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USE OF PHYTOECDYSTEROID (β -ECDYSONE) AS A CROP SAVER IN SERICULTURE INDUSTRY

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ABSTRACT
The effect of plant extract containing phytoecdysteroid (β-ecdysone) on the spinning behaviour of silkworms (NB_4D_2 × SH_6) and (SK_{28} × SK_{30}) was investigated in the present study. Larvae of 5th instar were fed mulberry leaves sprayed with different concentrations of plant extracts containing phytoecdysteroid. The control batches *i.e.* Control-I (leaf sprayed with branded hormone SAMPOORNA), and Control-II (without any treatment) were maintained in similar conditions. The phytocedysteroid was used as a crop saver under certain conditions which hastened the maturation process in silkworms when applied at a particular time during 5th instar. The effect of the administration during 5th instar from 72 h onwards was most remarkable as far as crop saving treatment is concerned. The administration of phytoecdysone at 72 h, and 120 h had a clear impact on the larval duration and obviously on other related characters such as larval weight and cocoon traits. In case of treatment made at 120 h (on 5th day), a shortest larval duration of 642.46 h was recorded in this time of application. In addition to this shortest 5th instar larval duration of 162.47 h was also recorded in this time of application. This decrease in 5th instar larval duration was observed due to plant extract application which contained phytoecdysteroid (β-ecdysone). A difference of nearly 18 h was observed in vth instar larval duration between control-II (179.47 h) and (162.47 h) in the treatment in which plant extract was used. This was the lowest larval duration. There was also significant difference between Control-I (164.38 h) and the lowest (162.47 h) recorded in plant extract during 5th instar. However the difference was only 2.3 h. This decrease in 5th instar larval duration was also measured in percentage as against the value of control-II. The highest decrease of 9.438 per cent was observed after applying plant extract of *Cupressus tularosa* Linn in a concentration of 1:100. In 120 h treatment maturation started at 128 h (8 h after the treatment) and cumulative maturation in this treatment was achieved at 162-164 h. It took nearly 32-36 h (from treatment to end) for culmination of mounting *i.e.* on the 7th day. During this treatment lowest number of feeds *i.e.* 26.5 were taken by treated batches as against 29.33 by the control-II (which was not treated). The application of plant extract containing ecdysteroid reduced 3-4 feeds and finished mounting earlier than other treatments. Although the quantity harvested was less as It was observed that in 120 h time of application there was a difference of about 8 grams between the lowest recorded weight of 10 mature larvae and that of Control-II, which resulted in deterioration of certain characters, however a crop was harvested earlier than normal harvesting time. This treatment would be recommended in a situation when there will be scarcity of leaf or due to any other unavoidable reason the worms are not fed well in time. Under such situation spraying of plant extract containing β-ecdysone would hasten maturation and save cocoon crop for the rearers.

KEY WORDS: β-ecdysone, Hastening, Maturation, *Bombyx mori* L.

INTRODUCTION

It is known that analogues of ecdysteroids also occur in certain proportions in plants. These plants synthesize these ecdysteroids as a defence mechanism and these occur in them in large quantities (Schmelz *et al*., 2000). The ecdysteroid derived from plant source is popularly known as Phytoecdysteroid. These phytoecdysteroids have been seen to be 20 times more active than Zoo-ecdysteroids (Nair *et al*., 2002). As a defence mechanism in plants it is also believed that they provide some degree of protection to the plant against non-adopted phytophagous insects (Bergamasco and Horn, 1983; Kubo and Hanke, 1986). Ecdysteroid (ES) in the context of sericulture can be any phyto-sterol structurally closer to the original insect ecdysteroid *i.e.* 20- hydroxyecdysone, which can induce a response in silkworm equal to that of the natural ecdysteroid and could be used for synchronizing the maturation activity among the silkworms in order to spin cocoon within short span of time (Rufaie *et al*., 2012). Ecdysterone or 20-hydroxyecdysone is the most widely occurring phytoecdysterone and many plants like *Taxus wallichiana* Zucc. (Himalayan Yew), *Cupressus tularosa* Linn (Cupreous) and Datura *stramonium* Linn. (Datura) have been identified to have shown reasonable concentration of 20-hydroxyecdysone (Rufaie *et al*., 2011). This Phytoecdysteroid can also advance the maturation activity and hasten cocoon spinning process in silkworms especially when a partial or complete crop loss is feared by the rearers either due to mulberry leaf shortage or disease attack.

