

EFFICACY OF NATIVE *BACILLUS THURINGIENSIS* BERLINER ISOLATES AGAINST DIAMOND BACK MOTH, *PLUTELLA XYLOSTELLA* (LINNAEUS) AND ITS COMPATIBILITY WITH COMMON INSECTICIDES

ABSTRACT

The comparative toxicity of native *Bacillus thuringiensis* (*B.t.*) strains against Diamond back moth, *Plutella xylostella* and their compatibility with some common insecticides were identified. In the bioassays five native strains NSC-1, NSC-3, NSC-9, COR-4 and GUR-5 were identified and proved as a novel isolate for controlling this pest. All the five native isolates were showed high toxicity and causing 100% mortality at 72 h after treatment. Compatibility of *B.t* isolates with chemical insecticides showed that all the tested insecticides (chlorpyrifos, alphamethrin, monocrotophos, multineem, spinosad and quinolphos) were compatible with the selected native *B.t* isolates except acetamiprid, DDVP and imidacloprid at all the field recommended dose, half the recommended dose and double the field recommended dose.

KEY WORDS: *Bacillus thuringiensis*, insecticidal activity, *Plutella xylostella*, bioassay

INTRODUCTION

Bacillus thuringiensis is a facultative anaerobic, gram-positive, motile, spore forming, rod shaped bacterium (Martin and Travers, 1989). It belongs to the order Eubacteriales and family Bacillaceae. It produces one or more crystalline inclusions during sporulation. The parasporal inclusions consist of one or more insecticidal proteins in the form of a crystal complex. These insecticidal proteins are commonly known as Insecticidal Crystal Proteins (ICP) or delta-endotoxin (Hofte and Whiteley, 1989; Crickmore *et al.*, 1998) which is very toxic to a wide variety of pests (Schnepf *et al.*, 1998; de Maagd *et al.*, 2001). *B.t* products are generally safe for vertebrates (Siegel and Shadduck, 1989; Kumar *et al.*, 2021) and beneficial to Arthropods (Flexner *et al.*, 1986), but they are frequently highly toxic to insect pests at low doses. Genes encoding these proteins were among the first to be used in plant genetic engineering for enhanced insect resistance (Vaeck *et al.*, 1987). These genes have been mainly used on major

crops such as cotton, maize, soybeans, potatoes, tomatoes, stored grains and on forest crops.

Cruciferous crops are highly nutritious vegetable crops, grown widely in India. It is attacked by several insect pests, among this diamond back moth (DBM), *Plutella xylostella* (Plutellidae; Lepidoptera) is the most serious, causing substantial damage. DBM has developed resistance to many conventional insecticides (Talekar *et al*, 1990, Navya *et al*, 2022 and Deivendran *et al*, 2007). Therefore in the present investigation, different native *Bacillus thuringiensis* isolates were evaluated for their insecticidal activity against *Plutella xylostella*.

One of the strategies used to exploit entomopathogens are the conservation of biological control agents within agro ecosystems. Inoculative and incremental introduction techniques are also important. In all cases, either to preserve the entomopathogens or to use it in combination with chemical pesticides, it is necessary to know the action of these productions on the micro-organism and then determine their compatibility. This interaction should be considered before recommending a given chemical agent and represents an important tool in programs of IPM. Pesticides can also act in a positive manner in combination with entomopathogens. At sub lethal doses they interact with the latter causing or activating infections, diseases by stress, or turning the insects more susceptible to the action of microbial toxins. In this respect, Tabashnik *et al*. (1990) reported that *Plutella xylostella* showed resistance to *Bt* formulations, which again emphasizes to exploit the joint action of *Bt* with Neem and other isolates of soil microbes, which could conceivably exert effective control of this pest. Compatibility of *B.t.* strains with chemical pesticides is very important for effective pest management. Enhanced effectiveness can be achieved by joint action of pathogens and chemical pesticides, which ultimately reduce the amount of total chemical insecticides used in field.

