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Effectiveness of Nuclear Polyhedrosis Virus and *Bacillus thuringiensis* alone and in Combination against *Spodoptera litura* (Fabricius)

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SM and MA conceived and designed the study. AA, MA, MSH, MIU and SM analyzed the data. SM and HAAK wrote the article. This work is a part of PhD thesis of SM.

Key words
Insect-pathogen, Microbial toxicity, Ecotoxicology.

ABSTRACT

Nuclear polyhedrosis virus (NPV) and *Bacillus thuringiensis* (*Bt*) are potential biological pest controller which can regulate an extensive range of bollworms larvae in both Agricultural and Horticultural crops. *Spodoptera litura* is a commercial pest of diverse field crops comprising cauliflower (*Brassica oleracea* var. botrytis). Current inspections were carried out to determine their impact on larval mortality, pupal and adult emergence by exposing three field strains of *S. litura* (Faisalabad, Chiniot and Sargodha), under laboratory conditions. Both NPV and *Bt* were applied using diet incorporation method. Two concentrations of a NPV based formulation (Somestar): $1x10^7$ and $1x10^8$ POB/ml, and three concentrations of a *Bt* based formulation (Dipel): *viz*., 0.1, 0.3 and 0.5 µg/g, were applied alone and in different combinations against 2nd and 4th instar larvae of *S. litura.* The highest mortality of larvae was recorded after 7 days of application when both insect pathogens were used in combination (SpltNPV 1×10^8 POB/ml + *Bt* 0.1 µg/g) *i.e.* 85.25, 79.31 and 92.11% mortality (in case of 2nd instar larvae) and 70.42, 63.64 and 80.88% (in case 4th instar larvae) of Faisalabad, Chiniot and Sargodha's population of *S. litura*, respectively. This study could be helpful to use NPV and *Bt* in intensive pest management under field conditions to control *S. litura* on cauliflower.

INTRODUCTION

Cauliflower (*Brassica oleracea* var. botrytis) is an essential crop cultivated in South-East Asia. It is the one of the best vegetable among various vegetables belong to family Brassicaceae (Campbell *et al*., 2012). It is usually grown as a winter crop as well as summer vegetable. A large number of insect pests damage this crop. Most serious pest is armyworm (*Spodoptera litura* F.) causing yield losses ranging from 31% to 100%. It invades more than 40 plant families (Lingappa *et al*. 2004). Conventional insecticides are being used for its management; however, growing environmental concerns and issues of insecticide resistance development necessitates the need to explore safe measures (Khan and Akram, 2017; Khan *et al*., 2016; Saleem *et al*., 2016).

Bio-rational pesticides based on Nuclear Polyhedrosis Virus (NPV) and *Bacillus thuringiensis* (*Bt*) are very effective tool to achieve the resistance problems and protect the natural enemies and environment. These pesticides are derived from biologically active substances like plants and microbes that affect growth and development of insects and provide protection against herbivores including lepidopteran pests (*Spodoptera litura* and *Helicoverpa armigera etc*.) (Senthil *et al*., 2005; Ignacimuthu *et al*., 2006; Baskar *et al*., 2011).

The main objective of this study was to check the potential of commercial formulations of NPV and *Bt* alone and in different combinations against *S. litura*. The use of microbial insecticides, especially bacterial formulations made from *B. thuringiensis* are in widespread use to manage the outbreak of several notorious insect pests because of their safe nature to humans including other mammals and non-target species. Mode of action of *Bt*-toxins is rather quite complex against target insects, and *Bt*-formulations often perform even better when integrated with other agents (Tabashnik, 1992). It is not a simple matter for insects to develop resistance against *Bt*-toxins as combined activity of several mechanisms is required for these mutations (Carlton and Gonzalez, 1986).

Nuclear Polyhedrosis Viruses (NPVs), belonging to family Baculoviridae, have attained great attention as a microbial insecticide, and it is being used to control different agricultural insect pests of different crops and

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vegetables (Black *et al*., 1997; Moscardi, 1999). Main advantages of NPVs are that they are host specific and they do not disturb the population of beneficial insects and pollinators are safer for health and environment (Federici, 1993; Lacey *et al*., 2001; Moscardi, 1999).

