INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

EFFECT OF PHYTOECDYSTEROID (β-ECDYSONE) ON SYNCHRONIZATION OF MATURATION IN SILKWORM *BOMBYX MORI* L.

Zia. H. Rufaie, Munshi, N.A., Sharma, R. K., Ganie, N.A. and Malik, G.N.
Temperate Sericulture Research Institute, Mirgund
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir
Post Box No: 674, G.P.O Srinagar, 190001, INDIA

ABSTRACT
The effect of plant extract containing phytoecdysteroids (β-ecdysone) on the spinning behavior of silkworms (NB_4D_2 × SH_6) and (SK_{28} × SK_{30}) was investigated in the present study. Fully grown larvae of 5th instar were fed with mulberry leaves sprayed with different concentrations of plant extracts. Also control batches (without any treatment) and a group receiving mounting hormone (branded hormone SAMPOORNA), were maintained in similar conditions. The time and number of spinning worms during mounting process and other parameters were recorded in all the batches. Silkworms of both the hybrids which took 36-40 hours in normal course for completion of mounting completed the same in 18-24 hours when treated. These hybrids treated silkworms showed spinning behavior within 6-10 hours where as mounting process took 24 hours in plant extract sprayed batches and also branded hormone sprayed batches.

KEY WORDS: Sampoorna, β-ecdysone, Synchronization, 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 defense 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 defense 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, 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. 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 showed reasonable concentration of 20- hydroxyecdysone (Rufaie *et al*.,2011) Ecdysteroid (ES) in the context of sericulture can be any phyto-sterol structurally closer to the original insect ecdysteroid, 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. It can also advance the maturation activities and hasten the cocoon spinning process especially when a partial or complete crop loss is feared either due to leaf shortage or due to disease attack. In addition to this it has been reported that Phytoecdysteroid is also used commercially to increase productivity in sericulture (Chou and Lu, 1980; Zhuang *et al*., 1992) and reported that phytoecdysteroid administration at a particular time also increases cocoon weight, shell weight and shell percentage.

MATERIALS AND 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 HPLC that three plant extracts are possessing 20 hydroxyecdysone like substance these were taken for further studies. The plant 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 of these plant extracts of each concentration containing phytoecdysteroid (20-E) was sprayed with an atomizer on equal quantity of mulberry leaves for *per os* administration to the larvae in each replication. Phytoecdysteroid was administered at the time of initiation of spinning behaviour. 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 recorded at regular intervals till the completion of mounting process in both experimental and control batches.

RESULTS AND DISCUSSION

The most common and appropriate use of phytoecdysteroid is to hasten the maturation and to induce simultaneous or synchronized cocoon spinning. The picking up ripe larval for seriposition is a cumbersome process. By using this technology labour requirement for picking up ripe larvae for mounting purposes is reduced. In the present study when plant extract containing phytoecdysteroid (20-hydroxyecdysone) was administered *per os* in the required concentrations at the onset of mounting when 3-5 per cent larvae were ripe in a batch, it resulted in maturation of 52.32 and 58.65 per cent larvae within 12 hrs of treatment as against 26.99 per cent observed in control-II (without treatment) within the same period (Table-1, Fig.-1). Although the control-I (Branded Hormone) was a purified product still then the crude plant extract containing phytoecdysteroid (20-hydroxycedysone) had neck to neck run with it for synchronization of maturation. All the remaining larvae matured quickly within next 24 hrs and all the larvae were transferred to mountages for seriposition. However in case of control-II (without treatment) full maturation took more time and finished within 36 hrs as compared to treated batches which were treated at the onset of spinning. The mounting duration was brought down from 36 hrs (under normal condition) to 24 hrs by the application of plant extract. Thus there was an average gain of 12 hours as a result of this treatment. It is therefore clear that when silkworms are treated at the onset of spinning with plant extracts containing 20-hydroxyecdysone, the mounting period can be shortened by about 40 to 50 per cent. The results were same for both the hybrids which were taken for the study.