MATERIALS AND METHODS

Bioassay against *Plutella xylostella*

The bioassay studies were conducted in the Department of Plant Protection, Allahabad Agricultural Institute-Deemed University, Allahabad, Uttar Pradesh. The cabbage variety of “Golden Acre” was sown and the seedlings of 30 days old were used to raise the crop and were planted at 60 cm x 45 cm spacing in plot size of 2.0 m x 1.0 m. Larvae or pupa of *Plutella xylostella* were collected from cabbage field with no previous application of any *B.t.* formulations and the stock culture was maintained at lab condition on cabbage leaves. The newly emerged adults were confined in semi transparent plastic jars containing 2-3 fresh cabbage leaves fixed in small vial (5x2.5 cm) containing water. The adults were fed with 10% honey solution fortified with multivitamins. Culturing was carried out at a temperature ranging from 25°C to 32°C and relative humidity 72 to 90 percent and five-day-old larvae were used for bioassay studies.

B. thuringiensis standard strains viz., *B.t.* subsp. *kurstaki* HD1, HD 73, *B.t.* subsp. *tolworthi*, *B.t.* subsp. *sotto*, *B.t.* subsp. *kenyae* and *B.t.* subsp. *israelensis* were obtained from *Bacillus* Genetic Stock Center (BGSC), Ohio State University, USA. Isolated native *B.t.* strains and the standard strains were cultured on nutrient agar at 30°C. Nutrient agar slants containing bacterial strains were also maintained at 4 °C until use. Pure cultures of *B.t.* strains and native isolates maintained on nutrient agar plates were used by inoculating a loopful in 250 ml sterile nutrient broth kept in 1 litre conical flasks. The flasks were incubated in an incubator shaker at 150 rpm for 72 h at 30 °C. After that, it was centrifuged at 6000 rpm for 10 min. The resulting pellet containing spore and parasporal protein crystals were washed in 20 ml sterile distilled water and centrifuged at 6000 rpm for 5 minutes and the washing was repeated twice. The pellets were re-suspended in 10 ml of sterile distilled water and kept at 4 °C (Carozzi *et al.* 1991).

The cabbage leaves collected from the field were washed with water containing 0.1% Triton x-100 thoroughly and air dried for 30-60 minutes. Leaf discs of 4.5 cm diameter were cut and 100 µl of spore crystal mixture of selected *B.t.* strains or native isolates were applied uniformly on both sides and air-dried for 1 h. They were placed individually into sterilized Petri plates (90mm). Ten numbers of six-days-old larvae was released in each petriplates and three replicates were maintained per treatment. Larval mortality was recorded after every 24 h Data collected in experiments were

analyzed by using Completely Randomized Design (CRD). The percentage values are converted in to corresponding angles (Arc sine transformation) for statistical interpretation. The treatment means were compared by Least Significant Difference (L.S.D) for their significance for all the experiments (Gomez and Gomez, 1984).

Compatibility of *Bacillus thuringiensis* strains with some insecticides

Compatibility of 10 common insecticides widely used to manage insect pests was studied with five selected native *B.t.* isolates at *in vitro* condition. The details of insecticides used and their concentrations are given in table 1.

Table-1. Pesticides utilized in the experiment

S.N o	Technical name	Commercial name	Concentrations used (%)		
			X	½ X	2X
1.	Chlorpyrifos 20% EC	Force	0.05	0.025	0.1
2.	Alphamethrin 10% EC	Viper-10	0.05	0.025	0.1
3.	Imidacloprid 200 SL	Confidor	0.02	0.01	0.04
4.	Acetamiprid 20% SP	Pride	0.05	0.025	0.10
5.	DDVP 76% EC	Badal	0.07	0.035	0.14
6.	Monocrotophos 36% SL	Mission	0.05	0.025	0.1
7.	Multi Neem 0.03% E.C.Azadiractin	Multiplex	3.00	1.50	6.00
8.	Spinosad 2.5% SC	Success	0.05	0.025	0.10
9.	Quinolphos 25% EC	Flash	0.05	0.025	0.10

Sterilized nutrient agar medium (20 ml) was poured into the previously sterilized petriplates (90mm). After solidification 1 ml of viable bacterial spores of each strain was over layed using glass spreader. All the test insecticides were tested at three different doses *viz.*, field recommended dose (x), half the field recommended dose (1/2 x) and double the field recommended dose (2x). Wells (0.7 cm D) were bored with sterile cup borer in the center of N-agar prepared and inoculated with bacteria and all the wells were filled with 0.1 ml of the respective insecticide test doses. The plates were kept in refrigerator for 30 minutes to allow diffusion of liquid and subsequently in