In the present study, we were interested to check the potential of commercial formulations of NPV and *Bt* alone and in different combinations against *S. litura* (army worm).

MATERIALS AND METHODS

Insect population

Different strains of *S. litura* were collected from host crop from three selected cauliflower growing localities (Faisalabad, Chiniot and Sargodha). The larvae collected from the field were reared on artificial diet in the plastic trays covered with fine muslin cloth to get the next generation for bioassays. The F1 adults were shifted in a transparent plastic jar and provided with a coarse tissue paper to facilitate the moths for egg laying. Artificial diet for adults was consisted of 50g sucrose solution, 1 gm ethyl-4-hydroxybenzoate, 1 ml ethanol (90%), 10 ml vitamin mixture and 500 ml distilled water. This artificial diet solution was placed in a petri dish plug with cotton swab to avoid the moths from drowning. After hatching, the young larvae were shifted on natural diet with the help of camel hair brush to avoid abrasive damage. Larvae were reared up to 2^{nd} and 4^{th} instar under controlled conditions at 25±2°C and 70±5% relative humidity.

Test chemicals

The commercial formulation of *Bt* (Dipel)® (Merck, UK) was applied at the rate of 0.1, 0.3 and 0.5 µg ml^{-1}. SpltNPV (Somestar)® (Agrilife, India) was applied @ 1×10^7 POB/ml and 1×10^8 POB/ml against 2^{nd} and 4^{th} instar larvae. *Bt* and SpltNPV formulations were applied alone and in combination.

Bioassay

Laboratory bioassays were carried out using the 2^{nd} and 4^{th} instar larvae of *S. litura* strains (F1). Freshly moulted larvae were exposed to *Bt* (0.1, 0.3 and 0.5 µg ml^{-1}) and SpltNPV (1×10^7 POB/ml and 1×10^8 POB/ml) mixed diets alone and in combination to observe their pathogenicity. Thirty larvae of each locality were considered as a treatment and replicated thrice. Mortality was assessed on 3^{rd}, 5^{th} and 7^{th} day after treatment. A diet piece of 1 cm^2 admixed with *Bt*/SpltNPV was offered to larvae to feed for 48 h and then shifted to normal diet. Artificial diet mixed with Tween-80 was used as control.

Statistical analysis

Collected data regarding mortality for both SpltNPV and *Bt* were corrected for control mortality by using Abbott's (1925) formula and analyzed statistically by the one-way analysis of variance (ANOVA) using Minitab software (Minitab, 2002). Means were compared by Tukey's (HSD) test at 5% level of significance (Sokal and Rohlf, 1995). Type of interaction was determined by the following equation:

$$CTF = (Oc-Oe)/Oe \times 100$$

Where, CTF is the co-toxicity factor, Oc is the observed % mortality resulted from the combined application, and Oe the expected % mortality calculated by the total % produced by each of the treatments used in the combination.

The effectiveness was categorized into three groups: a positive factor of 20 or more suggested synergism, a negative factor of 20 or more suggested antagonism, and any intermediate value (*i.e.,* between −20 and +20) was considered additive (Mansour *et al*., 1966; Wakil *et al*., 2012).

RESULTS

Mortality of 2nd instar larvae of S. litura

Mortality and co-toxicity factors are presented in Tables I, II and III. Integration of different levels of concentrations of *B. thuringiensis* and SpltNPV showed varied level of mortality against second instar larvae of *S. litura*. Significant differences regarding the mortality of 2^{nd} instar larvae of *S. litura* were recorded when they were fed on diet treated with *B. thuringiensis* and SpltNPV alone and in combinations after 3 days of application. All three kinds of interactions (Additive, Synergistic and Antagonistic) were observed when both pathogens were applied in combination. The results revealed that mortality of 2^{nd} instars larvae of *S. litura* increased with the increase in concentration of *B. thuringiensis* and SpltNPV. While significantly higher rate of mortality was observed when *B. thuringiensis* and SpltNPV were applied in combination than applied alone against all the field strains.

