Pakistan J. Zool., vol. 52(2), pp 609-616, 2020. DOI: https://dx.doi.org/10.17582/journal.pjz/20180619100621

Comparative Efficacy of *Bacillus thuringiensis* Commercial Formulations against Leaf Worm, *Spodoptera litura* Fabricius under Laboratory Conditions

Ammara Blouch[1], Ata ul Mohsin[2], Muhammad Naeem[2] and Rashid Mahmood[1]*

[1]*Honeybee Research Institute, National Agricultural Research Centre, Islamabad*
[2]*Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi*

ABSTRACT

A study was carried out in the Bio-control Laboratory, Department of Entomology at Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi to check the efficacy of commercial biopesticides under controlled environmental conditions. Bio pesticides are important alternates for chemical control of economically damaging insect pests like leaf worm, *Spodoptera litura* Fabricius. In this study, two commercial products including Dipel with *Bt* sub speciess *kurstaki* and Turex with *Bt* sub speciess *kurstaki* and *aizawai* were tested against three early larval instars of *S. litura* under laboratory conditions using leaf dip method. Mortality was recorded after three and seven days of exposure. The results indicated that larval mortality increased with time and Turex (*Bt* sub speciess *kurstaki* and *aizawai*) after 3 days of exposure caused significantly higher mortality i.e 46.43, 43.45 and 38.69 % as compared to Dipel (*Bt* sub speciess *kurstaki*) that caused 19.05, 6.55 and 4.76 % mortality for 1st, 2nd and 3rd instar, respectively. The data for 7th day also showed significantly higher mortality as 64.29, 60.71 and 45.24 % by Turex (*Bt* sub speciess *kurstaki* and *aizawai*) in comparison with 55.95, 57.74 and 42.86 % mortality by Dipel (*Bt* sub species *kurstaki*) for 1st, 2nd and 3rd instar, respectively. Susceptibility to both bio pesticides increased with increase in their concentration and decreased with increase in larval instar. Similarly LC_{50} values suggested Turex (*Bt* sub species *kurstaki* and *aizawai*) to be more toxic with less LC_{50} values as compared to Dipel (*Bt* sub species *kurstaki*). These results indicated that these bio pesticides if used at early insect stage can help to control this pest.

Article Information
Received 19 June 2018
Revised 01 May 2019
Accepted 10 June 2019
Available online 24 January 2020

Authors' Contribution
AB designed and carried out the study and wrote the manuscript. AM and MN supervised the research work. RM drafted the manuscript.

Key words
***Spodoptera litura, Bacillus thuringiensis*, Commercial formulations, Laboratory bioassays**

INTRODUCTION

Leaf worm, *Spodoptera litura* (Fab.) is one of the most voracious and damaging insect pest of more than one hundred host plants with important cultivated crops and vegetables in the South Asian countries (Qin *et al*., 2004). It is also known as leaf worm, common or tobacco cutworm and cluster or tobacco caterpillar. Under favourable environmental conditions, its population grows rapidly and it moves across the field like an army therefore it is called as "Armyworm". It causes major economic losses to crops and in severe situation, a total crop loss (Dhir *et al*., 1992; Singh and Sachan, 1992). Heavy losses in field crops have been estimated (25-50%) depending upon the population density of this pest (Patil *et al*., 1991). Warm and humid field conditions of South Asia favor its development, multiplication and resurgence (Ahmad *et al*., 2007). It has the ability to multiply at very fast rate, polyphagous in nature and can travel to long distances making it a very difficult pest to manage in outbreak situations (Ahmad *et al*., 2007). The infestation of *S. litura* in Pakistan usually starts at the end of March and continues till the end of November depending upon the cropping pattern (Sayyed *et al*., 2008). This pest is abundantly found during the months of September and October (Islam *et al*., 1984). Its outbreak occurs due to insecticide resistance, favorable weather conditions and heavy rainfall after a long dry period (Thanki *et al*., 2003).

Spodoptera litura is well known for its quick development of resistance to different groups of insecticides used to manage it (Kranthi *et al*., 2002). Different control methods including biological, physical and chemical are practiced for its management (Parera *et al*., 2000). However, chemical control method is the most common but its extensive use has resulted in serious resistance problems. Extensive use of synthetic insecticides is not only detrimental for the environmental but also results in high chemical and labour costs (Ding *et al*., 1998). Alternate host plants of *S. litura* like arum (*Arum maculatum)*, Elephant ear (*Colocasia esculenta)*

* Corresponding author: rashid_ento1@yahoo.com
0030-9923/2020/0002-0609 $ 9.00/0

and Desert Horsepurslane, *(Triamthema portulacasterum)* can help to reduce the development of pest on major crops (Ahmad, 2008).