B.O.D. incubator at 30 ± 2 °C for incubation. Observation on zone of inhibitions were recorded at 24 h interval up to 72 h Percent growth inhibition was calculated by the following formula given by Nene and Thapliyal (1979).

$$\% \text{ Inhibition} = \frac{\text{Diameter of zone of inhibition (cm)} \times 100}{\text{Diameter of petriplate}}$$

Results and Discussion

1. Insecticidal activity of *B.t.* strains against *Plutella xylostella*

In this study, the toxicity range is expressed as percent larval mortality of 70-100% (highly toxic), 50-70% (moderately toxic) and 20-50% (less toxic). A mortality of below 20 percent was taken negligible toxicity/non toxic. The distribution of mortality for the 16 soil, 7 ware houses and 3 insect cadaver of *B.t.* strains were separated into 20 groups ranging from 0-4 to 95-100% mortality according to the method described by Meadows *et al.* (1992). Isolates were considered as toxic if they caused 60% or more mortality (Hongyu *et al.*, 2000).

B.t. isolates obtained from different habitats were tested for their insecticidal activity against 5-days-old larvae of *Plutella xylostella* (Table 2). It was observed that there were variations in the toxicity of *B.t.* isolates when tested against the insects. Percent mortality caused by native *B.t.* isolates was ranging from 63.33-100 percent at 24 h of treatment. The results are in close agreement with that of Kaur *et al.* (2006) reported that four native *B.t.* strains, MTCC 868, *Bt5*, *Bt9* and 4D4 were found to cause 100 percent mortality to *P. xylostella* at 96 h after exposure. Asokan and Puttasamy (2007) revealed that 18 native isolates were toxic to the 5-day old larvae of *P. xylostella* out of 33 isolates tested and 3 isolates showed 100 percent mortality at 72 h after treatment. Malathi and Damodaran (1999) also reported that *B.t.* formulations were very effective in reducing the population of *P. xylostella* as compared to chemicals and other botanical insecticides. Among the standard *B.t.* strains HD-1 and HD-73 caused 100 percent mortality while *B.t. tolworthi* caused 96.67 percent mortality. Among the native *B.t.* isolates *viz.*, NSC-1 and NSC-9 caused 100 percent mortality even 24 h of treatment showing very high toxicity and at par with HD-1 and HD-73. An isolate, NSC-3 caused 96.67 percent mortality and was at par with *B.t. tolworthi* at 24h of treatment, while GUR-5 (from soils of arecanut plantation, Kerala) and COR-4 (from insect cadavers of *C. cephalonica*) caused moderate toxicity (63.33% both). At 48 h of

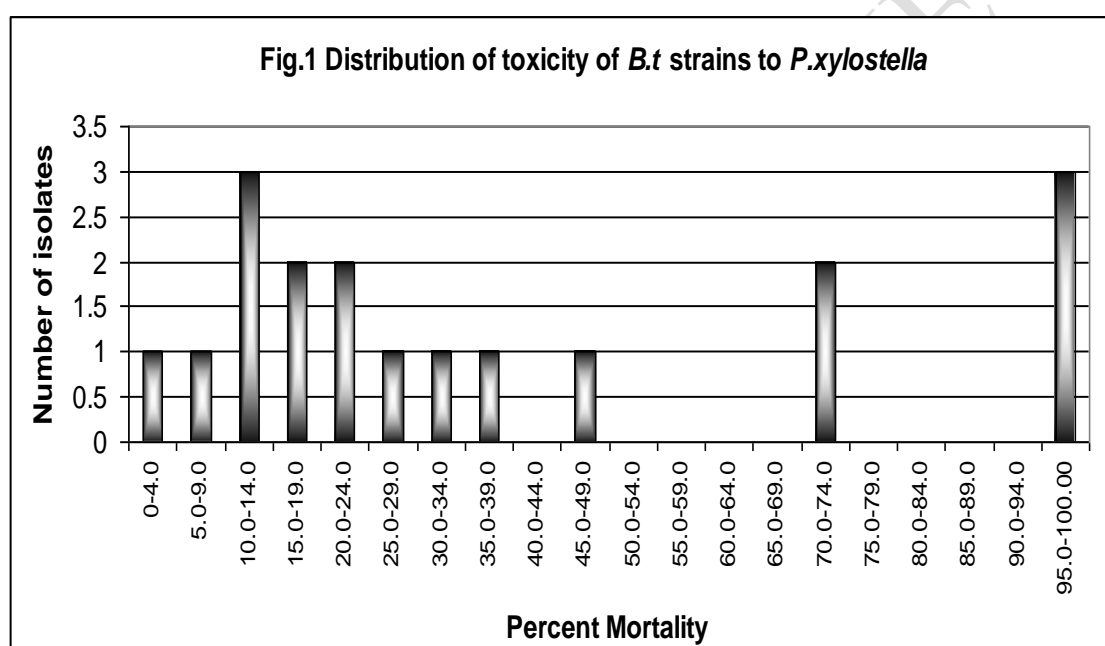
treatment, NSC-1, NSC-3 and NSC-9 also caused 100 percent mortality as like as HD-1, HD-73 and *B.t. tolworthi*. It was observed that isolate obtained from warehouse (NSC) caused high mortality to this insect as compared to soils and insect cadavers. Based on the cumulative mean percent mortality, Out of 18 isolates 7 isolates (38.88 %) were grouped as non-toxic as the toxicity ranged from 2.22-15.56 percent, 6 isolates (33.33 %) were causing 23.33- 46.67 percent mortality and were considered as less toxic, while 5 isolates (27.77 %) were considered as highly toxic as they caused 72.22-100.00 % mortality (Fig.1).

