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Comparative Evaluation of Selected Biorational Insecticides against *Spodoptera litura* (Fabricius) on Cauliflower

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ABSTRACT

Spodoptera litura is an economic pest of different field crops including cauliflower. In the present study three commercial formulations of biorational insecticides *viz*., *Bacillus thuringiensis* (DiPel®), NPV (SpltNPV) and Flubendiamide (Belt®) evaluated (alone and in combination) against *S. litura* under field condition. Minimum plant infestation (7.60±0.40%) was observed three days after application when *B. thuringiensis* and flubendiamide were applied in combination ((@0.5 kg/ha+75ml/ha, respectively). Maximum infestation (11.60±0.97%) was observed in SpltNPV @ 1.0×10^9 POB/ml + *B. thuringiensis* @1.00 kg/ha. Similarly, five days after application minimum plant infestation was observed in *B. thuringiensis* and flubendiamide @0.5 kg/ha+75ml/ha (5.20±0.49%) and maximum in SpltNPV @ 1.0×10^9 POB/ml + *B. thuringiensis* @1.00 kg/ha (8.80±0.49%). Whereas, minimum plant infestation was observed seven days after application where *B. thuringiensis* and flubendiamide were applied in combination @0.5 kg/ha+75ml/ha *i.e.* 2.80±0.49 % followed by *B. thuringiensis* @1.0 kg/ha + flubendiamide 480 SC @ 75ml/ha, SpltNPV @ 1.0×10^9 POB/ml + *B. thuringiensis* @0.5 kg/ha, SpltNPV @ 1.0×10^9 POB/ml + flubendiamide 480 SC @ 75ml/ha and SpltNPV @ 1.0×10^9 POB/ml + *B. thuringiensis* @1.00 kg/ha *i.e.* 4.00±0.63, 4.40±0.40, 4.80±0.48 and 5.60±0.40 %, respectively. Whereas, *B. thuringiensis* @1.0 kg/ha, flubendiamide @ 75ml/ha, SpltNPV @ 1.0×10^9 POB/ml and *B. thuringiensis* @0.5 kg/ha were gave plant infestation 6.40±0.40, 6.80±0.49, 8.80±0.48 and 9.20±0.49%, respectively. After second application, lowest plant infestation was recorded in the plot treated with *B. thuringiensis* @0.5 kg/ha+ flubendiamide 480SC @75ml/ha with plant infestation of 8.40±0.40, 5.60±0.40 and 2.20±0.40 % at three, five and seven days after application, respectively. However, all the insecticides reduced natural enemies (*Chrysoperla carnea*, ladybird beetles, and predatory bugs) populations in all the treatments. In conclusion, the results revealed the potential of *Bt*, NPV and flubendiamide for the management of *S. litura*. Further studies are needed to confirm the potential of these products against *S. litura* and negative impact on natural enemies under varying climatic conditions, and on different host crops.

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Authors' Contribution
SM performed the study, analyzed the data and wrote the manuscript. MA, MAA and WW designed and supervised the study. HAAK helped in the data analysis and writing of the manuscript.

Key words
Microbial insecticide, Ecotoxicology, Insect-pest management.

INTRODUCTION

Cauliflower (*Brassica oleracea* var. botrytis) is an important crop grown in South-East Asia. It is used as a vegetable as well as in different kinds of salads, throughout the year in homes and hotels of Pakistan. It is damaged by a large number of insect pests; of these, armyworm (*Spodoptera litura* F.) is one of the most serious pests causing yield losses ranging from 31% to 100%. It invades more than 40 plant families (Lingappa *et al*., 2004).

The status of *S. litura* is an admitted fact as a major pest of cauliflower crop. *Spodoptera litura* being an allopatric species is widely reported in Asia and other continents. It is a highly cosmopolitan and polyphagous pest which invades a wide host range of agricultural crops (Singh *et al*., 2015). Based on the crop damages, it is also known as Indian leaf worm, tobacco caterpillar and tobacco cutworm. Severe incidence of this pest may demand wide use of insecticides to safeguard the infested crops (Carasi *et al*., 2014).