An additive effect on mortality was observed when SpltNPV was combined at lower dose (1×10^7 POB/ml) with all three concentrations (0.1, 0.3 and 0.5 µg/g) of *B. thuringiensis.* Whereas, different interactions were recorded when SpltNPV was applied in combination at higher dose (1×10^8 POB/ml) along with all three concentrations of *B. thuringiensis*. When the highest dose (1×10^8 POB/ml) of SpltNPV was applied in combination with the lowest dose (0.1µg/g) of *B. thuringiensis*, mortality of *S. litura* larvae enhanced and synergistic effect was observed. Whereas, additive and antagonistic effects were observed when the highest dose of SpltNPV was applied in combination with *B. thuringiensis* (0.3 and 0.5 µg/g) against all strains after 3 days of application. After 3 days of application, mortality of 2nd instar larvae of *S. litura* ranged from 18.42 to 61.80% in the Faisalabad strain, 17.05 to 57.93% in the Chiniot strain and 19.54 to 65.39% in the Sargodha strain (Table I).

Table I.- Mortality of second instar larvae of *S. litura* after three days of exposure to *B. thuringiensis* and SpltNPV.

Localities	SpltNPV (POB/ml)	Bt (µg/g)	Actual mortality	Expected mortality	Co-toxicity factor	Type of effectiveness
Faisalabad	$1x10^7$	0.1	41.53±2.63 cd	39.11	6.18	Additive
	$1x10^7$	0.3	46.59±0.99 bc	42.33	10.06	Additive
	$1x10^7$	0.5	53.98±2.50 ab	51.75	4.30	Additive
	$1x10^8$	0.1	61.38±2.10 a	50.23	22.20	Synergism
	$1x10^8$	0.3	56.32±3.04 ab	53.45	5.37	Additive
	$1x10^8$	0.5	48.81±2.46 bc	62.87	-22.36	Antagonism
	$1x10^7$	0	18.42±1.13 g			
	$1x10^8$	0	29.54±1.02 ef			
	0	0.1	20.69±1.99 fg			
	0	0.3	23.91±2.19 efg			
	0	0.5	33.33±1.15 de			
HSD value			10.389			
F			54.2			
P			≤0.01			
Chiniot	$1x10^7$	0.1	37.09±1.97 de	35.25	5.21	Additive
	$1x10^7$	0.3	42.07±1.43 cd	38.62	8.93	Additive
	$1x10^7$	0.5	50.61±2.47 ab	48.89	3.53	Additive
	$1x10^8$	0.1	57.93±1.43 a	47.74	21.34	Synergism
	$1x10^8$	0.3	53.41±0.99 ab	51.11	4.49	Additive
	$1x10^8$	0.5	48.63±2.61 bc	61.38	-20.77	Antagonism
	$1x10^7$	0	17.05±0.19 g			
	$1x10^8$	0	29.54±1.01 ef			
	0	0.1	18.20±1.25 g			
	0	0.3	21.57±2.17 fg			
	0	0.5	31.84±1.35 e			
HSD value			8.5135			
F			74.9			
P			≤0.01			
Sargodha	$1x10^7$	0.1	43.18±2.98 de	41.11	5.04	Additive
	$1x10^7$	0.3	48.85±2.07 cd	44.56	9.63	Additive
	$1x10^7$	0.5	56.21±1.54 abc	54.02	4.05	Additive
	$1x10^8$	0.1	65.39±1.87 a	53.75	21.64	Synergism
	$1x10^8$	0.3	60.23±1.00 ab	57.20	5.29	Additive
	$1x10^8$	0.5	52.30±1.52 bcd	66.66	-21.55	Antagonism
	$1x10^7$	0	19.54±2.30 g			
	$1x10^8$	0	32.18±3.04 f			
	0	0.1	21.57±0.88 g			
	0	0.3	25.02±1.30 fg			
	0	0.5	34.48±1.99 ef			
HSD value			10.043			
F			65.8			
P			≤0.01			

Means sharing the same letters within columns are not significantly different.