Table-2. Toxicity of different isolates of *B. thuringiensis* against Diamond back moth, *Plutella xylostella*

S. No	Isolate No.	Cumulative percent mortality after			
		24h	48h	72h	Mean
Native isolates					
1.	DHD-2	16.67 (23.85) ^{ef}	26.67(30.99) ^e	26.67(30.99) ^{fg}	23.33 (28.61)
2.	AAI-DU-3	6.67 (13.25) ^{fg}	16.67(23.85) ^{fg}	23.33(28.77) ^{gh}	15.56 (21.96)
3.	AAI-DU-1	33.33 (35.22) ^{cd}	40.00(39.23) ^{cd}	43.33 (41.15) ^{de}	38.89 (38.53)
4.	PNN-2	23.33 (28.78) ^{de}	30.00(33.21) ^{de}	33.33(35.21) ^{ef}	28.89 (32.40)
5.	GUN-12	46.67 (43.08) ^{bc}	46.67 (43.08) ^c	46.67 (43.08) ^d	46.67 (43.08)
6.	RKH-2	6.67 (13.25) ^{fg}	13.33 (21.15) ^g	16.67 (23.85) ^{hi}	12.22 (19.42)
7.	DHD-3	10.00 (15.95) ^f	16.67(21.15) ^{fg}	16.67(23.85) ^{hi}	14.45 (20.31)
8.	TVD-5	26.67 (30.99) ^{de}	30.00 (33.21) ^{de}	33.33 (35.22) ^{ef}	30.00 (33.14)
9.	THR-5	20.00 (26.56) ^{de}	23.33 (28.78) ^{ef}	26.67 (30.99) ^{fg}	23.33 (28.77)
10.	GUR-5	63.33 (52.77) ^b	70.00 (56.79) ^a	76.67 (61.12) ^c	70.00 (56.89)
11.	SHL-5	6.67 (13.25) ^{fg}	10.00 (18.44) ^{gh}	13.33 (21.15) ⁱ	10.00 (17.61)
12.	NSC-1	100.00(87.14) ^a	100.00(87.14) ^a	100.00 (87.14) ^a	100.00 (87.14)
13.	NSC-3	96.67(81.95) ^a	100.00(87.14) ^a	100.00 (87.14) ^a	98.89 (85.41)
14.	NSC-9	100.00(87.14) ^a	100.00(87.14) ^a	100.00(87.14) ^a	100.00(87.14)
15.	COR-4	63.33 (52.75) ^b	70.00 (56.79) ^b	86.67 (68.85) ^b	73.33 (59.46)
16.	BAN-5	10.00 (18.44) ^f	10.00 (18.44) ^{gh}	13.33 (21.15) ^j	11.11(19.34)
17.	MAN-5	3.33(8.05) ^{gh}	6.67 (13.24) ^{hi}	6.67 (13.24) ^j	5.56 (11.51)