Variable levels of resistance to almost every group of insecticides have been observed in Pakistan, India and China in *S. litura* field populations. The resistance has been found to develop in both conventional insecticide groups like organochlorine, organophosphate, carbamates and pyrethroids as well as in new chemistry insecticides like indoxacarb, abamectin, and emamectin (Kranthi *et al*., 2002; Ahmad *et al*., 2008). The pesticides use not only results in such resistance problems but also causes health hazards to operators like farmers and the surrounding environment (Tinoco- Ojanguren and Halperin, 1998).

Currently, the use of microbes for controlling economically important pests has increased. *Bacillus thuringiensis* products have been tried on a very large scale because of their effectiveness against insects and safety to environment and humans (Falcon, 1971). It is a rod-shaped gram positive soil bacterium that produces crystal proteins which are toxic to certain insects but are harmless to the humans, wildlife and beneficial insects and considered to be the most important environmentally safe bio pesticides against agricultural pests (Butter *et al*., 1995; Puri *et al*., 1998). Keeping in view the importance of *B. thuringiensis*, two commercial formulations were tested against three first larval stages of *S. litura* under laboratory conditions because *S. litura* is a gregarious feeder and need to be controlled at three first larval stages to avoid extensive crop damage and economic losses and also due to the fact that *Bt* toxins are most effective for three first larval stages.

MATERIALS AND METHODS

Field collection and rearing of Spodoptera litura

The study was carried out in the Bio-control Laboratory, Department of Entomology at Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi under controlled environmental conditions. About 200 larvae of *S. litura* were collected from the cauliflower growing areas of Bahawalpur, where a number of insecticides are used for management of different insect pests. According to a study for the determination of Pesticide residues in Bahawalpur soil, the most widely detected pesticides which are being used heavily in Bahawalpur included mevinphos, endosulfan, fenitrothion, chlorpyriphos dichlorvos, dimethoate and methyl parathion (Anwar *et al*., 2014). *Spodoptera litura* larvae were kept in a plastic jar of about 2 litre volumes with some host plant leaves (Cauliflower). The jar was closed with a piece of muslin cloth and brought to laboratory for further rearing at 25±2°C, 50±10% relative humidity and 16 hr photoperiod. The collected larvae were reared in six hole Petri dishes on artificial wheat germ based diet (Ahmad *et al*., 2007). After 3-4 days larval diet was replaced with new one and the cells were cleaned for further rearing of larvae till pupation. Mature pupae were collected with the help of a forceps and were kept in separate plastic box lined with tissue paper. Emerged adult moths were shifted to plastic jars of 4 kg capacity covered with muslin cloth and were provided 10% sugar solution. Egg batches were collected daily from the tissue paper strips hanged inside the jars.

Test bioinsecticides and bioassays

Commercial formulations of two *Bacillus thuringiensis* strains including Dipel with *Bt* sub species *kurstaki* and Turex with *Bt* sub species *kurstaki* and *aizawai* were used for laboratory bioassays. Dipel potency was 16,000 i.u/mg. While the potency of Turex (WP) was 32,000 i.u/mg. Dipel was product of Valent Bio-Science U.S.A. and Turex was a product of Abbot Laboratories. Bioassays were conducted using leaf dip method against early three instars of *S. litura* (Anonymous, 1990). A stock solution based on preliminary bioassays of *Bt* insecticides was prepared in distilled water and diluted by 1/2 to 6 serial levels of concentration as 200, 100, 50, 25, 12.5 and 6.25 mg/ml. Leaf discs of 5 cm diameter were cut using 5cm diameter leaf cutter from the unsprayed host plant (cauliflower) and were washed with tap water and air-dried before use. These leaf discs were dipped in each test solution level for 10-15 seconds with gentle agitation and air-dried in fume hood. The treated leaf discs with their adaxial side upward were then placed in petri dishes of 5 cm diameter that contained moist filter paper at their bottom to avoid desiccation. Four leaf discs (replications) per concentration level with 20 larvae at each level were used (Total larvae=120). Five 1st, 2nd and 3rd instar larvae of *S. litura* were released in each Petri dish using camel hair brush. In case of control, the leaves were dipped in distilled water.

