weight, which was 9% more than that of the control, followed by a 6% increase over the control in T_2. Both these changes were statistically significant. There was no difference in the larval weight whatsoever between T_3 and T_0. In the multi×bivoltine hybrids as well, T_1 and T_2 recorded significant improvement in the larval weight compared to that of the control, but the level of improvement was definitely more (21 and 19%) compared to the bivoltines.

The positive difference in the larval duration and the larval weight can be attributed to the JHA treatment as reported in our earlier work (Nair *et al*., 2003). When the haemolymph JH titre is enhanced by the exogenous JH, larval duration tends to prolong as the moult inducing peak of the ecdysteroid makes a delayed appearance. The delayed peak also induces the larvae to consume more which is converted to extra body mass. Similar prolonged

Table 1: Effect of individual and combined administration of JHA and PE on the economic traits of CSR2×CSR4 hybrid of silkworm, *Bombyx mori* L.

Treatments	V instar larval duration (h)	Larval weight (g)	Survival (%)	Cocoon yield (kg)	Cocoon weight (g)	Shell weight (g)	Shell (%)
T_0	150	4.788	88.34	17.69	2.000	0.475	23.75
T_1	174* (+24)	5.226* (9.15)	89.46 (1.26)	19.93* (12.66)	2.215* (10.77)	0.517* (8.84)	23.34 (-1.72)
T_2	162* (+12)	5.087* (6.24)	92.94* (5.20)	19.45* (9.95)	2.226* (11.32)	0.520* (9.47)	23.36 (-1.64)
T_3	132 (-18)	4.788 (0.01)	92.81* (5.05)	18.32 (3.56)	2.007 (0.35)	0.475 (0.07)	22.94 (-3.41)
SE±	1.154	0.049	1.015	0.206	0.030	0.003	0.372
CD at 5%	3.990	0.169	3.512	0.710	0.104	0.009	NS

T_1: JHA; T_2: JHA+PE; T_3: PE; T_0: Control. Values in parentheses are percentage difference from the control except in larval duration which shows actual difference in hours. *Significant ($p<0.05$), NS: Non significant

Table 2: Effect of individual and combined administration of JHA and PE on the economic traits of PM×CSR2 hybrid of silkworm, *Bombyx mori* L.

Treatments	V instar larval duration (h)	Larval weight (g)	Survival (%)	Cocoon yield (kg)	Cocoon weight (g)	Shell weight (g)	Shell (%)
T_0	144	4.010	86.267	17.160	1.872	0.368	19.68
T_1	168* (+24)	4.859* (21.17)	91.267 (6.96)	19.88* (15.85)	2.186* (16.77)	0.425* (15.48)	19.44-1.22
T_2	156* (+12)	4.773* (19.02)	93.733 (8.66)	19.65* (14.51)	2.063* (10.21)	0.402* (9.24)	19.48-1.02
T_3	132 (-12)	4.050 (0.997)	89.867 (4.17)	18.05 (5.18)	1.884 (0.64)	0.361 (-1.90)	19.16-2.64
SE±	1.154	0.090	2.667	0.481	0.025	0.007	0.296
CD at 5%	3.990	0.312	NS	1.667	0.089	0.026	NS

T_1: JHA; T_2: JHA+PE; T_3: PE; T_0: Control. Values in parentheses are percentage difference from the control except in larval duration which shows actual difference in hours. *Significant ($p<0.05$), NS: Non significant

larval duration and resultant enhanced body weight have been previously reported on exogenous JHA administration (Akai *et al*., 1988). In this study, the prolonged duration was markedly reduced by PE administration just at the onset of spinning to those larvae which already obtained JHA. There was a reduction of 12 h in the duration in T_2 when compared to T_1 but the duration was still 12 h more when compared to the control. When PE was applied without JHA, the duration was reduced significantly. This is in agreement with the reports of Chou and Lu (1980).

Survival and cocoon yield: From the farmers' point of view, survival and cocoon yield are the most important parameters apart from labour requirement and these attributes are directly linked to monetary returns. In the bivoltine hybrid, T_2 and T_3 recorded considerable increase in the survival whereas, T_1 had no notable impact. The increase in survival is mainly due to PE effect. Although 7 to 9% rise was seen in survival in the multi×bivoltine hybrid, those were not statistically significant. Farmers' income from sericulture takes a beating when survival is hit due to various reasons because low survival generally leads to low yield. As JHA and JHA in combination with PE did not have any adverse effect on survival, the technique is well received.