18.	LAS-5	0.00(2.87) ^h	3.33 (8.05) ^{ij}	3.33 (8.05) ^{jk}	2.22 (6.32)
Standard isolates					
19.	HD-1	100.00(87.14) ^a	100.00(87.14) ^a	100.00(87.14) ^a	100.00(87.14)
20.	HD-73	100.00(87.14) ^a	100.00(87.14) ^a	100.00(87.14) ^a	100.00(87.14)
21.	<i>B.t.tolworthi</i>	96.67 (81.95) ^a	100.00(87.14) ^a	100.00(87.14) ^a	98.89 (85.41)
22.	Control	0.00 (2.87) ^h	0.00 (2.87) ^j	0.00 (2.87) ^k	0.00 (2.87)
	CD(P=0.05)	9.91	6.11	6.56	

Values in parenthesis are arc-sine transformations
Means followed by common alphabets are not significantly different at 5% level by LSD.



2. Compatibility of *B.t.* strains with insecticides

Compatibility of bio-agents with chemical pesticides is very important for effective pest management. Enhanced effectiveness can be achieved by joint action of pathogen and chemical pesticides, which ultimately reduce the amount of total chemical insecticides usage in crop protection. Many insecticides have been found compatible with *B.t.* having little or no effect on spore germination or cell multiplication. Hence, compatibility of 10 common insecticides widely used on agricultural crops was studied with native *B.t.* isolates.

2a. Compatibility of isolate NSC-1 with some insecticides

The sensitivity of native *B.t* isolate, NSC-1 was tested for compatibility with the common 10 insecticides used in pest management. The results showed that the isolate NSC-1 was compatible with all the doses of chlorpyriphos, alphamethrin, imidacloprid, monocrotophos, multineem, spinosad and quinolphos. It was found that DDVP at field and double the field recommended doses inhibited the growth of NSC-1. Field recommended dose (0.07%) inhibited 11.84, 9.26 and 5.73 percent area at 24, 48 and 72 h after treatment respectively. Double the field recommended dose (0.14 %) inhibited 28.88, 26.66 and 25.55 percent area at 24, 48 and 72 h after treatment respectively. A new insecticide acetamiprid was also found to inhibit the growth of this isolate double the field recommended dose (0.1%) as it showed 16.60, 15.55 and 14.44 percent inhibitory zone respectively. The diameter of the inhibitory zones gets reduced as duration of the inhibition increases (Table 3).

Table-3 Compatibility of isolate NSC-1 with different insecticides

S.No	Treatments	% Inhibition								
		24 hr			48hr			72hr		
		X	½ X	2X	X	½ X	2X	X	½ X	2X
1.	Chlorpyriphos	-	-	-	-	-	-	-	-	-
2.	Alphamethrin	-	-	-	-	-	-	-	-	-
3.	Imidacloprid	-	-	-	-	-	-	-	-	-
4.	Acetamiprid	-	-	16.60	-	-	15.55	-	-	14.44
5.	DDVP	11.84	-	28.88	9.26	-	26.66	5.73	-	25.55
6.	Monocrotophos	-	-	-	-	-	-	-	-	-
7.	Neem oil	-	-	-	-	-	-	-	-	-
8.	Spinosad	-	-	-	-	-	-	-	-	-
9.	Quinolphos	-	-	-	-	-	-	-	-	-
10.	Control	-	-	-	-	-	-	-	-	-

X- Field Recommended dose; ½X – Half the Field Recommended dose; 2X- Double the Field Recommended dose

2b. Compatibility of isolate NSC-3 with some insecticides

Native *B.t*. isolate NSC-3 was tested with the above same 10 insecticides at three different doses (field recommended dose, half the field recommended dose and double the field recommended). It is evident from the table-4 that NSC-3 was compatible with chlorpyriphos, alphamethrin, acetamiprid, monocrotophos, multineem, spinosad and quinolphos at all the three different doses. Imidacloprid showed inhibitory effect at double the field recommended dose (0.04%) as 17.22, 15.55 and 13.33 percent inhibition and also at field recommended dose (0.02%) as 14.44, 13.33 and 12.22 percent inhibition at 24, 48 and 72 h after treatment. DDVP was also showed inhibitory

effect at double the recommended dose (0.14%) Percent inhibition for the double the recommended dose was 25.55, 21.11 and 14.44 at 24, 48 and 72 h after treatment respectively.