Statistical analysis

Larval mortality was recorded after three and seven days of exposure period. Larvae that could not respond to stimulation with a blunt head needle or bodies deformed were considered as dead. Abbot's formula was used to calculate the corrected mortality (Abbot, 1925) and was analyzed by probit analysis (Finney, 1971). The results were interpreted using POLO-PC software (Russell *et al*., 1977) and means were compared using Duncan Multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

The data of mean mortalities after 3rd and 7th day of exposure showed 1st instar and 2nd instar larvae to be more susceptible to both the formulations; Dipel (*Bt* sub species *kurstaki*) and Turex (*Bt* sub species *kurstaki* and *aizawai*) as compared to 3rd instar. For 1st instar, Dipel caused 19.05 and 55.95 % mortality, while Turex caused 46.43 and 64.29 % mortality after 3rd and 7th day of application, respectively. For 2nd instar, Dipel caused 6.55 and 57.74 % mortality and Turex caused 43.45 and 60.7 % mortality after 3rd and 7th day, respectively. Similarly, for 3rd instar Dipel caused 4.76 and 42.86 % mortality while Turex caused 38.69 and 45.24 % mortality after 3rd and 7th day of exposure, respectively (Table I). Thus, mortality was higher for 1st and 2nd instar larvae as compared to 3rd instar larvae in case of both formulations. These results are in accordance with those of Puntambekar *et al*. (1997) who tested different Bt strains against certain lepidopteran pests and determined that use of 1018 spores per ml of Bt var. kurstaki (NCIM 2514) caused 85 % mortality in neonate larvae of *S. litura* and *Pthorimae operculella*. Sondos *et al*. (2000) also reported that Bt toxins were most effective for the newly hatched larvae of *S. littoralis*.

This Comparison of mean mortalities of the *S. litura* larvae through Duncan's Multiple Range test also indicated that the Turex formulation caused more mortality as compared to Dipel after both 3rd and 7th day of application. This further revealed that for all the three instars, there exists a significant difference between the mortality caused by both Insecticides on 3rd day. However, 7th day data of 1st and 2nd instars showed non-significant difference among the efficacy of two *Bt* formulations. DMR test also revealed that performance of each commercial formulation was statistically different at different levels of concentration. There is highly significant difference in mean mortality between highest and lowest concentration level of both insecticides i.e. 200 mg and 6.25 mg (Table I).

The toxicity data (Table II) also showed that 1st and 2nd instars were less significantly different regarding their susceptibility on 7th day as compared to 3rd instar which showed highly significant difference on both 3rd and 7th day for both commercial formulations. These results are in accordance with Loganathan *et al*. (2002) who also found that for the management of *S. litura* spraying with bio pesticides in the early stages is most effective.

The Toxicity values of both formulations (Table II) also suggested that Turex containing mixture of two strains was more toxic and was found to be more effective to control this pest with less LC_{50} values of 12.6, 15.9 and 26.0 on 3rd day and 3.50, 3.85 and 14.1 on 7th day as compared to Dipel with LC_{50} values of 144, 295 and 426 on 3rd day and 5.59, 3.45 and 19.81 on 7th day for 1st 2nd and 3rd instar respectively.

Graphical representation of data has also shown that with increasing level of concentration, mortality of larvae also increased. However, on 7th day mortality was high for all the instars irrespective of the dose level used showing that time factor plays a key role in the mortality in case of slow-acting insecticides like Bt (Figs. 1, 2 and 3).