Table II.- Mortality of second instar larvae of *S. litura* after five days of exposure to *B. thuringiensis* and SpltNPV.

Localities	SpltNPV (POB/ml)	Bt (µg/g)	Actual mortality	Expected mortality	Co-toxicity factor	Type of effectiveness
Faisalabad	$1x10^7$	0.1	48.21±1.93 cd	45.74	5.39	Additive
	$1x10^7$	0.3	55.71±1.55 bc	50.26	10.83	Additive
	$1x10^7$	0.5	64.10±2.61 ab	61.64	3.99	Additive
	$1x10^8$	0.1	70.11±2.29 a	58.01	20.87	Synergism
	$1x10^8$	0.3	65.52±1.99 ab	62.53	4.79	Additive
	$1x10^8$	0.5	57.93±1.43 bc	73.91	-21.62	Antagonism
	$1x10^7$	0	20.72±1.96 g			
	$1x10^8$	0	32.99±2.48 ef			
	0	0.1	25.02±1.30 fg			
	0	0.3	29.54±2.99 fg			
	0	0.5	40.92±2.04 de			
HSD value @ 5%			10.689			
F			68.8			
P			≤0.01			
Chiniot	$1x10^7$	0.1	44.94±0.96 de	42.41	5.97	Additive
	$1x10^7$	0.3	51.68±0.96 cd	45.82	12.80	Additive
	$1x10^7$	0.5	59.54±0.46 abc	57.16	4.16	Additive
	$1x10^8$	0.1	68.24±2.64 a	54.52	25.16	Synergism
	$1x10^8$	0.3	62.53±1.61 ab	57.93	7.94	Additive
	$1x10^8$	0.5	53.45±2.63 bcd	69.27	-22.84	Antagonism
	$1x10^7$	0	18.54±2.93 h			
	$1x10^8$	0	30.65±1.66 fg			
	0	0.1	23.87±2.01 gh			
	0	0.3	27.28±2.02 gh			
	0	0.5	38.62±2.10 ef			
HSD value @ 5%			9.9365			
F			74.0			
P			≤0.01			
Sargodha	$1x10^7$	0.1	54.56±2.08 d	51.23	6.50	Additive
	$1x10^7$	0.3	61.38±2.10 cd	56.82	8.02	Additive
	$1x10^7$	0.5	71.61±2.14 ab	68.24	4.94	Additive
	$1x10^8$	0.1	79.40±1.59 a	65.98	20.34	Synergism
	$1x10^8$	0.3	73.87±1.03 ab	71.57	3.20	Additive
	$1x10^8$	0.5	65.94±1.64 bc	82.99	-20.54	Antagonism
	$1x10^7$	0	23.91±2.19 g			
	$1x10^8$	0	38.66±1.40 ef			
	0	0.1	27.32±2.23 g			
	0	0.3	32.91±2.73 fg			
	0	0.5	44.33±2.05 e			
HSD value			10.013			
F			99.4			
P			≤0.01			

Means sharing the same letters within columns are not significantly different.

Table III.- Mortality of second instar larvae of *S. litura* after seven days of exposure to *B. thuringiensis* and SpltNPV.