Populations of *S. litura* in Pakistan have developed broad-spectrum resistance against conventional (chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids) and newer chemistry insecticides (spinosad, indoxacarb, fipronil, avermectins and insect growth regulators) (Abbas *et al*., 2012). Literature ensures

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its prime importance as first lepidopterous pest to develop insecticidal resistance in India (Srivastava and Joshi, 1965). Concerns related to human environment and development of resistance have inspired the researchers to investigate alternative options to conventional chemical application (Carlton and Gonzalez, 1986; Brousseau *et al.*, 1998). Now the pest management efforts are directed towards the use of bio-pesticides because of their promising potential for protection of economically important agricultural crops and environment (Nguyen, 2007; Inglis *et al*., 2010).

In Pakistan, the use of conventional insecticides has been the major choice to manage different insect pests of economic importance which ultimately lead to evolve insecticide resistance and field control failures (Khan *et al*., 2016a, b). Biorational insecticides are very effective tool to manage the resistance problems and protect natural enemies and environment (Khan and Akram, 2017; Yasoob *et al*., 2017). These insecticides are very effective against the target pests but less detrimental to natural enemies. Theses pesticides are usually derived from biologically active substances like plants and microbes that affect the growth and development of insects and provide protection against herbivores including lepidopteran pests (Senthil *et al*., 2005; Ignacimuthu *et al*. 2006; Baskar *et al*., 2011).

The main objective of this study was to check the efficacy of commercial formulations of NPV, *Bt* and flubendiamide alone and in different combinations against *S. litura* under field conditions.

MATERIALS AND METHODS

The experiment was carried out under field conditions on cauliflower crop grown in the Faisalabad region (Chak No. 208/R.B.).

Cauliflower nursery plants were transplanted in furrows following the randomized complete block design (RCBD) with four replications. All the recommended agronomic practices were undertaken for the purpose to gain healthy plants. Two spray applications, each with four replicates, of three bio-rational formulations, including flubendiamide (Belt), *B. thurengiensis* (DiPel) and NPV (SpltNPV) were carried out against *S. litura*. Data regarding %age plant infestation were recorded from 25 plants in each replication at three, five and seven days after application. While, the data regarding natural enemies' population (*Chrysoperla carnea*, ladybird beetle and predatory bugs) were also recorded from 25 randomly selected plants per replication at three, five and seven days after application. Whereas, mortality of *S. litura* caused by the application of insecticides was also recorded at the same time intervals. Following treatments were applied: T1, *B. thuringiensis* @0.5 kg/ha; T2, *B. thuringiensis* @1.0 kg/ha; T3, SpltNPV @ 1.0×10^9 POB/ml; T4, flubendiamide 480 SC @ 75ml/ha; T5, SpltNPV @ 1.0×10^9 POB/ml + *B. thuringiensis* @0.5 kg/ha; T6, SpltNPV @ 1.0×10^9 POB/ml + *B. thuringiensis* @1.00 kg/ha; T7, SpltNPV @ 1.0×10^9 POB/ml + flubendiamide 480 SC @ 75ml/ha; T8, *B. thuringiensis* @0.5 kg/ha + flubendiamide @ 480 SC @ 75ml/ha; T9, *B. thuringiensis* @1.0 kg/ha + flubendiamide @480 SC @ 75ml/ha and T10, control (untreated check).

Table I.- Plant infestation by *S. litura* on cauliflower after different time intervals at first application of different treatments.

