Int. J. Indust. Entomol. Vol. 18, No. 1, 2009, pp. 8~12

International Journal of
Industrial Entomology

Effect of Phytoecdysteroid on Disease Incidence, Melting and Economic Characters of the Mulberry Silkworm

Mithilesh Kar, P. Sudhakara Rao*, S. Kishore[1], T. Selva Kumar, Nisha Gopal, A. R. Narasimha Nayaka and K. Chandrasekaran

Central Sericultural Research and Training Institute, Mysore - 570 008, India
[1]S.V.University, Tirupati- Andhra pradesh, India

(Received 02 May 2008; Accepted 08 January 2009)

A study has been made to know the effect of a phytoecdysteroid 'Sampoorna' on uniform maturation of silkworms during spinning and its effect on diseased silkworms infected by major silkworm disease viruses, *Bombyx mori* nuclear polyhedrosis virus (BmNPV) and *Bombyx mori* infectious flacherie virus (BmIFV). In the present investigation, the effect of the phytoecdysteroid "Sampoorna" on Grasserie disease caused by BmNPV have shown an average cocoon melting of 11.91% with a disease incidence of 5.83%. The values of 't' test for different treatments of BmNPV indicated low survival rate and cocoon traits were drastically reduced. Another major disease Flacherie caused by BmIFV has shown considerable levels of larval disease incidence (22-32%) and cocoon melting (3-7.67%) with an average melting of 12.95% and 20.24% disease incidence. There is a drastic reduction in survival rate, cocoon yield and other economic traits. The control batches were indicated negligible values for disease incidence and cocoon melting with Sapoorna application and without the inoculation of the two disease-causing viruses. The application of Sampoorna on already infected batches with major pathogens triggered high mortality and disease incidence and melting percentage was also significantly increased with reduced economic traits. Hence, it is suggested that application of Sampoorna in infected batches should be done only in the extreme conditions of rearing. Application of Sampoorna on healthy batches led to uniform maturation and improvement in productivity with the added advantage of better quality cocoons and labour saving.

Key words: Silkworm, Phytoecdysteroid, BmNPV, BmIFV, Disease incidence

Introduction

Sericulture in India is an agro-based industry combining the features of rural and industry based activities providing gainful employment to the rural masses. Though India stands second the quantity of cocoon and quality of raw silk produced is low when compared to sericulturally advanced countries like China (Jayaramaiah *et al*., 1986). Centuries of domestication of mulberry silkworm *Bombyx mori* L. has made it highly susceptible to a number of diseases *viz*., pebrine. Grasserie, Flacherie and muscardine. These diseases cause extensive damage to silk cocoon crop leading to economic loss to the sericulturists. The crop loss due to different diseases was estimated by Selva Kumar *et al*. (2002) which ranged from 11.48 to 14.86 kg/100 dfls during different seasons.

Another major problem encountered by sericulture farmers in India is the non-uniform maturation of the silkworm during final phase of larval duration. Approximately 10% of total labour required for silkworm rearing is needed at the time of picking mature larvae for mounting. During inclement weather and poor rearing management, the duration of mounting prolongs up to 48-72 hrs. This leads to difficulties in mounting, increase in disease incidence, management of labour and harvesting of cocoons. However, by administering mounting hormone the larval period of 5th instar can be reduced and at the same time all the larvae will have uniform maturity and will spin uniform cocoons.

*To whom the correspondence addressed
Central Sericultural Research and Training Institute, Mysore - 570 008, India. Tel: +91 0821-2900571; E-mail:parimi_rao@yahoo.com

In China, Japan and South Korea phytoecdysteroids are being used commercially to increase productivity in sericulture (Zhuang *et al.,* 1992). In India too, this institute has come out recently with a phytoecdysteroid and commercialized as "Sampoorna" (Trivedy *et al*., 2003) and recommended for commercial use for early and uniform maturation of silkworm without affecting the cocoon quality. Sampoorna is not only effective for uniform maturation, but also in certain situations like leaf shortage, occurrence of non-cocooning silkworm and likely outbreak of diseases. But so far no information is available on disease occurrence, melting of cocoon and other economic traits after sampoorna application. Thus, an attempt has been made in the present investigation to asses the disease occurrence due to application of Sampoorna on two major silkworm diseases of silkworm, *viz.*Flacherie caused by *Bombyx mori* infectious flacherie virus (BmIFV) and Grasserie caused by *Bombyx mori* nuclear nucleopolyhedrovirus (BmNPV), its effect on melting and other economic characters.

