Influence of moisture on the toxicity of some biopesticides on *Bombyx mori* L.

Hirak Chatterjee

ABSTRACT

Adverse effect of moisture on some microbial insecticides such as Halt (*Bacillus thuriengiensis* var kurstaki, *B.t.k.*- 55000 S.U./mg), Biolep (*B.t.k.*- 32000 I.U. /mg), Vertimec (product of *Streptomyces avermitilis* - Avermectin-1.8% w/v) and Bassina (formulation of *Beauvaria bassiana*-1 x 10^7 spore / ml) were studied against third instar larvae of mulberry silkworm, *Bombyx mori* L. under four levels of relative humidity (30%, 60%, 80% and 90% R.H.) and constant temperature (20 ^{0}C ± 2 ^{0}C). The biocide avermectin at 1000 ppm caused 96.66% and 95.00% mortality after 72 h of treatment at 80% and 90% relative humidity respectively. Whereas, halt and biolep caused 95.00%, 90.00% and 81.66%, 80.00% mortality after 72 h at the two respective humidies level. Through fungal formulation of *B. bassiana* proved to be less harmful against *B. mori* at 30% and 60% R.H. it inflicted 40 – 43.33% mortality at higher moisture level (>70% R.H.). Relative humidity was found to play a major role towards pathogenecity of all the tested microbials against *B. mori*. Increasing mortality was recorded with increase in days after treatment and maximum effect was noticed at 80% R.H. The overall effect followed the same trend for all the microbial insecticides, and the observed descending order was Avermectin > Halt > biolep > *B. bassiana* at four levels of relative humidity, respectively.

Key words: Relative humidity, microbial insecticides, impacts, biosafety, *Bombyx mori*

INTRODUCTION

One of the most important components of bio-intensive pest management is the biological suppression of insect-pest by employing pathogen like bacteria, virus, and fungus, which was designated as microbial pesticides (Steinhaus, 1949). These biopesticides already established as an alternative to eco-destabilizing chemical insecticides especially against lepidopteran pests (Chatterjee, 2001). However, like other chemical pesticides a substantial portion of the applied biocides reach the soil ecosystem and also get contaminated in the vicinity area of the sprayed field where it could affect the non-target organisms. Among them mulberry silkworm is very important because of its immense value in natural silk production. The main limiting factor in large-scale use of *B.t.k.* and avermectin is their high toxicity against this beneficial insect *Bombyx mori* L. (Chatterjee and Choudhury, 2003) and other non-target species (Pramanik and Somchoudhury, 2001). Moreover, the humidity plays a major role in silkworm rearing (Upadhyay and Mishra, 1991) as well as affecting the pathogenecity of the microbial insecticides because of its biological origin. But this aspect was not adequately studied in the past. Therefore, the present study was conducted to assess the effect of some microbial insecticides on *B. mori* at varying levels of moisture under terai region of West Bengal, which is considered to be a new productive zone for bivoltine sericulture (Subba *et al*., 1987).

MATERIALS AND METHODS

Microbial formulations

Two formulations of *Bacillus thuringiensis* var *kurstaki* (*Btk*-55000 SU mg^{-1} (Halt) from Biostadt Agrisciences, Wockhardt Ltd. and *Btk* -32000 IU mg^{-1} (Biolep) from Biotech International Ltd. one formulation each of *Streptomyces avermitilis* (vertimec) [Avermectin-1.8% w/v from Novartis, Mumbai] and *Beauvaria bassiana* (Balsamo) Vuille. (Bassina- 1 x 10^7 spore ml^{-1}. from Agro Evo India Ltd.) were used in the present investigation.

Maintenance of insects

Bivoltine race of mulberry silkworm (*Bombyx mori* L) was reared in the laboratory following the procedure laid out by Krishnaswami (1978). Third instar larvae were used in the present study

Bioassays

To determine their adverse effect under varying moisture regime, freshly collected mulberry leaves were dipped in

1000 ppm microbial suspensions of the two *Btk* formulations and avermectin whereas, direct topical application was followed for *B. bassiana* spore suspension at a concentration of 2000 ppm. Treated leaves were shade dried and kept separately in a sterilized glass petri plate of 10 cm diameter which was then placed under four levels of relative humidity *viz*., 30%, 60%, 80% and 90% and the temperature of the growth chamber was maintained within 18^0C - 22^0C. Third instar larvae of *B. mori* were allowed to feed for 24 h, and thereafter fresh leaves were supplied. Mortality count was taken at 24 h interval for 3 days. Twenty larvae constituted one replication and all the treatments were replicated thrice. Only water was used for control and kept under normal laboratory conditions. The data thus obtained were subjected to two factors CRD analysis after correction by using Abbott's formula (1925).