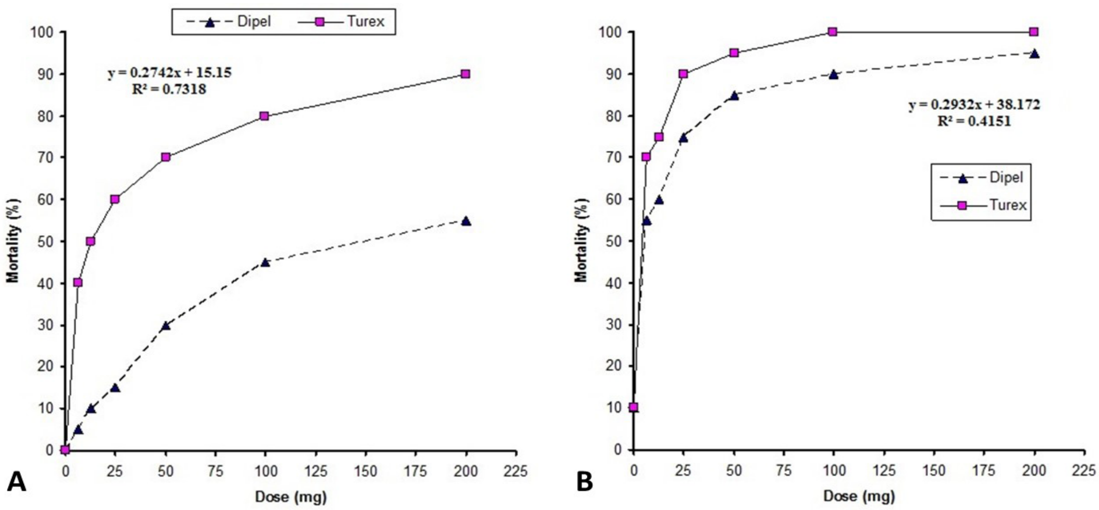

Fig. 1. Mortaity of 1st instar larvae *Spodoptera litura* against Dipel and Turex on 3rd day (A) and on 7th day (B) of application of insecticide.

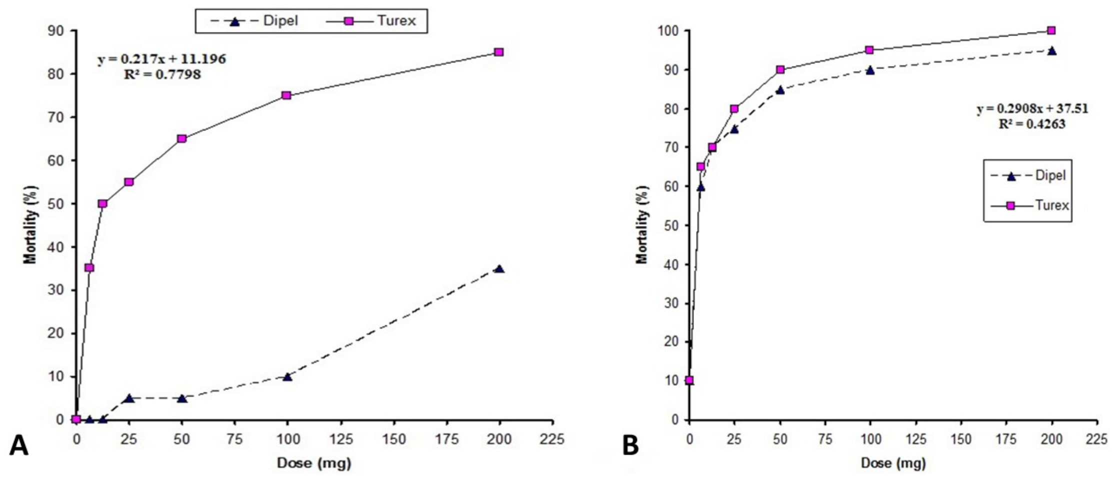

Fig. 2. Mortaity of 2nd instar larvae *Spodoptera litura* against Dipel and Turex on 3rd day (A) and on 7th day (B) of application of insecticide.

