Synergistic Effect of Azadirachtin and *Bacillus thuringiensis* against Bihar Hairy Caterpillar, *Spilarctia obliqua* Walker

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ABSTRACT The commercial formulation Delfin® containing *Bacillus thuringiensis* var. *kurstaki* (*Btk*) serotype 3A, 3B, SA11 (WG) and Margo Econeem™ Plus (EC) based botanical insecticide containing azadirachtin 1% (10,000 ppm) were evaluated alone and in different sublethal combinations against 3rd instars of *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae) under laboratory conditions. Leaf dip method was used for *Btk* whereas topical application for azadirachtin. The studies revealed that the LC_{50} value of azadirachtin after 72 h post-treatment was 0.153% whereas, for *Btk* it was 0.145%. No synergistic effect was observed when treatment was given at LC_{20} level of *Btk* (0.021%) and LC_{30} level of azadirachtin (0.06%) up to 72 h post-treatment. However, synergistic effect was evident from the combinations at LC_{25} level of *Btk* (0.003%) + LC_{25} level of a zadirachtin (0.004%) and LC_{30} treatment level of *Btk* (0.044%) + LC_{20} level of azadirachtin (0.031%) after 72 h. The studies suggest that combination of botanicals with microbes could be strategized for utilization in integrated management of *S. obliqua*.

KEY WORDS : Azadirachtin, *Bacillus thuringiensis*, Synergists, *Spilarctia obliqua*, toxicity

INTRODUCTION

Bihar hairy caterpillar, *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae), is a sporadic pest, widely distributed in India, China, Bangladesh, Myanmar, Nepal and Pakistan (CPC 2004). In India, it is a serious pest in West Bengal, Bihar, Madhya Pradesh, Uttar Pradesh, Punjab, Manipur, and other states. Due to its highly polyphagous nature, it attacks variety of bast fibre crops, pulses, oilseeds, cereals, certain vegetables, mulberry, medicinal, aromatic and other economically important plants, and causes severe economic damage (Gupta and Bhattacharya, 2008). It is one of the major pests of bast fibre crops like jute (*Corchorus* spp.) and mesta (*Hibiscus* spp.) and causes a total foliage loss up to 20–30% in jute plantations in West Bengal, India. However, jute is a more preferred host than mesta. *Tossa* jute (*Corchorus olitorius*) is more susceptible to *S. obliqua* than the white jute (*C. capsularis*) (Pandit, 1985). In recent years, outbreaks of this pest during 2011 in jute and 2012 in sunnhemp crop were reported and caused substantial loss to the fibre yield. The female moths lay eggs in groups and a single female may lay up to 1000 eggs. Upon hatching, the caterpillars feed gregariously during the early (first to third instars) larval stages and solitarily in the late (fourth to fifth instars) larval stages (Gupta and Bhattacharya, 2008).

Timely management of this pest is very important as delay may even lead to complete defoliation of

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crop if remains unchecked in the field. Chemical control of this pest is difficult due to dense hairiness and moreover, the acute toxicity and relative resistance of *S. obliqua* to chemical pesticides has also been reported (Perry *et al*. 1998; Dhingra *et al*, 2007). Chemical pesticides not only cause environmental and health hazards but also encourages pest resistance, resurgence and secondary pest outbreak. To overcome this, rotations of insecticides with different modes of action and use of insecticide mixtures have been the strategies to avoid resistance development (Roush and Miller, 1989; Tabashnik, 1989; Bharathi*, et al.,* 2010). Several studies conducted on combined toxicity of *Bacillus thuringiensis* (*Bt*) and chemical insecticides against *S. obliqua* are known (Bhattacharya and Pramanik, 2005; Khan *et al.,* 2010)

The use of biopesticides could effectively control insect pest complex associated with jute (Banerjee *et al.,* 2000). Biopesticides such as *Bt* and plant based insecticides like azadirachtin are safer viable options for eco-friendly management of *S. obliqua* in jute and allied fibre crops. *B. thuringiensis* has been in use as a safe stomach poison inducing immediate cessation of feeding, growth inhibition and eventually mortality against many lepidopteran pests. Several studies on the effect of *Bt* (Pawar and Charati, 2000; Pramanik and Somchoudhury, 2002; Khan *et al.,* 2012; Zeenat and Ajay, 2012) and neem (Chowdhury*et al.,* 2001; Mondal and Bhattacharya, 2003; Chowdhury *et al.,* 2012) against *S. obliqua* are known.