Treatments	%age plant infestation after application for		
	3 days	5 days	7 days
T1	14.40±0.74 b	11.60±0.75 b	9.20±0.49 b
T2	12.00±0.63 bcde	9.60±0.40 bcd	6.40±0.40 bc
T3	13.60±0.75 bc	10.80±0.80 bc	8.80±0.48 b
T4	12.40±0.74 bcd	10.00±0.63 bcd	6.80±0.49 bc
T5	9.60±0.98 efg	6.80±0.48 ef	4.40±0.40 cd
T6	11.60±0.97 cde	8.80±0.49 cde	5.60±0.40 cd
T7	10.40±0.74 def	7.60±0.24 def	4.80±0.48 cd
T8	7.60±0.40 g	5.20±0.49 f	2.80±0.49 d
T9	8.80±0.48 fg	6.40±0.40 ef	4.00±0.63 cd
T10	33.60±1.16 a	35.20±1.20 a	38.00±1.09 a
LSD value @ 5%	2.7314	2.6613	2.8470

Mean sharing the same letters within columns are not significantly different. T1, *B. thuringiensis* @0.5 kg/ha; T2, *B. thuringiensis* @1.0 kg/ha; T3, SpltNPV @ 1.0×10^9 POB/ml; T4, flubendiamide 480 SC @ 75ml/ha; T5, SpltNPV @ 1.0×10^9 POB/ml + *B. thuringiensis* @0.5 kg/ha; T6, SpltNPV @ 1.0×109 POB/ml + *B. thuringiensis* @1.00 kg/ha; T7, SpltNPV @ 1.0×109 POB/ml + flubendiamide480 SC @ 75ml/ha; T8, *B. thuringiensis* @0.5 kg/ha+ flubendiamide @ 480 SC @ 75ml/ha; T9, *B. thuringiensis* @1.0 kg/ha + flubendiamide @480 SC @ 75ml/ha and T10, control (untreated check).

RESULTS

Percentage plant infestation after first spray

Significant differences regarding plant infestation %age due to *S. litura* larvae were recorded on crop plants in the field, treated with SpltNPV, *Bt*. and flubendiamide alone and in combinations after three, five and seven days of 1st spray application (Table I).

Minimum plant infestation was observed three days after application where *B. thuringiensis* and flubendiamide were applied in combination T8 (7.60±0.40%) followed by T9, T5, T7 and T6 (8.80±0.48, 9.60±0.98, 10.40±0.74 and 11.60±0.97%, respectively). The plant infestation in T2, T4, T3 and T1 treatments (12.00±0.63, 12.40±0.74, 13.60±0.75 and 14.40±0.74%, respectively) was statistically at par. Whereas, maximum plant infestation was recorded in T10 (33.60±1.16 %) (Table I).

Five days after application, minimum plant infestation was observed where *B. thuringiensis* and flubendiamide were applied in combination T8 (5.20±0.49%) followed by T9, T5, T7, and T6 (*i.e.* 6.40±0.40, 6.80±0.48, 7.60±0.24 and 8.80±0.49%, respectively). Whereas, T2, T4, and T1 gave plant infestation 9.60±0.40, 10.00±0.63, 10.80±0.80 and 11.60±0.75%, respectively, and statistically at par with each other. While maximum plant infestation was recorded in T10 (control) *i.e.* 35.20±1.20. The similar trend was also observed seven days after application (Table I).

Table II.- Percent plant infestation by *S. litura* on cauliflower after different time intervals at second application of different treatments.

Treatments	%age plant infestation after application for		
	3 days	5 days	7 days
T1	16.40±0.74 b	13.20±0.80 b	9.00±0.80 b
T2	13.60±0.83 bcd	10.80±0.48 bcd	5.80±0.49 cd
T3	16.00±0.63 b	12.00±0.63 bc	8.20±0.63 bc
T4	14.40±0.97 bc	11.20±0.49 bcd	6.20±0.48 bcd
T5	11.20±0.63 cde	7.20±0.40 ef	3.80±0.40 de
T6	13.20±0.48 bcd	9.60±0.74 cde	5.40±0.48 cd
T7	11.60±0.78 cde	8.40±0.40 def	4.20±0.40 de
T8	8.40±0.40 e	5.60±0.40 f	2.20±0.40 e
T9	10.00±0.63 de	7.20±0.48 ef	3.80±0.40 de
T10	39.20±1.02 a	41.60±1.17 a	43.40±1.26 a
LSD value @ 5%	3.6618	3.1656	2.8752

Mean sharing the same letters within columns are not significantly different. For details of treatments, see Table I.