Table-4. Compatibility of isolate NSC-3 with different insecticides

S.N o	Treatments	% Inhibition								
		24 hr			48hr			72hr		
		X	½ X	2X	X	½ X	2X	X	½ X	2X
1.	Chlorpyriphos	-	-	-	-	-	-	-	-	-
2.	Alphamethrin	-	-	-	-	-	-	-	-	-
3.	Imidacloprid	14.44	-	17.22	13.33	-	15.55	12.22	-	13.33
4.	Acetamiprid	-	-	-	-	-	-	-	-	-
5.	DDVP	-	-	25.55	-	-	21.11	-	-	14.44
6.	Monocrotophos	-	-	-	-	-	-	-	-	-
7.	Neem oil	-	-	-	-	-	-	-	-	-
8.	Spinosad	-	-	-	-	-	-	-	-	-
9.	Quinolphos	-	-	-	-	-	-	-	-	-
10.	Control	-	-	-	-	-	-	-	-	-

X- Field Recommended dose; ½X – Half the Field Recommended dose; 2X- Double the Field Recommended dose

2c. Compatibility of isolate NSC-9 with some insecticides

Native isolate NSC-9 was also tested with the same 10 insecticides at the three different doses (field recommended dose, half the field recommended dose and double the field recommended). It was observed that (Table-5) NSC-9 was compatible with chlorpyriphos, alphamethrin, DDVP, monocrotophos, multineem, spinosad and quinolphos were compatible and found safer with the native isolate NSC-9 at all the three different doses. Imidacloprid at the double the field recommended dose (0.04%) showed that 15.00, 13.88 and 12.22 percent inhibition at 24, 48 and 72 h after treatment respectively. Acetamiprid at the double the field recommended dose (0.1 %) was found to show inhibitory effect as it caused 14.44, 13.33 and 12.77 percent inhibition at 24, 48 and 72 h after treatment respectively. It was observed from this study that as the duration of inhibition increases the diameter of the inhibitory zone gets reduced.

Table-5 Compatibility of *B.t.* isolate NSC-9 with different insecticides

S.No	Treatments	% Inhibition								
		24 hr			48hr			72hr		
		X	½ X	2X	X	½ X	2X	X	½ X	2X

1.	Chlorpyriphos	-	-	-	-	-	-	-	-	-
2.	Alphamethrin	-	-	-	-	-	-	-	-	-
3.	Imidacloprid	-	-	15.00	-	-	13.88	-	-	12.22
4.	Acetamiprid	-	-	14.44	-	-	13.33	-	-	12.77
5.	DDVP	-	-	-	-	-	-	-	-	-
6.	Monocrotophos	-	-	-	-	-	-	-	-	-
7.	Neem oil	-	-	-	-	-	-	-	-	-
8.	Spinosad	-	-	-	-	-	-	-	-	-
9.	Quinolphos	-	-	-	-	-	-	-	-	-
10.	Control	-	-	-	-	-	-	-	-	-

X- Field Recommended dose; ½X – Half the Field Recommended dose; 2X- Double the Field Recommended dose

2d Compatibility of isolate COR-4 with some insecticides

Compatibility of the native isolate COR-4 with the same common 10 insecticides was tested and it was observed that (Table-6) only acetamiprid (0.1%) and DDVP (0.14) at the double the recommended dose inhibit the growth of the *B.t* native isolate COR-4. At the double the field recommended dose of acetamiprid showed that 11.84, 9.20 and 5.70 percent inhibition at 24, 48 and 72 h after treatment DDVP also showed inhibitory effect on COR-4 at the double the field recommended dose and the percent inhibition was 21.60, 19.88 and 12.44 at 24, 48 and 72 h after treatment respectively.