In fact, the efficacy of neem-based products is determined by the amount of azadirachtin and the type of formulation (Stark and Walter, 1995; Koul and Wahab, 2004). Therefore, this compound could be useful in combination with *Bt* to increase efficacy and host range as well as to reduce the resistance development. There are some studies available where the efficacies of *Bt* and neem have been evaluated against *S. obliqua* (Trisyono and Whalon, 1999; Chahal *et al.,* 2003; Devaki and Krishnayya, 2004).The objective of the present study was to determine the specific levels of combination of *Bt* with azadirachtin that will provide synergistic formulation to control *S. obliqua* larvae judiciously.

MATERIALS AND METHODS

Insects

The newly hatched gregarious larval masses of *S. obliqua* were collected from Research Farm of Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, India. The larvae were reared in the rearing container (13 × 13 cm diameter) on jute under controlled condition of 27 ± 2°C and relative humidity of 75–80% in BOD incubator. Third instars (10- days-old) having average body weight of 98 mg were used for bioassays.

Bioassay for *Bacillus thuringiensis*

The leaf dip method was used for bioassay with Delfin® commercial formulation of *B. thuringiensis* var. *kurstaki* (WG) to determine the mortality of *S. obliqua*. Five concentrations (0.002, 0.001, 0.0005, 0.00025 and 0.000125%) of *Btk* were prepared in water by making serial dilution during study along with control. The fresh five jute leaves with uniform age were dipped for two minutes in each concentration and air dried under room temperature for about 30 min. Distilled water was used in controls. Larvae (*n* = 50) starved for 4 h were used in 5 replicates of 10 larvae each and released on to the treated leaves in Petri dishes (7.5 × 1.5 cm). Petri dishes were kept in BOD incubator at 26 ± 1°C and subsequently mortality was recorded.

Bioassay for Azadirachtin

Leaf dip method was used for bioassay with *Margo Econeem*™ Plus based botanical insecticide containing 1% azadirachtin. Econeem was applied to the leaves and third instars released on to the leaves in similar fashion as mentioned above. Five test concentrations of Econeem were used based on azadirachtin content along with controls. The test concentrations for determination of LC_{50} were 0.003, 0.002, 0.001, 0.005 and 0.0025%. Remaining procedures were similar to *B. thuringiensis* treatments.

Synergistic Combination for *Bacillus thuringiensis* and Azadirachtin

The synergistic effect was calculated using the

modified method of Singh *et al.* (2006). The LC_{20}, LC_{25} and LC_{30} of both the neem and *Btk* were calculated using the SPSS software. To test the synergistic effect three combinations were made, i.e. i) LC_{25} *Btk* + LC_{25} azadirachtin; ii) LC_{20} *Btk* + LC_{30} azadirachtin; iii) LC_{30} *Btk* + LC_{20} azadirachtin. Five replicates of each combination with control were used for the bioassay. Leaf dip method was followed for treating the third instars as mentioned above. The larval mortality was recorded after 24, 48 and 72 h after treatment. Once the mortality values were obtained Post Hoc test was done to determine the significance in the mean difference of mortality among the synergistic combinations.

Statistical Analysis

The larval mortality was observed after 24 and 48 h of *Bt* treatment and 24, 48 and 72 h after azadirachtin treatment and corrected by adjusting mortality in controls using Abbott's formula (Abbott's, 1925). The moribund larvae were also counted as dead. Probit analysis was used to calculate LC values using SPSS programme (16.0 version).