Percentage plant infestation after second spray

After second application, a similar pattern of plant infestation %age was recorded in all treatments. Lowest plant infestation was recorded in the plot treated with T8 (*B. thuringiensis* @0.5 kg/ha+ flubendiamide 480SC @75ml/ha) with plant infestation of 8.40±0.40, 5.60±0.40 and 2.20±0.40% at three, five and seven days after application, respectively. Whereas, combination treatments *i.e.* T9, T5, T7 and T6 also gave significant results as applied alone (10.00±0.63, 7.20±0.48 and 3.80±0.40%); (11.20±0.63, 7.20±0.40 and 3.80±0.40%); (11.60±0.78, 8.40±0.40 and 4.20±0.40 %) and (13.20±0.48, 9.60±0.74 and 5.40±0.48 %) at three, five and seven days after application, respectively. While maximum plant infestation was recorded in T10 (control) *i.e.* 39.20±1.02, 41.60±1.17 and 43.40±1.26 % at three, five and seven days after application, respectively (Table II).

Effect on population fluctuation of natural enemies

The results regarding the effect of different treatments on population fluctuation of natural enemies of *S. litura* revealed that NPV was proved toxic against all the natural enemies applied alone as well as in combination. *B. thuringiensis* has insecticidal activity reduced the population of all the natural enemies applied alone as well as in combination, especially with flubendiamide, but less toxic than insecticide (flubendiamide). Flubendiamide proved more toxic to natural enemies as compared with NPV and *B. thuringiensis*.

Although all the treatments reduced the population of natural enemies, including *C. carnea,* ladybird beetle and predatory bugs, but their effects were different on these natural enemies. Lady bird beetle and *C. carnea* were comparatively less susceptible to toxic action of treatments while predatory bugs were comparatively more susceptible to mortal action due to their delicate body. Among all the three observations recorded for each natural enemy, the maximum population of *C. carnea* (1.52 individuals/10 plants) ladybird beetle (1.68 individuals/10 plants) and predatory bugs (1.28 individuals/10 plants) was recorded in T3 (NPV) except control treatment. Whereas, the minimum population of *C. carnea* (0.48 individuals/10 plants) ladybird beetle (0.56 individuals/10 plants) and predatory bugs (0.32 individuals/10 plants) was recorded in T4 (flubendiamide 480 SC) during the first observation (Fig. 1A).

In second and third observations, similar pattern was observed where NPV was found less toxic against natural enemies with maximum population density of *C. carnea* (1.36 individuals/10 plants) ladybird beetle (1.52 individuals/10 plants) and predatory bugs (1.20 individuals/10 plants) was recorded for second observation and during the third observation maximum population density of *C. carnea* (1.20 individuals/10 plants) ladybird beetle (1.44 individuals/10 plants) and predatory bugs (1.04 individuals/10 plants) was recorded respectively. While lowest population of *C. carnea* (0.32 individuals/10 plants) ladybird beetle (0.48 individuals/10 plants) and predatory bugs (0.24 individuals/10 plants) was recorded for second observation and during the third observation minimum population density of *C. carnea* (0.24 individuals/10 plants) ladybird beetle (0.40 individuals/10 plants) and predatory bugs (0.16 individuals/10 plants) was recorded, respectively (Fig. 1B, C).