Localities	SpltNPV (POB/ml)	Bt (µg/g)	Actual mortality	Expected mortality	Co-toxicity factor	Type of effectiveness
Faisalabad	1x10^7	0.1	61.38±2.10 c	57.13	7.45	Additive
	1x10^7	0.3	67.43±2.07 bc	61.68	9.32	Additive
	1x10^7	0.5	73.91±2.78 ab	71.27	3.70	Additive
	1x10^8	0.1	85.25±2.21 a	70.42	21.06	Synergism
	1x10^8	0.3	79.81±1.76 a	74.98	6.45	Additive
	1x10^8	0.5	67.01±2.48 bc	84.56	-20.75	Antagonism
	1x10^7	0	27.59±1.98 f			
	1x10^8	0	40.88±1.57 de			
	0	0.1	29.54±2.99 ef			
	0	0.3	34.10±2.02 def			
	0	0.5	43.68±3.04 d			
HSD value			11.755			
F			81.2			
P			≤0.01			
Chiniot	1x10^7	0.1	52.30±1.52 d	49.90	4.80	Additive
	1x10^7	0.3	60.27±2.68 cd	55.61	8.38	Additive
	1x10^7	0.5	68.16±1.35 bc	66.26	2.87	Additive
	1x10^8	0.1	79.31±1.99 a	62.57	26.76	Synergism
	1x10^8	0.3	72.76±1.70 ab	68.27	6.57	Additive
	1x10^8	0.5	61.40±2.57 cd	78.93	-22.20	Antagonism
	1x10^7	0	24.88±2.10 g			
	1x10^8	0	37.55±2.33 ef			
	0	0.1	25.02±1.30 g			
	0	0.3	30.73±2.25 fg			
	0	0.5	41.38±1.99 e			
HSD value @ 5%			10.278			
F			92.0			
P			≤0.01			
Sargodha	1x10^7	0.1	67.01±2.48 c	64.52	3.86	Additive
	1x10^7	0.3	76.17±1.73 bc	70.04	8.75	Additive
	1x10^7	0.5	85.36±2.87 ab	81.91	4.20	Additive
	1x10^8	0.1	92.11±2.89 a	74.71	23.28	Synergism
	1x10^8	0.3	87.55±2.82 ab	80.23	9.12	Additive
	1x10^8	0.5	73.06±1.69 c	92.11	-20.68	Antagonism
	1x10^7	0	34.14±2.29 ef			
	1x10^8	0	44.33±2.05 de			
	0	0.1	30.38±2.26 f			
	0	0.3	35.90±2.61 def			
	0	0.5	47.78±2.43 d			
HSD value			12.243			
F			92.4			
P			≤0.01			

Means sharing the same letters within columns are not significantly different.

After 5 and 7 days of application, similar pattern of interaction was observed in tested population of all three localities (Faisalabad, Chiniot and Sargodha). After 5 days of application, mortality of 2nd instar larvae of *S. litura* ranged from 20.72 to 70.11% in the Faisalabad strain, 18.54 to 68.24% in the Chiniot strain, and 23.91 to 79.40% in the Sargodha strain (Table II). While after 7 days of application, mortality of 2 instar larvae of *S. litura* varied from 27.59 to 85.25%, 24.88 to 79.31% and 30.38 to 92.11% for the Faisalabad, Chiniot and Sargodha strains of *S. litura*, respectively (Table III).

Table IV.- Mortality of fourth instar larvae of *S. litura* after three days of exposure to *B. thuringiensis* and SpltNPV.

Localities	SpltNPV (POB/ml)	Bt (µg/g)	Actual mortality	Expected mortality	Co-toxicity factor	Type of effectiveness
Faisalabad	1x10^7	0.1	27.32±2.23 cde	25.01	9.21	Additive
	1x10^7	0.3	31.84±1.35 bcd	28.62	11.25	Additive
	1x10^7	0.5	37.47±1.61 ab	35.29	6.19	Additive
	1x10^8	0.1	42.07±1.43 a	34.14	23.24	Synergism
	1x10^8	0.3	38.66±1.40 ab	37.74	2.44	Additive
	1x10^8	0.5	35.21±2.12 abc	44.41	-20.72	Antagonism
	1x10^7	0	11.38±1.21 g			
	1x10^8	0	20.50±2.15 efg			
	0	0.1	13.64±1.97 g			
	0	0.3	17.24±1.91 fg			
	0	0.5	23.91±2.19 def			
HSD value			9.2490			
F			33.8			
P			≤0.01			
Chiniot	1x10^7	0.1	22.49±1.26 cd	21.49	4.67	Additive
	1x10^7	0.3	27.28±2.01 bc	25.05	8.90	Additive
	1x10^7	0.5	33.75±2.29 ab	31.72	6.41	Additive
	1x10^8	0.1	38.62±0.69 a	30.73	25.69	Synergism
	1x10^8	0.3	35.98±1.37 a	34.29	4.92	Additive
	1x10^8	0.5	31.11±1.11 ab	40.96	-24.05	Antagonism
	1x10^7	0	10.11±0.10 e			
	1x10^8	0	19.35±2.40 d			
	0	0.1	11.38±1.21 e			
	0	0.3	14.94±1.15 de			
	0	0.5	21.61±1.28 cd			
HSD value			7.5795			
F			43.4			
P			≤0.01			
Sargodha	1x10^7	0.1	32.60±1.34 de	30.88	5.59	Additive
	1x10^7	0.3	38.62±0.69 cd	35.21	9.70	Additive
	1x10^7	0.5	45.48±1.46 abc	43.94	3.50	Additive
	1x10^8	0.1	51.72±1.98 a	42.26	22.39	Synergism
	1x10^8	0.3	49.43±1.14 ab	46.59	6.09	Additive
	1x10^8	0.5	43.33±1.92 bc	55.33	-21.68	Antagonism
	1x10^7	0	13.64±0.15 i			
	1x10^8	0	25.02±1.30 fg			
	0	0.1	17.24±1.99 hi			
	0	0.3	21.57±0.88 gh			
	0	0.5	30.31±1.66 ef			
HSD value			7.2527			
F			84.3			
P			≤0.01			