RESULTS

Toxicity of *Bacillus thuringiensis* and Azadirachtin

The LC_{50} value of *Btk* after 24 h was 1.71% and 0.145% after 48 h treatment (Table 1). The effect being more after 48 h, therefore, this concentration was used to determine the synergistic relation between the two insecticides. The χ^2 values for both periods were 4.74 and 3.51, respectively with significance level > 0.15, which indicates the fast action of *Bt*k in killing the pest larvae. The LC_{20}, LC_{25} and LC_{30} values of *Btk* were 0.26 and 0.21% (after 24 and 48 h); 0.37 and 0.031% (after 24 and 48h) and 0.512 and 0.044% (after 24 and 48 h), respectively. Azadirachtin mortality on the other hand for 24 and 48 h was not significant but LC_{50} values could be calculated after 72 h treatment and was 1.53%. The significance level for χ^2 at 72 h was 5.04 and more than 0.150 obtained with *Btk*, which indicates that quick mortality is relative to duration of treatment. The LC_{20}, LC_{25} and LC_{30} values of azadirachtin at 72 h were 0.312, 0.441 and 0.603%, respectively.

Synergistic Effect of *B. thuringiensis* and Azadirachtin

LC_{30} *Btk* + LC_{20} azadirachtin showed the highest larval mortality of 70%, followed by LC_{25} *Btk* + LC_{25} azadirachtin of 64% and LC_{20} *Btk* + LC_{30} azadirachtin of 30% (Table 2). There was no significant mean difference in test insect response between LC_{30} *Btk* + LC_{20} azadirachtin and LC_{25} *Btk* + LC_{25} azadirachtin, thus any of these two treatments can be used for obtaining synergistic effect. However, two treatments LC_{30} *Btk* + LC_{20} azadirachtin were better than LC_{20} *Btk* + LC_{30} azadirachtin and LC_{25} *Btk* + LC_{25} azadirachtin. It is evident from the result that the concentration of *Bt* in the mixture is in proportion to the insect mortality, or in other terms insecticidal property of the mixture is enhanced by higher

Table 1. Toxicity of *Bacillus thuringiensis* and azadirachtin on Bihar hairy caterpillar, *Spilarctia obliqua*

Treatment	Observation Time	DF	LC_{50} (%)	Regression equation	χ^2
Bacillus thuringiensis	24 h	4	1.71	Y = 1.226 + 1.189X R^2 = 0.9384	4.74
	48 h	4	0.14	Y = 2.852 + 0.996X R^2 = 0.9833	3.51
Azadirachtin	24 h	4	8.05	Y = 0.315 + 1.167X R^2 = 0.7836	6.37
	48 h	4	4.70	Y = 0.734 + 1.120X R^2 = 0.8558	5.08
	72 h	4	1.79	Y = 1.027 + 1.207 X R^2 = 0.9351	5.04

concentration of *Bt* in the mixture. There was no synergistic effect observed between LC_{20} *Btk* + LC_{30} azadirachtin treatments, but synergistic effect was evident from the combinations of LC_{25} *Btk* + LC_{25} azadirachtin and LC_{30} *Btk* + LC_{20} azadirachtin (Table 2).