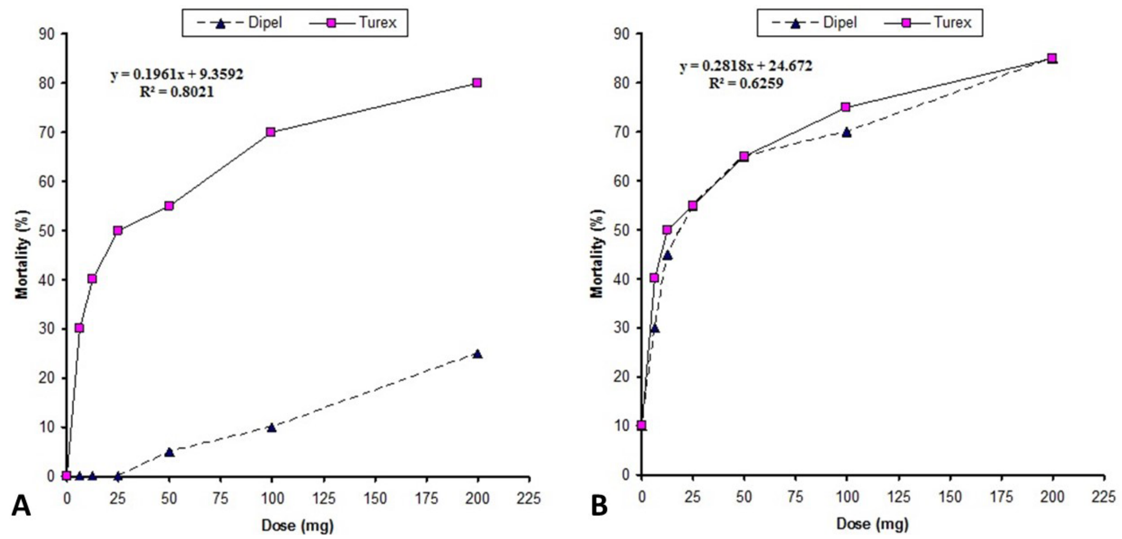

Fig. 3. Mortaity of 3rd instar larvae *Spodoptera litura* against Dipel and Turex on 3rd day (A) and on 7th day (B) of application of insecticide.

These results are in agreement with those of other researchers; Dulmage and Cooperators (1981) revealed that *B. thuringiensis* strains that are active against the lepidopteron larvae differ greatly in their insecticidal spectra and potency. Murthy *et al*. (2014) found out that Bt results in higher larval mortality owing to improved solubility of crystal toxins in the alkaline midgut fluid due to their smaller size thus more toxin becomes available for binding with receptors on the surface of midgut epithelium resulting in rapid midgut paralysis. Pandey *et al*. (2009) reported that highest mortality (73.3%) of third instar larvae of *S. litura* was caused at 10% concentration of commercial Btk formulation Biolep.

The highest mortality rates shown by Turex seems to be due to its high potency i.e., 32000 i.u/mg which was greater as compared to the other formulation i.e. Dipel having the potency of 16,000 i.u/mg. The other reason for the highest performance of Turex may be its active ingredient i.e., the strain which is a mixture of subsp. *Bt kurstaki* and *Bt aizawai*. The active ingredient, in case of the other formulation Dipel is *Bt kurstaki*. The results not only concluded the efficacy of *Bt* as a good bio pesticide against *Spodoptera litura* (Fab.) but also revealed that *Bt* potency can be increased and it can be made more effective bio pesticide by using it in combination with other *Bt* strains or different insecticides (Saleem *et al*., 1995, 1996). Nathan *et al*. (2006) also found that bacterial toxins and botanical insecticides in combination were more effective against the rice leaf folder, *Cnaphalocrocis medinalis* even at low concentration as compared to their effect independently. Sharma *et al*. (2001) performed leaf dip bioassay for a commercial formulation of *Bt* var. kurstaki and aizawai and evaluated that both the formulations caused 100 and 93.7 per cent mortality of *S. litura* larvae, respectively.

Table I. Mean mortalities of 1st, 2nd and 3rd instar larvae of *Spodoptera litura* by Dipel and Turex after 3rd and 7th day of application (n=4).

Time (Days)	Dose (mg)	Dipel (Bt sub species kurstaki)			Turex (Bt sub species kurstaki and aizawai)		
		1st instar mortality (mean±SE)	2nd instar mortality (mean±SE)	3rd instar mortality (mean±SE)	1st instar mortality (mean±SE)	2nd instar mortality (mean±SE)	3rd instar mortality (mean±SE)
3	0	0.00±0.00g	0.00±0.00e	0.00±0.00h	0.00±0.00g	0.00±0.00e	0.00±0.00h
	200	45.83±4.17bcd	29.17±4.1d	20.83±4.17efg	75.00±4.81 a	70.83±4.17 a	66.67±6.80 a
	100	37.50±7.98cde	8.33±4.81e	8.33±4.81fgh	66.67±6.80 ab	62.50±4.17 ab	58.33±4.17ab
	50	25.00±10.7d-g	4.17±4.17e	4.17±4.17 gh	58.33±4.81abc	54.17±4.17 abc	45.83±4.17bc
	25	12.50±4.17efg	4.17±4.17 e	0.00±0.00 h	50.00±6.80 a-d	45.83±4.17bcd	41.67±4.81bcd
	12.5	8.33±4.81 fg	0.00±0.00 e	0.00±0.00 h	41.67±4.81bcd	41.67±4.81 cd	33.33±6.80cde
	6.25	4.17±4.17 g	0.00±0.00 e	0.00±0.00 h	33.33±6.80 c-f	29.17±4.17 d	25.00±4.81def
	Mean	19.05±3.71 B	6.55±2.16 B	4.76±1.68 B	46.43±4.80 A	43.45±4.40 A	38.69±4.29 A
7	0	8.33±4.81	8.33±4.81	8.33±4.81	8.33±4.81	8.33±4.81	8.33±4.81
	200	79.17±4.17	79.17±4.17	70.83±4.17	83.33±0.00	83.33±0.00	70.83±7.98
	100	75.00±4.81	75.00±4.81	58.33±4.81	83.33±0.00	79.17±4.17	62.50±4.17
	50	70.83±4.17	70.83±4.17	54.17±7.98	79.17±4.17	75.00±4.81	54.17±7.98
	25	62.50±7.98	62.50±7.98	45.83±7.98	75.00±4.81	66.67±6.80	45.83±4.17
	12.5	50.00±6.80	58.33±4.81	37.50±7.98	62.50±4.17	58.33±4.81	41.67±10.76
	6.25	45.83±4.17	50.00±6.80	25.00±4.81	58.33±4.81	54.17±4.17	33.33±6.80
	Mean	55.95±4.71B	57.74±4.65 A	42.86±4.32A	64.29±4.90 A	60.71±4.79 A	45.24±4.36 A