Means sharing the same letters within columns are not significantly different.

Table V.- Mortality of fourth instar larvae of *S. litura* after five days of exposure to *B. thuringiensis* and SpltNPV.

Localities	SpltNPV (POB/ml)	Bt (µg/g)	Actual mortality	Expected mortality	Co-toxicity factor	Type of effectiveness
Faisalabad	$1x10^7$	0.1	42.07±1.43 c	40.03	5.09	Additive
	$1x10^7$	0.3	48.89±1.48 bc	43.44	12.54	Additive
	$1x10^7$	0.5	56.32±3.04 ab	52.14	8.02	Additive
	$1x10^8$	0.1	62.53±1.61 a	51.15	22.25	Synergism
	$1x10^8$	0.3	59.59±1.25 a	54.56	9.22	Additive
	$1x10^8$	0.5	50.54±1.48 b	63.25	-20.10	Antagonism
	$1x10^7$	0	18.42±1.13 f			
	$1x10^8$	0	29.54±1.01 de			
	0	0.1	21.61±1.28 f			
	0	0.3	25.02±1.30 ef			
	0	0.5	33.71±0.38 d			
HSD value			7.7272			
F			108.0			
P			≤0.01			
Chiniot	$1x10^7$	0.1	37.09±1.97 de	34.32	8.05	Additive
	$1x10^7$	0.3	41.99±2.54 cd	37.66	11.51	Additive
	$1x10^7$	0.5	48.21±1.93 bc	46.40	3.91	Additive
	$1x10^8$	0.1	57.93±1.44 a	46.62	24.25	Synergism
	$1x10^8$	0.3	53.95±2.02 ab	49.96	7.98	Additive
	$1x10^8$	0.5	44.83±1.99 bcd	58.70	-23.62	Antagonism
	$1x10^7$	0	16.09±1.15 g			
	$1x10^8$	0	28.39±2.14 ef			
	0	0.1	18.23±1.24 g			
	0	0.3	21.57±2.17 fg			
	0	0.5	30.31±1.66 ef			
HSD value			9.5386			
F			58.3			
P			≤0.01			
Sargodha	$1x10^7$	0.1	46.09±1.45 de	43.45	6.08	Additive
	$1x10^7$	0.3	52.22±2.43 cd	48.28	8.18	Additive
	$1x10^7$	0.5	60.23±1.01 abc	57.47	4.80	Additive
	$1x10^8$	0.1	69.31±2.02 a	56.09	23.56	Synergism
	$1x10^8$	0.3	64.37±1.15 ab	60.92	5.66	Additive
	$1x10^8$	0.5	54.60±2.50 bcd	70.11	-22.13	Antagonism
	$1x10^7$	0	19.54±2.30 h			
	$1x10^8$	0	32.18±3.04 fg			
	0	0.1	23.91±2.19 gh			
	0	0.3	28.74±3.03 fgh			
	0	0.5	37.93±1.99 ef			
HSD value			11.125			
F			60.1			
P			≤0.01			

Means sharing the same letters within columns are not significantly different.