MATERIALS & METHODS

Two hybrids NB_4D_2 × SH_6 and SK_{28} × SK_{30} were reared on mulberry leaves under recommended environmental conditions (Krishnaswami, 1986) up to the end of fifth instar. The larvae were grouped into five batches each containing 250 larvae in triplicate. After it was confirmed under high performance liquid chromatography (HPLC)

that three plant extracts were possessing 20 hydroxyecdysone like substance and these were taken for further studies. These plants extracts of Taxus *wallichiana* Zucc. (Himalayan Yew), *Cupressus tularosa* Linn (Cupreous) and *Datura stramonium* Linn. (Datura) were re-dissolved in methanol to prepare 10% stock solution (10g in 100ml) which was used to prepare further dilution of 1:100 and 1:50 (w/v) concentrations of each extract in distilled water (Jayapaul *et al.*, 2003) for further study during silkworm rearing. A uniform quantity (@ 10 ml on 50 g of leaf) of these plant extracts of each concentration containing phytoecdysteroid (20-E) was sprayed with an atomizer for *per os* administration to the larvae in each replication. The phytocedysteroid was used as a crop saver under certain conditions which hastened the maturation process in silkworm hybrids when applied at 72 and 120 h during 5^{th} instars of silkworm larva. In addition to these plant extracts a commercial formulation of plant extract (branded hormone SAMPOORNA) was also used as control-I as per its recommended concentration. A control without any treatment was also maintained side by side for evaluating the performance of plant extracts containing 20-hydroxyecdysone like substance. The time and number of worms mounted was recorded at regular intervals till the completion of mounting process in both experimental and control batches.

RESULTS & DISCUSSION

The most common and appropriate use of phytoecdysteroid is to hasten the maturation and to induce simultaneous or synchronized cocoon spinning. However phytocedysteroid can be used as a crop saver under certain conditions when applied at a particular time during 5^{th} instar of silkworm larva. The effect of the administration from 72 hours onwards was most remarkable as far as crop saving treatment is concerned. The treatments done at 72, and 120 hours had a clear impact on the larval duration and obviously on other related characters such as larval weight and cocoon traits. In case of treatment at 120 hours, it was observed that shortest larval duration of 642.46 h was recorded in this time of application. In addition to this shortest V^{th} instar larval duration of 162.47 h was also recorded in this time of application. This reduction in 5^{th} instar larval duration was observed due to *per os* administration of plant extract containing 20-hydroxyecdysone like substance to the larvae in each replication. A difference of nearly 18 hours was observed in Vth instar larval duration between control-II (179.47 h) and plant extract fed batches (162.47 h). This 162.47 h was the lowest V^{th} instar larval duration. There was also significant difference in 5^{th} instar larval duration between Control-I *i.e*. 164.38 h and the lowest recorded after application of plant extract *i.e.* 162.47 h. However the difference was only 2-3 hours. This decrease in 5^{th} instar larval duration was also measured in percentage as against the value of control-II. The highest decrease of 9.43 per cent was observed after applying plant extract of *Cupressus tularosa* Linn at a concentration of 1:100. in 120 h treatment, where maturation of worms for mounting started at 128 h (*i.e*. 8 hours after the treatment) and cumulative maturation in this treatment was achieved at 162-164 hours in 5^{th} instar. It took nearly 32-36 hours (from treatment to end) for culmination of mounting *i.e.* on the 7^{th} day. During this treatment lowest number of feeds *i.e*. 26.5 were taken by treated batches as against 29.33 by the control-II (which was not treated). The application of plant extract containing ecdysteroid reduced 3-4 feeds and finished mounting earlier than other treatments. Similarly in case of 72 h treatment it was observed that maturation was also hastened as compared to control. However it progressed slowly as compared to 120 h treatment. The maturation process started 32 h after treatment (at 104 hours) and progressed slowly and completed mounting of silkworms for seriposition within 64 h (at 168 hours) as compared to 120 h treatment. Here also a difference of nearly 12-13 h was observed between control-II and the lowest value recorded in this treatment. The possible reason for this was that treatment at 120 h *i.e*. on 5^{th} day of 5^{th} instar was more effective due to the reason that more number of feeds (more than 20) were taken by this batch as compared to 72 h treatment. In 72 h treatment also 2-3 feeds were saved and a cocoon crop was harvested. However the quantity harvested was less as there was a difference of nearly 8 grams in larval weight of these treatments and other normal treatments, which resulted in deterioration of certain characters. However a crop was harvested earlier than normal harvesting time. A moderate pupation was observed in these batches. The highest pupation was recorded in control batches where worms were fed fully as compared to treated batches where plant extract was applied, due to which less feeds were taken by the larvae as they matured earlier. Nair *et al.*, 2005 has also reported that when larvae were treated at 72 h, the economic traits were adversely affected but the larval duration was shortened considerably. While as the present study indicated that economic traits get adversely affected in 72 h as well as in 120 h treatments, however in case of 120 h treatment the larval duration gets shortened by nearly 18 hours and in 72 h treatment by nearly 12-13 h. It is evident from results that this technology can't be utilised on seed crops as there should be a pupation of more than 90 per cent which is not achievable under this technology. Although the larval duration and the survival are inversely related, the survival as such was good in almost all the treatments. This also gives an impression that treatments did not influence the survival adversely.