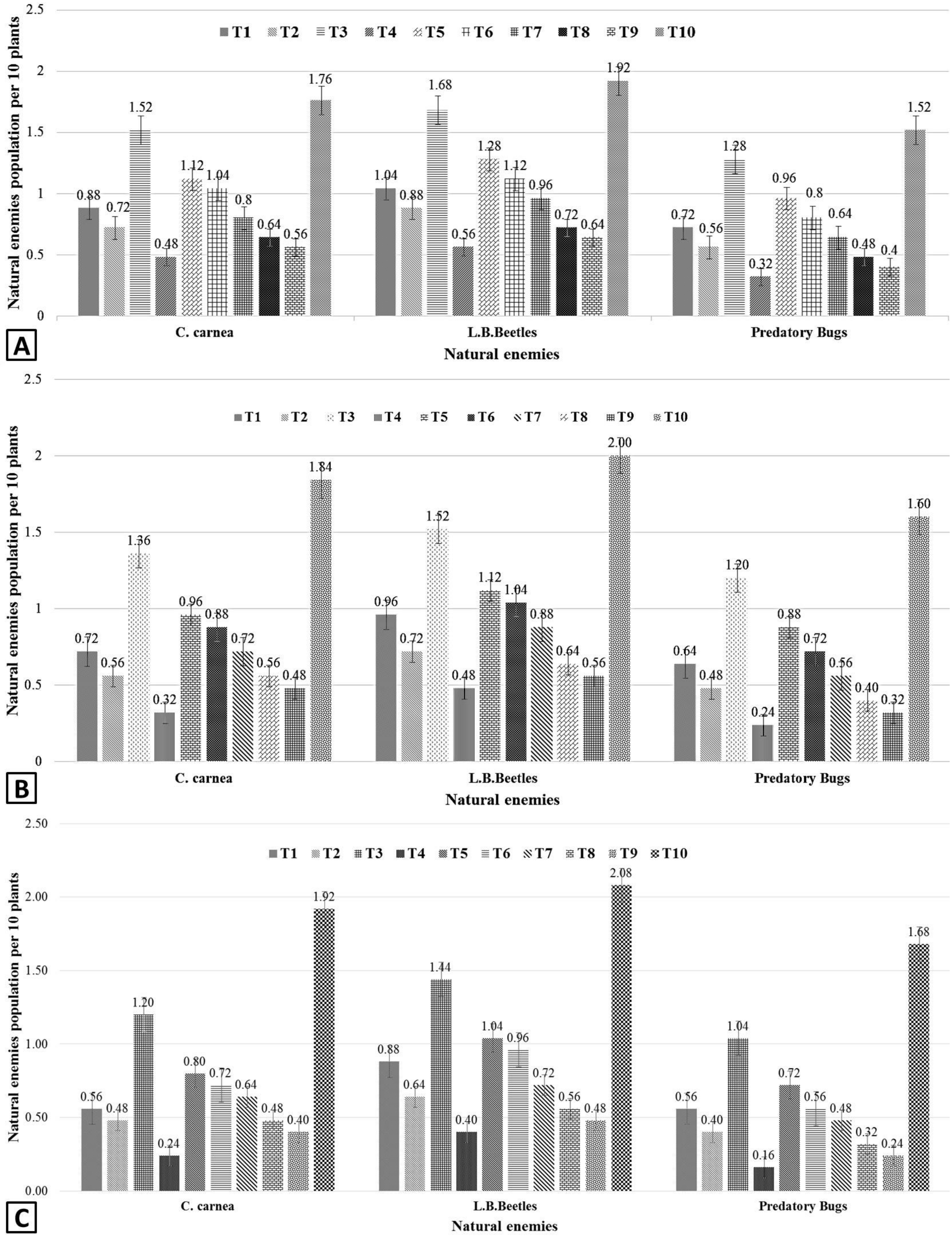

Fig. 1. Population density of natural enemies per 10 plants at first (A), second (B) and third (C) observation after spray applications.

Table III.- Mean %age mortality of *S. litura* on cauliflower after different time intervals at first application of different treatments.