Mortality of 4th instar larvae of S. litura

In the case of 4^{th} instar larvae of all the field strains, different kinds of interactions were recorded when both pathogens were applied in combination. An additive effect on mortality was observed when SpltNPV was combined at the lowest dose ($1×10^7$ POB/ml) along with all three concentrations (0.1, 0.3 and 0.5 µg/g) of *B. thuringiensis.* Whereas, different interactions were recorded when SpltNPV applied in combination at the highest dose ($1×10^8$ POB/ml) along with all three concentrations of *B. thuringiensis*. When the highest dose ($1×10^8$ POB/ml) of SpltNPV was applied in combination with the lowest dose (0.1µg/g) of *B. thuringiensis*, the mortality of *S. litura* larvae enhanced and synergistic effect was observed.

Table VI.- Mortality of fourth instar larvae of *S. litura* after seven days of exposure to *B. thuringiensis* and SpltNPV.

Localities	SpltNPV (POB/ml)	Bt (µg/g)	Actual mortality	Expected mortality	Co-toxicity factor	Type of effectiveness
Faisalabad	1x10^7	0.1	47.71±2.38 cd	44.86	6.35	Additive
	1x10^7	0.3	54.56±2.08 bc	50.26	8.54	Additive
	1x10^7	0.5	63.68±2.71 ab	62.10	2.54	Additive
	1x10^8	0.1	70.42±2.46 a	57.13	23.27	Synergism
	1x10^8	0.3	65.86±2.29 ab	62.53	5.34	Additive
	1x10^8	0.5	58.43±0.96 bc	74.37	-21.43	Antagonism
	1x10^7	0	20.72±1.96 g			
	1x10^8	0	32.99±2.48 ef			
	0	0.1	24.14±1.99 fg			
	0	0.3	29.54±2.98 fg			
	0	0.5	41.38±1.99 de			
LSD value @ 5%			11.465			
F			60.5			
P			≤0.01			
Chiniot	1x10^7	0.1	40.92±2.04 def	38.58	6.07	Additive
	1x10^7	0.3	47.74±2.05 cde	42.87	11.35	Additive
	1x10^7	0.5	56.67±1.92 abc	54.94	3.14	Additive
	1x10^8	0.1	63.64±2.98 a	52.22	21.87	Synergism
	1x10^8	0.3	60.69±2.04 ab	56.51	7.39	Additive
	1x10^8	0.5	51.15±2.07 bcd	68.58	-25.42	Antagonism
	1x10^7	0	17.01±1.79 h			
	1x10^8	0	30.65±1.66 fg			
	0	0.1	21.57±2.17 gh			
	0	0.3	25.86±1.29 gh			
	0	0.5	37.93±1.99 ef			
LSD value @ 5%			10.329			
F			62.1			
P			≤0.01			
Sargodha	1x10^7	0.1	53.33±1.92 ef	49.85	6.99	Additive
	1x10^7	0.3	59.54±1.97 de	54.33	9.59	Additive
	1x10^7	0.5	70.46±1.01 bc	66.90	5.32	Additive
	1x10^8	0.1	80.88±1.24 a	63.29	27.79	Synergism
	1x10^8	0.3	75.29±1.02 ab	67.78	11.08	Additive
	1x10^8	0.5	64.10±2.61 cd	80.35	-20.22	Antagonism
	1x10^7	0	22.53±2.53 i			
	1x10^8	0	35.98±1.37 gh			
	0	0.1	27.32±2.23 hi			
	0	0.3	31.80±2.13 hi			
	0	0.5	44.37±2.40 fg			
LSD value @ 5%			9.8311			
F			109			
P			≤0.01			

Means sharing the same letters within columns are not significantly different.