Materials and Methods

Fifty disease free layings of popular hybrid of CSR2× CSR4 were collected from the Silkworm seed production centre, Mysore and rearing was conducted up to third instar as per standard procedure (Datta, 1992). The stock inoculum of BmNPV and BmIFV were collected from the silkworm pathology laboratory of this institute. The polyhedral occlusion bodies of BmNPV were counted using haemocytometer under phase contrast microscope (Cantwell, 1974) and serially diluted to obtain required concentration (1×10^8 POB/ml). The stock inoculum of BmIFV containing 10^{-1} dilutions was diluted to required concentration of 10^{-7}. These concentrations were choosed based on the earlier studies conducted by this institute which will cause 70% mortality. The batches were inoculated with respective pathogens immediately after fourth moult. 50 ml of Sampoorna (Phytoecdysteroid) was collected from the Silkworm physiology division of the institute and as per the recommendations of Trivedy *et al*. (2003), 40 ml of Sampoorna was diluted with four liters of water. Then, the diluted solution was sprayed on mulberry leaves using hand sprayer and fed to 4th, 5th and 6th day of final instar larvae as the treatments mentioned below.

T1: Larvae were inoculated with BmNPV immeadiately after fourth moult and Sampoorna was sprayed on 4th day of 5th instar and rearing was continued till cocooning.

T2: Larvae were inoculated with BmNPV immeadiately after fourth moult and sampoorna was sprayed on 5th day of 5th instar and rearing was continued till cocooning.

T3: Larvae were inoculated with BmNPV immeadiately after fourth moult and sampoorna was sprayed on 6th day of 5th instar and rearing was continued till cocooning.

T4: Larvae were inoculated with BmIFV immediately after fourth moult and sampoorna was sprayed on 4th day of 5th instar and rearing was continued till cocooning.

T5: Larvae were inoculated with BmIFV immediately after fourth moult and sampoorna was sprayed on 5th day of 5th instar and rearing was continued till cocooning.

T6: Larvae were inoculated with BmIFV immediately after fourth moult and sampoorna was sprayed on 6th day of 5th instar and rearing was continued till cocooning.

T7: Sampoorna was sprayed on larve of 4th day of 5th instar (without any inoculation) and rearing was continued up to cocooning.

T8: Sampoorna was sprayed on larvae of 5th day of fifth instar (without any inoculation) and rearing was continued up to cocooning.

T9: Sampoorna was sprayed on larvae of 6th day of fifth instar (without any inoculation) and rearing was continued up to cocooning.

T10: Larvae were inoculated with BmNPV immediately after fourth moult and rearing was continued till cocooning.

T11: Larvae were inoculated with BmIFV immediately after fourth moult and rearing was continued till cocooning.

T12: Larvae were reared up to cocooning without any inoculation and spray of sampoorna which was served as a control batch.

In all the treatments, data were collected on incidence of different diseases during rearing and on mounting. The harvested cocoons were assessed for melting and other economic traits. Three replications with 300 larvae each were maintained in each treatment and the experiment was repeated two times. The average data on larval mortality, melting and other economic trials of two rearings were pooled and analysed statistically using ANOVA and 't' test.