Table-6 Compatibility of *B.t.* isolate COR-4 with different insecticides

S.No	Treatments	% Inhibition								
		24 hr			48hr			72hr		
		X	½	2X	X	½	2X	X	½	2X
1.	Chlorpyriphos	-	-	-	-	-	-	-	-	-
2.	Alphamethrin	-	-	-	-	-	-	-	-	-
3.	Imidacloprid	-	-	-	-	-	-	-	-	-
4.	Acetamiprid	-	-	11.84	-	-	9.20	-	-	5.70
5.	DDVP	-	-	21.60	-	-	19.88	-	-	12.44
6.	Monocrotophos	-	-	-	-	-	-	-	-	-
7.	Neem oil	-	-	-	-	-	-	-	-	-
8.	Spinosad	-	-	-	-	-	-	-	-	-

9.	Quinolphos	-	-	-	-	-	-	-	-	-
10.	Control	-	-	-	-	-	-	-	-	-

X- Field Recommended dose; ½X – Half the Field Recommended dose; 2X- Double the Field Recommended dose

2e Compatibility of isolate GUR-5 with some insecticides

Native *B.t.* isolate GUR-5 was tested with the above same 10 insecticides at three different doses for compatibility. The results showed that all the insecticides were compatible with the *B.t.* isolate of GUR-5 except DDVP. It is evident from the table-7, that DDVP showed inhibitory effect at double the recommended dose and the percent inhibition were 17.89, 15.11 and 14.00 at 24, 48 and 72 h after treatment. It was observed that the diameter of the inhibitory zones get reduced as duration of the inhibition increases.

Table-7 Compatibility of *B.t* isolate GUR-5 with different insecticides

S. N o	Treatments	% Inhibition								
		24 hr			48hr			72hr		
		X	½ X	2X	X	½ X	2X	X	½ X	2X
1.	Chlorpyriphos	-	-	-	-	-	-	-	-	-
2.	Alphamethrin	-	-	-	-	-	-	-	-	-
3.	Imidacloprid	-	-	-	-	-	-	-	-	-
4.	Acetamiprid	-	-	-	-	-	-	-	-	-
5.	DDVP	-	-	17.89	-	-	15.11	-	-	14.00
6.	Monocrotophos	-	-	-	-	-	-	-	-	-
7.	Neem oil	-	-	-	-	-	-	-	-	-
8.	Spinosad	-	-	-	-	-	-	-	-	-
9.	Quinolphos	-	-	-	-	-	-	-	-	-
10.	Control	-	-	-	-	-	-	-	-	-

X- Field Recommended dose; ½X – Half the Field Recommended dose; 2X- Double the Field Recommended dose

Morris (1977) reported that carbamates were generally more compatible with *Bacillus thuringiensis* than the other insecticides viz., acephate, trichlorophan, methomyl, carbaryl and diflubenzuron. Filho *et al.* (2001) reported that thiamethoxam, imidacloprid and acephate were compatible with *Bacillus thuringiensis*, at the same

time, monocrotophos and deltamethrin were the insecticides that most affected *B. thuringiensis*. Bhattacharya *et al.* (2004) reported that endosulfon at all three concentrations, imidacloprid and carbaryl at higher concentrations showed inhibitory effect on *Bacillus thuringiensis*.

Conclusion

Under field conditions, slow mortality caused by *B. thuringiensis* is a major limitation, which prevents their large-scale use in agricultural crops. A combination of *B.t* formulations with reduced dosages of chemical pesticides may prove useful under such situations to provide quick mortality and reduce the chemical pesticides load in the environment. Moreover, the use of *B.t* with certain synthetic pesticides can help overcome the resistance development in insects towards these pesticides (Pree and Daly, 1996).

Disclaimer (Artificial intelligence): Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

- Asokan, R and Puttaswamy (2007). Isolation and characterization of *Bacillus thuringiensis* from soil, leaf, seed dust and insect cadaver. *J. Biol. control*, **21**(1): 83-90.
- Bhattacharya, S., Dutta, S., & Dhar, T (2004). In vitro compatibility of different entomopathogens to pesticides, plant growth regulators and micronutrients. *Annals of Plant Protection Sciences*, *12*(1), 199-202.
- Carozzi, N.B., Kramer, V.C., Warren, G.W., Evola, S. and Koziel, M.G (1991). Prediction of insecticidal activity of *Bacillus thuringiensis* strains by PCR product profiles. *Applied and Environmental Microbiology*, **57**: 3057-3061.
- cereus* in soil supplemented with grass or manure. *Plant and soil*, **83**:389-398.
- Crickmore, Neil, D. R. Zeigler, J. Feitelson, E. S. C. H. E. R. I. C. H. I. A. Schnepf, J. Van Rie, Didier Lereclus, J. Baum, and DH98935 Dean (1998). Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiology and molecular biology reviews* 62, no. 3 : 807-813.