Treatments	%age mortality after application for		
	3 days	5 days	7 days
T1	55.91±1.63 f	68.34±1.35 h	75.89±1.25 f
T2	65.92±1.79 cde	73.22±1.65 ef	83.28±1.42 de
T3	59.91±2.48 ef	70.06±0.69 gh	77.41±1.57 f
T4	63.11±1.10 de	72.16±1.05 fg	81.92±1.16 e
T5	72.08±1.51 abc	80.53±1.18 bc	88.64±1.22 b
T6	66.87±1.36 cd	75.65±1.06 de	85.29±1.46 cd
T7	70.65±1.81 bc	78.09±1.18 cd	87.30±1.21 bc
T8	77.26±1.12 a	85.40±0.94 a	92.67±1.30 a
T9	74.39±0.93 ab	81.57±1.20 b	89.65±1.24 b
T10	0.00 g	0.00 i	0.00 g
LSD value @ 5%	6.3853	2.6749	2.4210

Mean sharing the same letters within columns are not significantly different. For details of treatments, see Table I.

Percentage mortality of S. litura *on cauliflower*

Significant differences regarding %age mortality of *S. litura* larvae were recorded on the crop plants in the field, treated with NPV, *Bt*. and flubendiamide alone and in combinations after 3, 5 and seven days of 1st spray application (Table III).

Three days after application, maximum mortality of larvae was recorded where *B. thuringiensis* and flubendiamide were applied in combination T8 (0.5 kg/ha+75ml/ha) *i.e.* 77.26 % followed by T9, T5, T7, T6, T2, T4 and T3 (*B. thuringiensis* @0.5 kg/ha) *i.e.* 81.57, 72.08, 70.65, 66.87, 65.92, 63.11 and 59.91%, respectively. Among them T8, T9 and T5 were statistically at par with each other as well as T6 and T2 were also statistically at par with each other. Apart from T10 (control), minimum mortality was recorded in T1 *i.e.* 55.91%. Similar trend was observed five days after application, mortality ranged from 68.34 to 85.40%. Maximum mortality of larvae was observed where *B. thuringiensis* and flubendiamide were applied in combination T8 (0.5 kg/ha+75ml/ha) *i.e.* 85.40%. T9 and T5 were statistically at par with each other gave larval mortality 81.57 and 80.53 %, respectively followed by T7, T6, T2, T4 and T3 *i.e.* 78.09, 75.65, 73.22, 72.16 and 70.06%, respectively. Apart from T10 (control) minimum mortality was recorded in T1 *i.e.* 68.34 %, respectively.

Whereas, at seven days after application mortality ranged from 75.89 to 92.67 %. T7 treatment gave more than 80% mortality. Maximum mortality of larvae was observed where *B. thuringiensis* and flubendiamide were applied in combination (0.5 kg/ha+75ml/ha) *i.e.* 92.67%. T9, T5 and T7 were statistically at par with each other and caused larval mortality 89.65, 88.64 and 87.30 %, respectively, followed by T6, T2 and T4 *i.e.* 85.29, 83.28 and 81.92%, respectively. Apart from T10 (control), minimum mortality was recorded in T1 (75.89%) and T3 (77.41%) and both treatments were statistically at par with each other.

After second spray application, similar pattern of larval mortality was recorded in all treatments. Maximum mortality was recorded in plot treated with T8 (*B. thuringiensis* @0.5 kg/ha+ flubendiamide 480SC @ 75ml/ha) with larval mortality of 78.65, 86.63 and 93.75% at three, five and seven days after application, respectively. Whereas, the combinations T9, T5, T7 and T6 also revealed significant results (75.82, 83.56 and 90.77%); (73.17, 82.18 and 89.96%); (71.07, 80.10 and 88.79%) and (67.35, 76.68 and 86.47%) at three, five and seven days after application, respectively. While except from control treatment, minimum mortality was recorded in T1 *i.e.* 58.28, 67.74 and 76.53 % at three, five and seven days after application, respectively (Table IV).

Table IV.- Mean %age mortality of *S. litura* on cauliflower after different time intervals at second application of different treatments.