TABLE-1: Synchronization in maturation at the end of 5th age

S. No.	Time (hrs)	Maturation percentage after application of plant extract (Phytoecdysteroid) at the initiation of spinning		
		Control-I (Hormone) (%)	Plant extract containing (Phytoecdysteroid) (%)	Control-II (%) (without treatment)
1.	6	11.66	14.33	9.33
2.	12	58.65	52.32	26.99
3.	18	89.66	82.98	39.77
4.	24	100	100	77.80
5.	30			89.45
6.	36			100
7.	42			
8.	48			

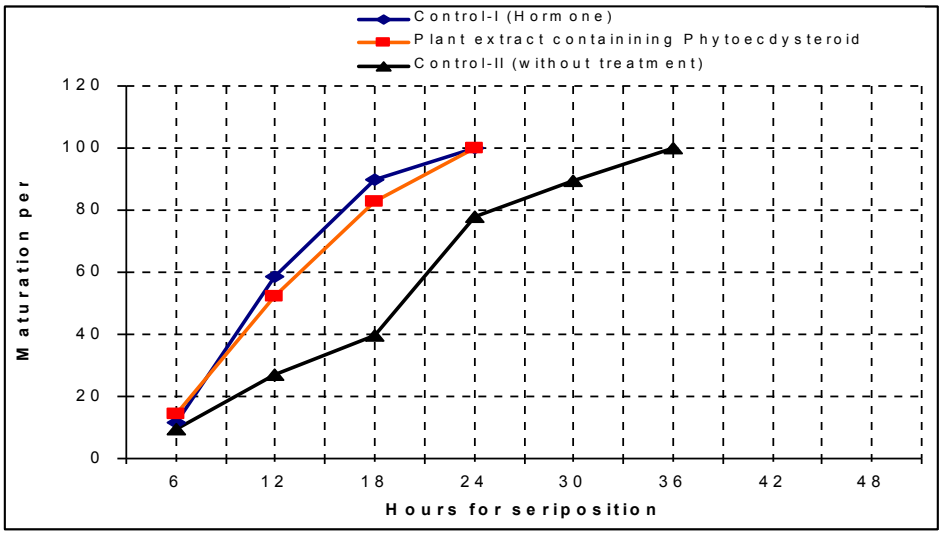

FIGURE-1: Effect of oral administration of plant extract (phytoecdysteroid) on the hastened maturation events and synchronized cocoon spinning in silkworm, *Bombyx mori* L.

The present study showed a clear difference in mounting duration between the control and the treated ones. More than 80 per cent larvae matured in about 18 hrs after the treatment, where as in control-II, only 30 to 40 per cent larvae matured within same period when application was done at the onset of spinning. The present study is almost in agreement with the findings of Philip *et al.* (2007) who have also reported that 80 per cent larvae were ready for mounting in about 16 hrs after treatment. Whereas in control only 15-30 per cent larvae matured by that time.

A similar result was reported by using phytoecdysteroid extracted from *Sesuvium portulacastrum* on silkworm hybrids (Nair *et al.,* 2002) and that from caryophyllaceae family of plants on pure silkworm breeds (Trivedy *et al.*, 2003c). This difference in the larval and mounting duration is because of a physiological role played by the exogenous ecdysteroid on the insect development system. The feeding larvae always contain a baseline level or low titre of ecdysone but reach to pupation inducing peak before pupation (Sehnal, 1989). By giving an extra dose of plant based ecdysteroid at the critical time, the pupation inducing peak of ecdysteroid content in silkworm is advanced and maturation of worms is achieved within a stipulated period of time and thereby change the larval behaviour as such. Due to its advantages, the formulations containing phytoecdysteroid have been accepted quickly by the farmers in some parts of the country and it has given them the desired results in the form of uniform maturation.

REFERENCES

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., 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 (1) 2011-104-107.

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.