RESULTS AND DISCUSSION

Effect of Halt

It was revealed from the table-1 that *Btk*-55000 SU mg^{-1} caused maximum mortality at 80% followed 90%, 60% and 30% relative humidity. There was significant difference ($P < 0.05$) in mortality under four levels of relative humidity as well as percentage of mortality increases with the progression of days after treatment. Highest cumulative mortality (95.00%) was obtained at 80% R.H. after 72 h of treatment. Considering the DMRT test, there was no significant difference among the different levels of R.H. after 24 and 48 h of treatment but mortality percentage after 72 h of treatment significantly increased at 80-90% R.H. This may be ascribed due to increased pathogenecity of *B. thuringensis* under humid condition as compared to dry one. However the finding is in confirmation with that of Pramanik and Somchoudhury (2001).

Effect of Biolep

Table 2 showed that biolep caused a maximum of 71.55% at 80% R.H. followed by 68.88%, 64.99% and 61.55% mortality at 90%, 60% and 30% R.H. respectively. There was significant difference in effect under four levels of relative humidity as well as the percentage of mortality increases with the progression of days after treatment. Highest cumulative mortality was obtained at 80% R.H. after 72 h of treatment. DMRT test revealed that, there was no significant difference in mortality after 24 and 48 h of treatment at four different levels of R.H. but mortality percentage after 72 h of treatment significantly increased at 80-90% R.H. This may be due to decreasing pathogenecity of *B. thuringiensis* under dry condition. The result under present investigation was not in agreement with the finding of Ignoffo (1962) where relative humidity was reported to have no role in inhibition or activation of the patogenecity of *B.t.* products. Recent report expressed that *Bt* affects *M. mori* vigorously (You *et al.*, 2008; Yao *et al*., 2008).

Table 1. Effect of Halt-55 and Biolep on the larval mortality (in %) of *B. mori* at different levels of relative humidity (in %)

Relative huminity (in %)	Hours of treatments			
	24	48	72	Overall
Halt-55				
30	50.00 ^{d}	63.33 ^{bcd}	70.00 ^{bc}	61.11
60	55.00 ^{cd}	68.33 ^{bc}	75.00 ^{b}	66.11
80	65.00b ^{cd}	76.66 ^{b}	95.00 ^{a}	78.88
90	61.66 ^{bcd}	65.00 ^{bcd}	90.00 ^{a}	72.22
Biolep				
30	53.33 ^{e}	61.33 ^{cde}	70.00 ^{abcd}	61.55
60	53.33 ^{e}	66.66 ^{bcde}	75.00 ^{abc}	64.99
80	61.66 ^{cde}	71.33 ^{abcd}	81.66 ^{a}	71.55
90	58.33 ^{de}	68.33 ^{abcde}	80.00 ^{ab}	68.88

Means followed by a same letter are not different at 5% level of significance by DMRT.

Table2. Effect of Avermectin-1.8% on the mortality (in %) of *B. mori* larvae at different levels of relative humidity (in%)

Relative Huminity (in %)	Hours of treatments			
	24	48	72	Overall
30	68.33 [d]	78.33 [cd]	85.00 [c]	77.22
60	73.33 [cd]	81.66 [cd]	86.66 [bc]	80.55
80	78.33 [cd]	86.66 [bc]	96.66 [a]	87.21
90	76.66 [cd]	83.33 [cd]	95.00 [ab]	84.99

Means followed by a common letter are not different at 5%level of significance by DMRT

Effect of Vertimec

Avermectin-1.8% w/v was emerged as the most toxic compound to *B. mori* at 80% R.H. followed by 90% R.H. and even at 30% R.H, it inflicted 72.22% mortality (Table 2). Otherwise similar trend was noticed regarding its effect on this insect. Moreover, a maximum of 96.66% motility was observed after 72 h of feeding at 80% R.H. followed by 95% at 90% R.H. which were statistically different from the other observations. The observation made in this experiment corroborates with the findings of Chatterjee and Choudhury (2003).

Effect of Bassina

Beauavria bassiana was found to be less harmful against this beneficial insect at least under dry condition as the fungus inflicted only 15.55% and 27.22% mortality at 30% and 60% R.H. respectively (Table 3). However, adverse effect was noticed under 80-90% R.H. when 37.22% - 39.44% overall mortality was recorded which again substantiate that for fungal infection high relative humidity > 70% and moderate temperature (20-30 ^{0}C) is necessary (Dhaliwal and Arora, 2001). In all the four levels of relative humidity, mortality per cent after 72 h of treatment significantly differs from the other two observations. This is due to some incubation period required for manifestation of fungal pathogenecity.