Treatments	%age mortality after application for		
	3 days	5 days	7 days
T1	58.28±0.91 g	67.74±1.37 g	76.53±1.32 f
T2	64.68±0.80 de	74.27±1.32 de	85.16±1.79 de
T3	60.81±1.23 fg	71.50±0.75 f	79.20±1.93 f
T4	62.57±0.96 ef	73.22±0.71 ef	83.49±1.44 e
T5	73.17±1.12 bc	82.18±1.52 bc	89.96±1.68 b
T6	67.35±1.23 d	76.68±1.39 d	86.47±1.50 cd
T7	71.07±1.69 c	80.10±1.13 c	88.79±1.58 bc
T8	78.65±1.04 a	86.63±0.95 a	93.75±1.32 a
T9	75.82±0.85 ab	83.56±1.49 b	90.77±1.27 b
T10	0.00 h	0.00 h	0.00 g
LSD value @ 5%	3.6851	2.7529	2.9061

Mean sharing the same letters within columns are not significantly different. For details of treatments, see Table I.

DISCUSSION

Pakistan has a diversity of weather conditions which enable farmers to grow cauliflower throughout the year, but different insect pests caused 20 to 40 % yield losses annually (FAOSTAT, 2013). Among different insect pests, *S. litura* is the most serious pest which caused 31% to 100% yield loss (Lingappa *et al*., 2004). To overcome this pest, farmers totally relay on insecticides in Pakistan

(Basit *et al*., 2013). Keeping in view the adverse effects of pesticides on human health, environment and beneficial insects (Khan *et al*., 2017; Arshad *et al*., 2015, 2017), the present study was designed to minimize the bad effects on human health and save our environment and conserve beneficial insects by using biopesticide and microbes. Now the world is also following this trend to control the insect pests (Crickmore *et al*., 2014; Khan *et al*., 2016; Iqbal *et al*., 2016; Ilyas *et al*., 2017).

In the current study flubendiamide has been proved very effective against this pest due to its novel mode of action and selective activity. Previous studies (Tohnishi *et al*., 2005; Shaurub *et al*., 2014; Nasution *et al*., 2015) have also reported that flubendiamide significantly control a broad range of lepidopterous pests, and relatively safer for predators, parasites and pollinators and environment.

The use of *Bt* insecticides is another safe option to control this pest because it effectively control the lepidopterous larvae, its action is fast, easy to produce at low cost, long shelf life, safer for the 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 microbes significantly control this pest. Previous studies (Hokkanen and Hajek, 2003; Lacey and Merritt, 2003; Wu *et al*., 2005; Romeis *et al*., 2006; Marvier *et al*., 2007; Kumar *et al*., 2008; Birch *et al*., 2011; Fuentes and Jackson, 2012) are also supported our results, they reported that *B. thuringiensis* is a safe option and effectively control this pest by applying alone and in combination with different safer bio-pesticides.

Among entomo-pathogen viruses, SpltNPV is very important microbe, in this study SpltNPV gave hopeful results against this pest but in combination its efficacy was improved significantly. The result of current study supported by Sutanto *et al*. (2014), they found that SpltNPV effectively controls the larval as well as pupal stage of this pest and also controls the adult emergence.

In the current study, the results regarding plant infestation %age, population density of natural enemies and %age mortality of *S. litura* larvae revealed that *Bt* gave significant results in combination with flubendiamide and SpltNPV rather than applied alone gave significant results when applied in combination rather than alone. The current finding were parallel to the findings of previous studies (Reddy and Manjunatha, 2000; Nathan and Kalaivani, 2006; Kandalkar and Men, 2006; Singh *et al*., 2007, 2009; Khanna *et al*., 2009; Kalantari *et al*., 2014) they reported that *Bt* gave significantly higher results when applied in combination with insecticides and SpltNPV rather than alone.

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 observed 93.33 % mortality of larvae when *Bt*. applied in combination with plant extract of *Datura stramonium* four days after application. Kalantari *et al*. (2014) reported synergistic action by combining *Bt* at lower concentration and SpltNPV at higher concentration. In conclusion, the tested chemicals could be helpful in the management of *S. litura*, however, further trials should be conducted in different agro-ecological zones.

Statement of conflict of interest

Authors have declared no conflict of interest

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