- De Maagd, R. A., Bravo, A., and Crickmore, N (2001). How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. *TRENDS in Genetics*, 17(4), 193-199.
- Deivendran, A, G.S. Yadav and H.R. Rohilla. 2007. Efficacy of some insecticides against *Plutella Xylostella* on cauliflower, *J. Insect Sci.* **20** (1): 102-105.
- Filho Batista, A., Almeida, J. E., and Lamas, C. (2001). Effect of thiamethoxam on entomopathogenic microorganisms. *Neotropical Entomology*, 30, 437-447.
- Flexner, J., Lighthart, B., and Croft, B. A. (1986). The effects of microbial pesticides on non-target, beneficial arthropods. *Agriculture, ecosystems & environment*, 16(3-4), 203-254.
- Gomez, K.A. and Gomez. A.A. Statistical procedures for Agricultural Research. (Eds.), John Wiley and Sons, New York 1984; 7-20.
- Höfte, H., and Whiteley, H. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological reviews*, 53(2), 242-255.
- Hongyu, Z., Ziniu, Y. and Wangxi, D. 2000. Isolation, distribution and toxicity of *Bacillus thuringiensis* from ware houses in China. *Crop protection*, **19**: 449-454.
- Kaur, P., Joshi, N and Brar, K.S. 2006. Morphological and biochemical characterization of *Bacillus thuringiensis* Berliner isolates and their evaluation against *P. xylostella*. *J. Biol. Control*, **30**(2): 191-195.
- Kumar, P., Kamle, M., Borah, R. (2021). *Bacillus thuringiensis* as microbial biopesticide: uses and application for sustainable agriculture. *Egypt J Biol Pest Control* 31, 95 <https://doi.org/10.1186/s41938-021-00440-3>.
- Malathi, A., and Damodaran, A. (1999). Stress due to exams in medical students-a role of Yoga. *Indian journal of physiology and pharmacology*, 43, 218-224.
- Martin, P.A.W. and Travers, R.S. (1989). Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Applied and Environmental Microbiology*, **55**: 2437-2442.

- Meadows, M.P., Ellis, D.J., Jarrett, P. and Burges, H.D. (1992). Distribution, frequency and diversity of *Bacillus thuringiensis* in an animal feed mill. *Applied and Environmental Microbiology*, **58**: 1344-1350.
- Morris, O. N. (1977). Compatibility of 27 chemical insecticides with *Bacillus thuringiensis* var. kurstaki. *The Canadian Entomologist*, *109*(6), 855-864.
- Navya, R.N.S., Balasubramani,V and Raveendran, M. (2022). Characterization of indigenous *Bacillus thuringiensis* isolate RM11 toxic to the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *Egypt J Biol Pest Control* *32*, 56. <https://doi.org/10.1186/s41938-022-00553-3>
- Nene, Y. L., and Thapliyal, P. N. (1979). Fungicides in plant disease control. 2nd Edition, Oxford and IBH Pub. Co., New Delhi.
- Pree, D. J., and Daly, J. C. (1996). Toxicity of mixtures of *Bacillus thuringiensis* with endosulfan and other insecticides to the cotton boll worm *Helicoverpa armigera*. *Pesticide science*, *48*(3), 199-204.
- Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D. R. and Dean. D.H. (1998). *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.*; *62*: 775-806.
- Siegel, J. P., and Shaddock, J. A. (1989). Safety of microbial insecticides to vertebrates, Chapter 8. *Safety of microbial insecticides*. CRC Press, Boca Raton.
- Tabashnik, B. E., Cushing, N. L., Finson, N., and Johnson, M. W. (1990). Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of economic entomology*, *83*(5), 1671-1676.
- Talekar, N.S, Yong, J.C. and Lee, S.T. (1990). Annotated Bibliography of diamond back moth, Vol. 2. Asian Vegetable Research and Development Center, Shanhua, Taiwan, 603.
- Vaeck, M., Reynaerts, A., Höfte, H., Jansens, S., De Beuckeleer, M., Dean, C and Leemans, J. (1987). Transgenic plants protected from insect attack. *Nature*, *328*(6125), 33-37.