The most important effect of these treatments at 120 h and 72 h during 5^{th} instar was reduction in larval duration and formation of a cocoon crop. In this treatment the longest total larval period of 659.47 h was recorded in control-II (without treatment), and as such there was a difference of about 18 h between the shortest larval period (642.46 hours) and the control-II (659.47 hours). This has happened due to the application of plant extract containing 20-hydroxyecdysone like substance in this crop saving use. The reduction in the period is almost one day in both the trials. This means a quantum reduction in the leaf consumption *i.e*. 3-4 feeds were saved by application of plant based ecdysteroid at these application timings. The reduction in the larval duration and consumption of mulberry leaves manifests a rather strong adverse effect on the quantitative traits like yield etc. Usually no farmer would go for such a drastic reduction of yield as well as in cocoon characters unless he is compelled to do so under adverse conditions, like safety of entire crop from diseases and unforeseen shortage of mulberry leaf. However the

farmer would naturally prefer a reduced yield to a much bigger crop loss. Due to this reason, phytoecdysteroid emerges as a crop saver. In case of untimely leaf shortage and sudden disease outbreak, phytoecdysteroid can be applied at any time after 3rd day of Vth instar (after 72 hours) which is obligatory feding period to reduce larval duration. The present study recommends the application of extracts of *Taxus wallichiana* Zucc. *Cupressus tularosa* Linn and *Datura stramonium* Linn containing 20-hydroxyecdysone like substance at the onset of spinning, however under stress conditions the application can be done after 5th day of 5th instar *i.e*. after 120 h (Table-1, Fig. 1).

TABLE-1: Effect of Phytoecdysteroid on maturation of silkworm *Bombyx mori* L. (Mean of two Years)

S. No.	Duration in 5th Instar (h)	Maturation percentage (72 h)	Maturation percentage (120 h)	Control-I (Hormone) maturation %age	Control-II (without treatment) maturation %age
1	72				
2	80				
3	88				
5	96				
6	104	7			
7	112	16			
8	120	27			
9	128	46	8	12	
10	136	57	21	26	
11	144	73	37	49	
12	152	90	64	71	
13	160	94	100	100	12
14	168	100			33
15	176				74
16	184				93
17	192				100
18	200				
19	208				
20	216				