Results

The effect of the phytoecdysteroid on Grasserie caused by nuclear polyhedrosis virus (BmNPV), one of the major diseases of silkworm is presented in Table 1. When silk-

Table 1. Effect of Sampoorna on BmNPV inoculated population of silkworm

Treatment	Yield/10,000 larvae	Yield (kg)	Cocoon (g)	Shell weight (g)	Shell ratio (%)	Melting (%)	Disease incidence (%
T1	7517	10.800	1.446	0.297	20.57	14.67	8.50
T2	8117	11.620	1.434	0.293	20.41	11.00	7.83
T3	8600	13.570	1.577	0.325	20.63	10.67	3.33
T10	8500	12.470	1.715	0.382	22.27	11.33	3.67
T12 (control)	9650	20.740	1.948	0.431	22.16	2.67	1.33
SE ±	110.66	0.83	0.013	0.004	0.31	1.47	0.76
CD at 5%	383.00	1.13	0.052	0.017	1.07	--	2.46

Table 2. Comparative values of Sampoorna effect on BmNPV inoculated population of silkworm

Treatment	Yield/10,000 larvae	Yield (kg)	Cocoon (g)	Shell weight (g)	Shell ratio (%)	Melting (%)	Disease incidence (%
T1	7517	10.800	1.446	0.297	20.57	14.67	8.50
T7	9100	17.080	1.723	0.405	23.50	3.33	3.33
't' test	−9.50**	−12.89**	−19.15**	−17.67**	−10.59**	3.56*	5.81**
T2	8117	11.620	1.434	0.293	20.41	11.00	7.83
T8	9733	18.030	1.830	0.433	23.45	1.67	1.50
't' test	−12.96**	−30.63**	−15.09**	−17.91**	−13.18**	5.29*	6.52**
T3	8600	13.570	1.577	0.325	20.6	10.67	3.33
T9	9650	17.890	1.850	0.420	22.97	1.67	1.83
't' test	−18.19**	−14.86**	−10.29**	−9.33**	−6.99**	19.09**	3.18*

Table 3. Effect of Sampoorna on BmIFV inoculated population of silkworm

Treatment	Yield/10,000 larvae	Yield (kg)	Cocoon (g)	Shell weight (g)	Shell ratio (%)	Melting (%)	Disease incidence (%
T4	6983	11.180	1.620	0.350	21.52	7.33	22.83
T5	6883	10.980	1.626	0.325	20.00	7.67	22.50
T6	6467	11.900	1.602	0.333	20.76	3.00	32.33
T11 (control)	5217	6.670	1.264	0.265	21.20	33.83	3.33
SE±	183.0	0.92	0.09	0.006	0.36	0.68	1.45
CD5%	632.0	3.18	0.030	0.021	--	2.36	--

worms are inoculated with BmNPV, an average cocoon melting of 11.91% with a disease incidence of 5.83% was recorded. In T1 cocoon melting and disease incidence was increased considerably (14.67%; 8.50% respectively). In T2, a melting percent of 11.00 with a disease incidence of 7.83. In control batch (T10) 11.33% of melting and 3.67% disease incidence was recorded. In the normal batch, without any pathogen inoculation (T12) showed only negligible melting (2.67%) and disease incidence (1.33%) (Table1). The values of 't' test for different treatments of BmNPV is presented in Table 2 and when compared between T1 and T7 survival rate and cocoon traits were drastically reduced in T1 where pathogen load is already existing. It also true for 5^{th} and 6^{th} day treated inoculated batches. Another major disease is infectious flacherie virus is presented in Table 3. In T4, T5 and T6 were showed considerable levels of larval disease incidence (22-32%) with a cocoon melting ranging from 3 to 7.67%. When silkworms inoculated with BmIFV, an average larval disease incidence level of 20.84% with a cocoon melting of 12.95%. The data pertaining to BmIFV inoculated and sampoorna sprayed batches (T4, T5 and T6) when compared with non-inoculated and sampoorna sprayed batches (T7, T8 and T9) presented in Table 4 indicating significant disease incidence and cocoon melting. There is a drastic reduction in survival rate, cocoon yield and other economic traits. The comparative data pertaining to T4, T5 and T6 with that of T11 is presented in Table 5 also indicating significant differences for disease incidence and cocoon melting. But when comparison made between

Table 4. Comparative values of Sampoorna effect on BmIFV inoculated population of silkworm