Additive and antagonistic effects were observed when the highest dose of SpltNPV was applied in combination with medium and higher doses of *B. thuringiensis* (0.3 and 0.5µg/g) in all localities, after 3 days of application. After 3 days of application, mortality of 4th instar larvae of *S. litura* ranged from 11.38 to 42.07% in the Faisalabad strain, 10.11 to 38.62% in the Chiniot strain and 13.64 to 51.72% in the Sargodha strain (Table IV). After 5 days of application, mortality of 4th instar larvae of *S. litura* ranged from 18.42 to 62.53% in the Faisalabad strain, 16.09 to 57.93% in the Chiniot strain and 19.54 to 69.31% in the Sargodha strain (Table V). While after 7 days of application, mortality of 4th instar larvae of *S. litura* varied from 20.72 to 70.42%, 17.01 to 63.64% and 22.53 to 80.88% for the Faisalabad, Chiniot and Sargodha strains of *S. litura*, respectively (Table VI).

DISCUSSION

Pakistan has a diversity of weather conditions which enables the farmers to grow cauliflower (*Brassica oleracea* var. botrytis) throughout the year, but different insect pests caused 20 to 40 % yield losses annually (FAOSTAT, 2013). Among different insect pests, *S. litura* is one of the most serious pest which caused 31% to 100% yield losses (Lingappa *et al*., 2004). To overcome urban and agricultural pests, farmers mostly relay on conventional insecticides in Pakistan (Basit *et al*., 2013; Khan *et al*., 2017, 2018a, b). Keeping in view the adverse effects of pesticides on human health, environment and beneficial insects (Yasoob *et al*., 2017; Ilyas *et al*., 2017), the present study was designed to minimize the bad effects on human health and save our environment and conserve the beneficial insects by using bio pesticide and microbes. Now the world is also following this trend to control the insect pests (Crickmore *et al*., 2014).

In the current study, the results revealed *Bt* insecticide as a safe option to control this pest because it has a significant effect on mortality of *S. litura* larvae. Previous studies have reported this insecticide as quick in action, easy to produce at low cost, long shelf life, safer for environment and beneficial insects and can be applied with novel pesticides in combination (Marvier *et al*., 2007; Kumar *et al*., 2008; Birch *et al*., 2011). In the current study *B. thuringiensis* in combination with other microbial insecticide significantly control *S. litura* under laboratory conditions.

Among entomo-pathogen viruses, SpltNPV is very important microbe. In this study SpltNPV gave hopeful results against this pest and in combination with *Bt* insecticide its efficacy was improved significantly. The result of current study are in agreement with Sutanto *et al*. (2014) who found that SpltNPV effectively controls the larval as well as pupal stage of this pest and also controls the adult emergence. Rajguru and Sharma (2014) evaluated the effectiveness of *B. thuringiensis* alone and in combination with water based extracts of eight plant species against *S. litura* larvae and observed 93.33% mortality of larvae when *Bt*. applied in combination with plant extract of *Datura stramonium* after 4 days of application. Kalantari *et al*. (2014) reported synergistic action by combining *Bt* at lower concentration and SpltNPV at higher concentration.

In the gut of the larvae both microbial insecticides synergize, which is the common site of infection. Cytoplasmic membranes become unsettle when *Bt* toxin adheres in the lining of midget. Knaak and Fiuza (2005) reported that the intensified infection of SpltNPV in the lepidopterous larvae after 6 hours of SpltNPV-*Bt* combined application. The combined action of SpltNPV-*Bt* creates some physiological abnormalities due to the suppression of detoxification enzymes in the lepidopterous larvae (Duraimurugan *et al*., 2009).

Bt insecticides are host specific and kill the target insects rapidly after ingestion, while NPVs are slow in action and take more time to kill the larvae but their joint action enhanced their efficacy against insect pests. These microbes have a potential to control the lepidopterous larvae effectively. These microbes are safer for natural enemies, environment and play a vital role in integrated pest management (IPM) program. Further studies are needed to check efficacy of these insecticides under field conditions.

CONCLUSION

The study revealed the toxic potential of NPV and Bt product against *S. litura* under laboratory conditions. Studies should further be extended under field conditions before considering these products in management plan for *S. litura*.

Statement of conflict of interest

The authors declare no conflict of interest.

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