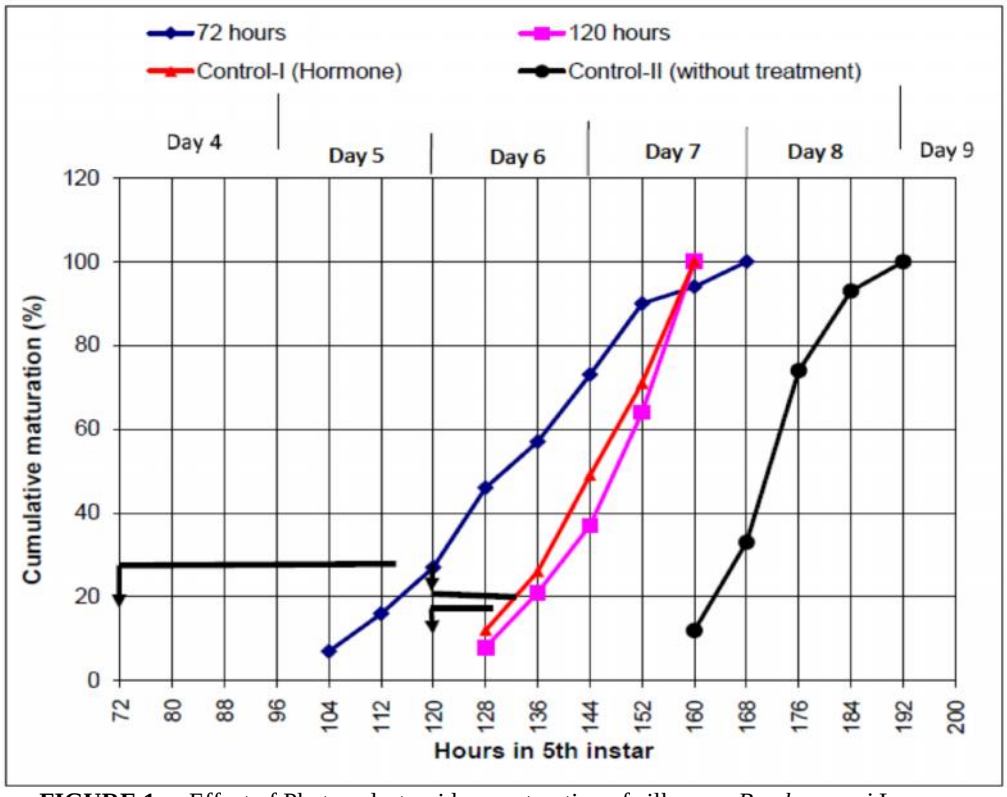

FIGURE 1: Effect of Phytoecdysteroid on maturation of silkworm *Bombyx mori* L.

PREFERENCES

Bergamasco, R. and Horn, D.H.S. (1983) Distribution and role of insect hormone implants. **In** : *Endocrinology of Insects* (Ed. A.R. Iss). Inc. New York, pp 627-654.

Chou, W.S. and Lu, H.S. (1980) Growth regulation and silk production in *Bombyx mori* L. from phytogenous ecdysteroids. **In** : *Progress in ecdysone Research* (Ed. J.A. Hoffman) Elsevier/North Holland Biomedical press, Amesterdam, pp. 281-297.

Jayapaul, C., Padmalatha, C., Singh, A.J.A.R., Murugesan, A.G. and Dhasarathan, P. (2003) Effect of plant extracts on nutritional efficiency in mulberry silkworm *Bombyx mori* L. *Indian J. Seric.* **42**(2): 128-131.

Krishnaswami, S., Narasimhanna, M.N., Suryanarayanan, S.K. and Kumararaj, S. (1973) *Manual on Sericulture*, Vol. 2, Agriculture Service Bulletin, 15, Food and Agriculture Organization, Rome.

Kubo, I. and Hanke, F.J. (1986) Chemical method for isolating and identifying phytochemicals biologically active in insect. **In** : Insect Plant Interactions. (Eds. J.R. Miller and T.A. Miller). Springer Verlag. New York, pp. 225-249.

Nair, K.S., Trivedy, K., Shyam, R., Chintalwar, G.J., Chinya, P.K., Datta, R.K., Chattopadhyay, S. and Banerji, A. (2002) Ecdysteroid from *Sesuvium portulacastrum* for synchronization of cocoon spinning in silkworm, *Bombyx mori* L. **In**: *Advance in Indian Seric. Res.* [Eds. S.B. Dandin and V.P. Gupta) CSRTI, Mysore, pp. 247-251.

Philip, T., Qadri, S.M.H. and Kumar, S.N. (2007) Field evaluation of phytoecdysteroid (Sampoorna) for maturity and spinning in silkworm larvae, *Bombyx mori* L. *Indian J. Seric.* **46**(1): 83-85.

Rufaie, Zia H., Munshi, N.A., Sharma R.K., Ganie, N.A., and Malik G.N. (2012) Effect of phytoecdysteroid (β-ecdysone) on synchronization of maturation in silkworm *Bombyx mori* L. *International Journal of Advanced Biological Research* Vol.2 (1) 2011-104-107.

Rufaie Zia. H., Munshi, N.A., Sharma R.K., Khursheed Ahmed., Malik. G.N. and Raja. T.A. (2011) Occurrence of insect moulting hormone (β- ecdysone) in some locally available plants. *International Journal of Advanced Biological Research* Vol.1 (2) 2012-238-240.

Schmelz, E.A., Grebenok, R.J., Ohnmeiss, T.E. and Bowers, W.S. (2000) Interactions between *Spinacia oleracea* and *Bradysia impatiens*: A role for phytoecdysteroids. *Arch. Insect Biochem. Physiol.* **51**(4) : 204-221.

Sehnal, F. (1989) Hormonal role of Ecdysteroids in insect larvae and during metamorphosis. **In**: Ecdysone : From Chemistry to Mode of action. (Ed. J. K

Trivedy, K., Dhar, A., Kumar, N.S., Nair, K.S., Ramesh, M. and Gopal, N. (2003c) Effect of phytoecdysteroid on pure breed performance of silkworm, *Bombyx mori* L. *Int. J. Indust. Entomol.* **7**: 29-36.

Zhuang, D.H., Xiang, M. and Gui, Z.Z. (1992) The practical studies on the growth and development by insect hormones in the silkworm *Bombyx mori* XIX Int. Cong. Entomol. Beijing, China.