In each row or column means with similar letter are statistically non-significant at 5% level according to Duncan Multiple range test. Small letters represent mean comparisons in each row and capital letters are used for mean comparisons between columns.

Table II. Toxicity of Dipel and Turex against 1st, 2nd and 3rd instar larvae of *Spodoptera litura* after 3rd and 7th day of application (n=4).

Insecticides	Time (Days)	Instar	LC_{50} (mean±SE)	FL at 95%	Chi-Square	DF	n	p
Dipel (*Bt* sub species *kurstaki*)	3	1st	144± 49.70	84.2-419.2	0.204	4	140	0.995
		2nd	295± 102.6	185.3-1617.4	0.504	4	140	0.973
		3rd	426± 232.75	218.5-16441.2	0.393	4	140	0.983
	7	1st	5.59±2.77	0.8-11.3	0.249	4	140	0.993
		2nd	3.45± 2.47	0.1-8.8	0.133	4	140	0.998
		3rd	19.8±6.45	7.9-35.4	0.385	4	140	0.984
Turex (*Bt* sub species *kurstaki* and *aizawai*)	3	1st	12.6± 4.59	3.9-22.6	0.175	4	140	0.996
		2nd	15.9± 5.84	4.9-29.6	0.228	4	140	0.994
		3rd	26.0± 8.34	11.1-49.7	0.251	4	140	0.993
	7	1st	3.50± 1.74	0.4-6.8	0.962	4	140	0.915
		2nd	3.85± 2.15	0.4-8.1	0.904	4	140	0.924
		3rd	14.1± 5.84	3.2-27.6	0.245	4	140	0.993

LC_{50}: lethal concentration at 50% level; FL: fiducial limit at 95% level; SE: significant error; n: Total no. of larvae/conc. level for all treatments + control.

These results are supported by the findings of other workers who have determined the toxicity and specificity of pathogens against different insect groups (Jaquet *et al*., 1987; Dong *et al*., 2004). Jayanthi and Padmavathamma (2001) found that the microbial pesticides themselves and along with chemical insecticides revealed them best in controlling *S. litura* under glasshouse condition. *B. thuringiensis* 1×10^7 spores/ml+ fenvelerate 0.005 per cent was proved best in respect of highest larval population reduction (89.90 %) and lowest leaf damage (20.15 %). The highest pod yield (15.03 g/pant) was also recorded with the same treatment. Shahid *et al*. (2003) checked the efficacy of fungus (*Metarhizium anisopliae*) and bacterium (*Bacillus thuringiensis*) against rice stem borer and leaf folder and found a decrease in the population in both laboratory and field without any harmful effects on predators and thus proved the usefulness of bio-pesticides.

Discovery of the potent *Bt* strains in Diptera (Goldberg and Margalit, 1977) and Coleoptera (Krieg *et al*., 1983) also demonstrated that the spectrum of potential uses of *Bt* is wider than initially believed. Further investigations are required to determine the efficacy of different strains of *Bt* against other pests and also the effect of different combinations of *Bt*.

ACKNOWLEDGEMENT

Author is heartily thankful to Dr. Munir Ahmad Associate Professor, Dr. Muhamad Asif Aziz Assistant Professor and Dr. Imran Bodlah Assistant Professor, Department of Entomology, Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi for their cooperation and guidance in this research work.

Statement of conflict of interest

The Authors declares there is no conflict of interest.

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