Treatment	Yield/10,000 larvae	Yield (kg)	Cocoon (g)	Shell weight (g)	Shell ratio (%)	Melting (%)	Disease incidence (%)
T4	6983	11.180	1.620	0.350	21.52	7.33	22.83
T7	9100	17.080	1.723	0.405	23.50	3.33	3.33
't' test	−6.09**	−4.41*	−19.15**	−17.67**	−10.59**	0.86NS	1.11NS
T5	6883	10.980	1.626	0.325	20.00	7.67	22.50
T8	9733	18.030	1.830	0.433	23.45	1.67	1.50
't' test	−12.96**	−30.63**	−15.09**	−17.91**	−13.18**	5.29**	6.52**
T6	6467	11.900	1.602	0.333	20.76	3.00	32.33
T9	9650	17.890	1.850	0.420	22.97	1.67	1.83
't' test	−18.19**	−14.86**	−10.29**	−9.33**	−6.99**	19.09**	3.18*

Table 5. Effect of Sampoorna on BmIFV inoculated population of silkworm compared to control

Treatment	Yield/10,000 larvae	Yield (kg)	Cocoon (g)	Shell weight (g)	Shell ratio (%)	Melting (%)	Disease incidence (%)
T4	6983	11.180	1.620	0.350	21.52	7.33	22.83
T11 (control)	5217	6.670	1.264	0.265	21.20	16.00	33.33
't' test	6.09**	9.19**	25.43**	7.15**	0.46NS	−4.11*	−11.66**
T5	6883	10.980	1.626	0.325	20.00	7.67	22.50
T11 (control)	5217	6.670	1.264	0.265	21.20	16.00	33.33
't' test	10.38**	28.29**	4.81**	−1.57NS	--	−3.20*	−8.14**
T6	6467	11.900	1.602	0.333	20.76	3.00	32.33
T11 (control)	5217	6.670	1.264	0.265	21.20	16.00	33.83
't' test	8.39**	3.33*	28.33**	5.33**	−0.59NS	−10.47**	−1.77NS

T4, T5 and T6 with that of T12 (normal control), there is a drastic reduction in survival and cocoon traits with significant levels of disease incidence and cocoon melting in treated batches. The control (T12) batch indicated negligible values for disease incidence and cocoon melting (Table 1).

Discussion

The impact of phytoecdysteroid on the larval growth and development of mulberry silkworm *Bombyx mori* has been investigated by many workers. The exogenous administration of phytoecdysteroids was found to increase the cocoon yield and enhance the productivity of silkworm rearing without significant variations in the quality of cocoons (Ito, 1970; Kobayashi, 1978). It was observed in the present investigation that on application of phytoecdysteroid on the silkworm batches, which were already infected, the disease incidence and melting percentage was significantly increased (Table 2 and 3). This may be due to the presence of pathogen load and increased intake of phytoecdysteroid, which can influence hormonal imbalance particularly when administered on 4th day of 5th instar (T4). The normal batches administered with phytoecdysteroid (T7-T9) had shown little variation in cocoon yield with reduced level of cocoon mortality and disease incidence. Ninagi and Maruyama (1995) clearly showed the application of phytoecdysteroid one day after first feeding in 5th instar, prolonged the larval duration, while application on the 4th day of 5th instar shortened the larval duration. In the present study, the administration of phytoecdysteroid after the middle stage of 5th instar on 4th day (T7) resulted in shortening of larval duration by 24 hrs and slight reduction in cocoon yield. The fifth day of 5th instar is a very crucial period in larval development as the larvae may be preparing for spinning according to the prevailing climatic conditions. At this instance, the larvae will reduce feeding and its digestive capacity reduces as the amalyze level drops at the on set of spinning (Abraham *et al*., 1992). This may also due to the decrease in juvenile hormone titer and corresponding increase in moulting hormone level as indicated by authors like Cao *et al*. (1985) and Plantein *et al*. (1987). These findings are in parallel with the findings of Zhuang *et al*. (1992). But this method of application on 4th day of 5th instar can only