DISSCUSSION

The mode of action of *B. thuringiensis* insecticidal crystal toxins include ingestion, proteolytic activation of protoxins to release active toxins favoured by midgut pH, binding of toxin to midgut receptors, pore formation in the brush border membrane vesicles (BBMV's) resulting in Na+/K+ imbalance, feeding cessation, septicaemia and eventual death. During the present investigations, *S. obliqua* larvae infected by *Bt* become inactive and stop feeding. The caterpillar becomes pale yellow and black, flaccid, eventually dies, usually within days. The body contents turn brown to black as they decompose. Such symptoms are known to occur as early as two days after larval treatments with high concentrations of *Btk* and mortality occurs on or before the 2nd day after treatment depending on the age and stage of the larvae when they were exposed to the toxin (Miao, 2002; Navon, 2000). The insect didn't die immediately after feeding on the treated leaves but stopped feeding. The rate of damage to the gut cells is enhanced with high concentration of parasporal protein in the larval feed, thus higher mortality is achieved faster with higher concentrations. The potentiating effect observed for *B. thuringiensis* from our experiment was in agreement with the earlier observations (Morris *et al*., 1995; Salama and Salem, 2000; Latha *et al*. 2002) against *Spodoptera littoralis*, *Agrotis ipsilon* and *M. cenfigurata*. The LC_{50} values obtained in present study are better than those obtained for *Plutella xylostella* (Singh *et al*., 2000). The direct and indirect effects to treatment efficacy through reduced larval feeding and fitness need to be better understood in order to improve the use of *Btk* for management of S. *obliqua* in bast fibre crops.

Azadirachtin causes in a stage and dose-dependent manner, a cessation of feeding, delay of moulting and death of larvae. The cadaver body turned black and died in the mid process of ecdyses. Microbial activity was enhanced either by causing insect toxicity or by potentiation of the crystal protein. Similar observations were reported by Chowdhury *et al*. (2012). The synergistic relation between *Btk* and azadirachtin may be due to the direct effect of *Btk* and azadirachtin on enzyme regulation of the insect larvae. The present study showed that no synergistic effect was observed in

Table 2. Synergistic effect of *Bacillus thuringiensis* and azadirachtin on Bihar hairy caterpillar, *Spilarctia obliqua*

Treatment	Mortality (%)	Treatment	Mean difference	SE	Significance	Fiducial limit (95%) Lower	Upper
LC_{25} *Bt* + LC_{25} Neem	64	LC_{20} *Bt* + LC_{30} Neem	(0.39)*	0.1130	0.005	0.1460	0.6384
		LC_{30} *Bt* + LC_{20} Neem	- (0.08)	0.1130	0.476	-0.3294	0.1631
LC_{20}*Bt* + LC_{30} Neem	30	LC_{25} *Bt* + LC_{25} Neem	- (0.39)*	0.1130	0.005	-0.6384	-0.1460
		LC_{30} *Bt* + LC_{20} Neem	- (0.47)*	0.1130	0.001	-0.7216	-0.2291
LC_{30} *Bt* + LC_{20} Neem	70	LC_{25} *Bt* + LC_{25} Neem	(0.08)	0.1130	0.476	-0.1631	0.3294
		LC_{20} *Bt* + LC_{30} Neem	(0.47)*	0.1130	0.001	0.2291	0.7216

* The mean difference is significant at the 0.05 level.

the combination of LC_{20} *Btk* and LC_{30} azadirachtin on mortality of *S. obliqua* after 72 h of treatment which is in close agreement with Singh *et al* (2006) on *Helicoverpa armigera* (Salama and Salem, 2000) and *Plutella xylostella*. On the other hand synergistic effect was evident from the combinations of LC_{25} *Btk and* LC_{25} azadirachtin and LC_{30} *Btk* and LC_{20} azadirachtin treatments after 72 h, which is similar to the observations of Nathan *et al* (2004) for *Cnaphalocrocis medinalis* a major insect pest of rice. The botanicals had an enhancing influence on the *Bt*, the combination treatments being more efficient than the individual botanical or *Bt* treatments alone (Facknath and Lalljee, 1999).

The toxicity of neem and *Bt* combinations against second instar *Bt*-susceptible and resistant *L. decimpunctata* larvae suggests that combined effects could be useful for resistant pests as well (Altre *et al.*, 1996; Trisyono and Whalon, 1999). Hence, it can be concluded that LC_{25} *Btk* + LC_{25} azadirachtin and LC_{30} *Btk* + LC_{20} azadirachtin are best synergistic combinations for *S. obliqua* larvae causing significant mortality. The studies also suggest that combination of botanicals with microbes could be strategized for utilization in integrated management of *S. obliqua* for large scale use.

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