Comparative effect of all the microbial insecticides against *B. mori* at four levels of relative humidity shows that maximum and minimum larval mortality was obtained at 80% and 30% relative humidity, respectively. Among the biopesticides, vertimec was the most toxic biopesticide followed by Halt, Biolep and Bassina. Moreover, the difference in mortality was more distinct at 80-90% R.H. from the other levels for fungal formulation than the other microbials. The interaction among the different biocides and hours after treatment were found significant. From the above discussion it can be concluded that the effect of all the biopesticides were more pronounced within a particular range of relative humidity (80-90%) and a moderate temperature of 25-30 ^{0}C. This is again a favourable condition required for silkworm rearing (Upadhyay and Mishra, 1991). Hence, any contamination in the mulberry leaves from the vicinity field may jeopardize the sericulture industry from this promising zone. Therefore, the use of avermectin should be restricted and can be suggested in barest minimum requirement. The major limiting factor in large-scale use of *B.t.k.* is its high

Table 3. Effect of *Beauvaria bassiana* on *B. mori* at different levels of relative humidity

Relative Huminity (in %)	Percentage of mortality after different hours of treatments			Overall effect
	24	48	72	
30	3.33 [e]	16.66 [d]	26.66 [bcd]	15.55(20.71)
60	23.33 [cd]	28.33 [abc]	30.00 [abc]	27.22(31.32)
80	35.00 [abc]	36.66 [abc]	40.00 [ab]	37.22(37.56)
90	36.66 [abc]	38.33 [ab]	43.33 [a]	39.44(38.88)

Means followed by a common letter are not different at 5%level of significance by DMRT

toxicity against *B. mori* So wide spread use can't be advocated unless *B.t.k*. proves its safety towards this high valued insect.

REFERENCES

Abbott, W.S. 1925. A method for computing the effectiveness of insecticides. *Journal of Economic Entomology*, **18**: 265-267.

Chatterhee, H. 2001. Investigation on potentialities of microbials on some important lepidopteran pests in terai zone of West Bengal. *Ph.D. Thesis*, Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal, 54-123.

Catterjee, H. and Choudhury, P.P. 2003. Persistent toxicity of some microbial pesticides against *Bombyx mori* (L) feeding on mulberry leaves in Terai region of West Bengal. *Pesticide Research Journal*, **15**(1): 80-84.

Dhaliwal, G. S and Arora, R. 2001. In: *Integrated pest Management: Concepts and Approaches*, Kalyani, Pub. Ludhiana 220 **P**.

Ignoffo, C. M. 1962. The effects of temperature and humidity on mortality of larvae of *Pectinophora gossypiella* (Saunders) infected with *Bacillus thuringiensis* Berliner. *Journal of Insect Pathology,* **4** (1): 63-71.

Krishnaswami, S. 1978. *New technology of silkworm rearing*. Bulletin No.2, Central Sericultural Research and Training Institute, Central Silk Board, India. 1-23 **PP**.

Pramanik, A and Somchoudhury, A.K.2001. Adverse effect of *Bacillus thuriengiensis* Berliner on silkworm, *Bombyx mori* and other beneficial non-target organisms. *Journal of Entomological Research,* **25**(1): 53-57.

Steinhaus, E.A. 1949. *Principles of Insect Pathology*. McGraw Hill Book Cp. Inc. New York, 757 **PP.**

Subba RAO, G.; Chakraborty, N.G.; Nath, S.; Nath, T. N.; Shamsuddin, M. and Sen, S.K. 1987. Scope of bivoltine rearing in the district of Jalpaiguri (West Bengal). Seminar on prospect of mulberry sericulture in the Eastern Region of India, May 5-6, Berhampore.

Upadhyay, V.B. and Mishra, A.B. 1991. Nutritional ability of bivoltine silkworm, *Bombyx mori* L. larvae at higher temperature regimes. *Journal of Advnced Zoology*, **12** (1): 56-59.

You, T.H., Lee, M.K., Jenkins, J., Alzate, O. and Dean, D.H. 2008. Blocking binding of Bacillus thuringiensis cry 1A2 to *Bombyx mori* cadherin receptor results in only a minor reduced activity. *BMC Biochemistry*, **9** (3): 2091 – 2093.

Yao, H.W., Cai-Ying Jiang, Gong-Yin Ye, Cui Hu, and Yu-Fa Peng. 2008. Toxicological Assessment of Pollen from Different *Bt* Rice Lines on *Bombyx Mori* (Lepidoptera: Bombyxidae). *Environmental Entomology*, **37**(3):825-837. 2008.

Hirak Chatterjee

Department of Plant Protection, Palli Siksha Bhavana, Visva-Bharati,Sriniketan- 731236, West Bengal, India.
E-mail: hc65@radiffmail.com