The perusal of cocoon yield shows that JHA alone and in conjugation with PE enhanced the cocoon yield significantly. In the case of T_1 and T_2, the upsurge is 13 and 10%, respectively in bivoltine hybrid and 16 and 15% in multi×bivoltine. A close look makes it clear that 10% improvement in Bivoltine and 15% improvement in multi×bivoltine could be achieved with a prolongation of 12 h on combined administration of JHA and PE. But, for 13 and 16% increase in the same order, a prolongation of 24 h was required which was resulted on JHA administration alone. A comparison here shows that the combined administration is beneficial as this would ensure enhanced yield along with synchronized cocoon spinning albeit an extended period of 12 h. This can ultimately turn out to be a promising management tool in sericulture. PE administration as expected recorded a marginal rise. In fact, a rise in cocoon yield is not expected here since the duration of 5th instar is reduced significantly by PE treatment, the ultimate objective being labour and mulberry leaf saving through advanced and synchronized cocoon spinning.

Cocoon traits: In the bivoltine hybrid, the improvement in cocoon weight was 11% and that in shell weight was 9% in both T_1 and T_2. These were statistically significant. T_1 and T_2 did not show much difference between themselves in their effect on cocoon traits. In both the cases, the shell percentage marginally went down. In T_3, there was no impact either on the cocoon or shell weight. Since the objective of this treatment was mainly hastened and synchronized cocoon spinning, any escalation in the cocoon traits is not expected but considered as an added advantage. Similar results were seen in the multi×bivoltine hybrid also but the rise was obviously much more in T_1 compared to T_2. This is in line with the trend of change in other traits. JHA alone brings about positive changes in cocoon traits and in this study, it was 17 and 15% for cocoon and cocoon shell weight, respectively whereas, the combined administration of JHA and PE could elevate these traits by 10 and 9%, respectively. As already stated, the higher improvement of course was accompanied by a prolonged duration. In T_3, there was no much difference in cocoon traits compared to the control. In all the treatments, shell percentage was marginally affected but

statistically insignificant. Increment in cocoon and shell weight on JHA treatment (Mamatha *et al*., 2006) and unaffected or marginally affected cocoon traits on PE administration (Trivedy *et al*., 2003) has already been reported.

Role of JHA and PE in crop management/yield enhancement: The results of this study give us a fairly clear idea where we stand when we need to decide on silkworm crop management physiologically. Substantial rise in the yield (10-20%) is ensured by JHA application but with a prolonged larval feeding period. This prolonged period can be cut short at least by 50% if PE is administered at the critical time which would also ensure synchronized larval maturation and cocoon spinning. As farmers are anyway adopting PE administration (T_3) gleefully due to its visible positive impact, JHA application in addition would provide enhanced yield and additional returns.

In silkworm rearing, the physiological processes happening during every stage have got direct or indirect relation with the crop performance and more so that happening in the 5th instar. The crop quality and quantity can be tailor-made effectively by exogenous administration of insect hormone mimics which may either prolong the larval period or shorten it. A prolonged period would make the larvae consume more and produce heavier and bigger cocoons (as evidenced by T_1) and the shortened period would naturally result in smaller and lighter cocoons. PE treatment which does not affect the cocoon yield as such brings about uniform larval maturation and cocoon spinning. This may not have a significant effect on cocoon traits (as seen in T_3). There are definite advantages in either of these techniques and selection of the technique is need-based. Non-uniform silkworm maturation and staggered cocoon spinning is a serious problem at the farmers' place which can be effectively managed by PE administration at the onset of spinning. This will remarkably bring down the requirement of skilled labour for picking up ripe larvae as all the larvae can be transferred to the cocoon building frames almost simultaneously. This is apart from saving good quantity of mulberry leaf. On the contrary, farmers who possess additional mulberry leaf or who can afford to manage extra mulberry leaf can make use of JHA administration and get handsome increase in the yield. The combined administrations of these two bioactive compounds provide the advantage of both the techniques. This study eradicates one of the main apprehensions regarding the use of both these technique on the same larvae indicating that the larvae which are treated with JHA, will mature uniformly and spin heavier cocoons if treated with PE as well.

It can be surmised that JHA or PE administrations are need based technologies of sericulture for enhanced yield or to physiologically manage the silkworm crop. When both these technologies are simultaneously used on same crop, it brings in the advantages of both these technologies. This includes enhanced yield as well as synchronized cocoon spinning.

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