be employed to avert total crop loss in extreme conditions when the inoculated batches are already having pathogen load. In the present investigation application of Sampoorna on 4th day of 5th instar (T1 and T3) triggered higher mortality. Chandrakala *et al*. (1998) suggested that phytoecdysteroid could also be used to reduce the larval duration and to reduce crop loss due to diseases. Zhuang *et al*. (1992) suggested that the phytoecdysteroid will induce early spinning and giving less time for the spread of the diseases in order to avoid total crop loss. However, the timing of application of Sampoorna is critical especially under diseased conditions as it is investigated in the present study. The application could be after the 4th day of final instar to explore the benefit of phytoecdysteroid. And our study suggests that this should be done only in the extreme conditions of rearing. When labour force is becoming scarce in the developing countries, any labour saving method like application of phytoecdysteroids will lead to uniform maturation and improvement in productivity with the added advantage of getting better quality cocoons.

Acknowledgement

The authors are thankful to Dr. Kanika Trivedy, Scientist of Silkworm physiology laboratory for providing Phytoecdysteroid “Sampoorna” sample and Mr. A. K. Palit, Assistant Director (Statstics) Silkworm breeding laboratory of this institute for statistical analysis.

References

Abraham E, Nagaraju GJ, Datta RK (1992) Biochemical studies of analysis in the silkworm, *Bombyx mori* L.; Comparative analysis in diapausing and non-diapausing strains. Insect Biochem Mol Biol 22, 867-873.

Cao M, Jiang RJX, Li XY (1985) Effect of JHA on ecdysteroid titers in haemolymph of fifth instar larvae of *Bombyx mori* L. (Lepidoptera). J Shangai Institute of Entomology, 5, 25-31.

Cantwell GE (1974) Laboratory exercise 1.In;Insect diseases II, (Ed) G. E. Cantwell, Marcel Dekker Inc., New York, PP. 539-547.

Chandrakala M, Maribasetty VG, Jyothi HK (1998) Application of phytoecdysteroid in Sericulture, Curr Sci, 74, 341-348.

Datta RK (1992) Guidelines for bivoltine rearing. *Central Silk Board, Bangalore,* India pp.25.

Ito T, Horie Y, Watanabe K (1970) Effect of phytoecdysteroids on the length of the fifth instar and the quality of cocoons in silkworm *Bombyx mori*. Ann Zool Jpn, 43,175-181.

Jayaramaiah M, Kuberappa GC, Devaiah MC, Kotikal YK (1986) White muscardine disease of silkworm and its management. Indian Silk, 25, 15-16.

Kobayashi M (1978) Insect Endocrinology. In: The silkworm. An important laboratory tool. (ed) Tazima, Y. Kodansha Ltd., Tokyo, pp159-188.

Ninagi O, Maruyama M (1995). Utilisation of 20 hydroxy ecdysone extracted from a plant in sericulture. JARQ, 30, 123-128.

Plantein G, Boszuet C, Calvez B, Nardon C (1987). Relationship between juvenile hormone levels and synthesis of major haemolymph proteins in *Bombyx mori* larvae. Compendium of Biochemistry and Physiology, B; Compendium Biochemistry, 86, 501-508.

Selva Kumar T, Nataraju B, Balavenkatasubbaiah M, Sivaprasad V, Baig M, Kumar V, Sharma SD, Thiagarajan V, Datta RK (2002). A report on the prevalence of silkworm diseases and estimated crop loss. In. Advances in Indian sericulture research (Eds.) S. B. Dandin, and V. P. Gupta, *Proc. of. Nat. conf. on strategies for Sericulture Research and Development*, CSR&TI., Mysore, Karnataka, India, Nov.16-18, p.354-357.

Trivedy K, Sashindran Nair K, Ramesh M, Gopal N, Nirmal Kumar S (2003). Early and uniform maturation in silkworm, *Bombyx mori* L. by phytoecdysteroid extracted from a plant of family Caryopillaceae. Int J Indust Entomol, 7, 65-68.

Zhuang D, Xiang HM, Gui ZZ (1992) The practical studies on the growth and development by insect hormones in the silkworm *Bombyx mori* L.. XIXint.Cong. Entomol, Jun 28th—July2nd, Beijing, China.