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Field Evaluation of Bio-pesticide for Control of Chickpea Pod Borer *Helicoverpa armigera,* a Major Pest of Chickpea Crop

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Abstract.- Studies conducted on application of biological insecticide (with and without adjuvants) revealed that *Bacillus thuringiensis* (*Bt)* field tests conducted over 3 successive chickpea seasons indicated consistently a significant ($p<0.05$) increase in yield between the DiPel 2X® alone/DiPel® + adjuvant treatments and control **(**.in the 1st year trial the calculated grain yield in Karate and DiPel 2X® + pottasium carbonate (K_2CO_3) treatments were 1822.0 Kg/Ha^{-1} (the highest) and 1535.0 Kg/Ha^{-1}, respectively. The grain yield was noted to be 2.0 to 2.5 times higher in all the DiPel 2X® + adjuvants treatments as compared to the grain yield (591.0 Kg/Ha^{-1}) of control, in 2nd year trial, the highest calculated grain yield (1157.0 Kg/Ha^{-1}) and (1022.0 Kg/Ha^{-1}) were noted in chemical insecticide and DiPel 2X®+ Powder milk (P. milk) treatments, respectively. Application of DiPel 2X® with adjuvants significantly increased grain yield 4-6 times as compared to control plot yield (171.0 Kg/Ha^{-1}), and in 3rd year trial, the overall calculated grain yield obtained in chemical insecticide (Karate®) and DiPel 2X® + K_2CO_3 were 934.0 and 693.0 Kg/Ha^{-1}, respectively being 3 to 4 times higher than the control grain yield (206.0 Kg/ha^{-1}). The grain yield in all the *Bt* treatments was at least 2 to 3 times higher than control plot yield. The application of microbial insecticide (DiPel 2X®) @ 1.6 kg Ha^{-1} (51.2 BIU Ha^{-1}) alone or with adjuvants costs more than the cost of chemical insecticides application in sub-tropical climate at temperature ranges between 16 to 34°C**.** The objective behind the use of adjuvant (heavy organic matters other than K_2CO_3) was to increase the *B.t.* persistency. *Bt* field test results indicated that microbial insecticides can be used (with and without adjuvants) for management of *H. armigera* populations infesting chickpea and their use would reduce reliance on toxic chemical released into the agro-ecosystem of Pakistan. This study in providing an alternate tool with eco-friendly approach as part of an Integrated Insect Pest Management (IIMP) programmes.

Key words: *Bacillus thuringiensis* Berliner, *Helicoverpa armigera,* microbial insecticide, chemical insecticides.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important grain legume crop grown on a large in the districts of Khushab, Layeiah, Bhakkar and Mianwali (in Punjab) as well as Shikarpur, Khairpur and Larkana (in Sindh) Pakistan. The total areas under chickpea crop in 2010 was 1093.9 thousand hectares with chickpea production of 868.2 thousand tones (Anonymous, 2010). Johansen *et al.* (2000) reported that every year, this insect cause heavy economic losses in areas where chickpea is grown and the crop is normally grown rainfed in the postrainy season (Oct-Mar). Production of chickpea in the Pakistan could not keep pace with demand, as evidenced by increasing imports of the crop. The crop continued to be grown largely as a subsistence crop by resource-poor farmers. The major constraints leading to low and unstable yields of chickpea are drought stress, foliar diseases (*e.g.*, *Ascochyta* blight caused by *Ascochyta rabiei* (Pass.) Labr. and botrytis gray mold caused by *Botrytis cinerea* Pers. Ex Fr., soil-borne diseases (*e.g.*, *fusarium* wilt caused by *Fusarium oxysporum* Schl.) and insect pests (Johansen *et al.*, 1994). Chickpea can be host of a wide range of insect pests (Reed *et al.*, 1987; Ranga Rao and Shanover, 1999) but acid exudation from above-ground plant parts probably acts as a partial deterrent to many of these (Reed *et al.*, 1987). By far the most economically important insect pest of chickpea is the pod borer, *Helicoverpa armigera* Heubn (Abbasi *et al*., 2007; Ahmed *et al*., 1997, 2000a,b). Substantial yield losses due to this

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pest have been reported in areas where the crop is grown in Pakistan. Among the valuable contributions pertaining to this subject are those of Ahmed *et al*. (2006) who reported detailed studies conducted on cross resistance of Cry1Ac resistant cotton bollworm *H. armigera* to various spore-delta-endotoxins of *Bt*. Extensive studies were reported by Khalique and Ahmed (2003) on impact of *Bt* on biology of *H. armigera*. Ahmed and Khalique (2002) did experimentation and reported forecasting adult populations of *H. armigera* on chickpea through pheromone traps and its role in management of this insect. Khalique and Ahmed (2001a,b) studied synergistic interaction between *Bt* and pyrethroid-insecticide, they have also conducted studies evaluation of toxicity of *Bt* and its sub-lethal effect on the development of *H. armigera*. Ahmed *et al*. (1998a,b, 1996) evaluated synergistism of *Bt* and malic acid on susceptibility of larval instars of *H. armigera*. Abbasi *et al*. (2007) developed tapioca-based artificial diet for mass rearing of *H. armigera* for conduction of bioassay of *Bt* using different instars of the test insect. Abbas and Young (1993) also conducted studies on *Bt* var. *kurstaki* activity against larvae of *Helicoverpa zea* and *Heliothis virescens*. Khalique and Ahmed (2005) reported compatibility of bio-pesticide with chemical insecticide for management of *H. armigera* (Huebner). Khalique and Ahmed (2002) studied retarded affect of spore-δ-delta endotoxin complex of *Bt* subsp. *Kurstaki* Berliner strains on the development of *H. armigera* (Huebner) and reported significant retarded development in the larvae of *H. armigera*.

The microbial (*Bt* based) insecticides can be used as component of integrated pest management (IPM) approach to provide an environmentally safe and suitable alternative to generally hazardous, broad spectrum chemical insecticides used against *H. armigera* (Hubner). As far as environmental protection is concerned, there is need for complimentary use of microbial (*Bt* based) and botanical insecticides in support of IPM. The biologically-derived insecticides, such as *Bt*-based biopesticide have provided a commercial alternative to broad spectrum chemical insecticide because of their specificity in killing target insect pest. The present study has been done in Pulses Programme, National Agricultural Research Center, Islamabad, which elaborates on the use of *Bt* based commercial bio-pesticide/microbial insecticides and compared with chemical insecticide for managing *H. armigera* population infesting chickpea.

MATERIALS AND METHODS

Material and products

Chickpea variety CC11514XILC482 was sown in all the three successive years and an exotic microbial commercial preparation of Abbott Laboratory, USA (DiPel 2X®, contained *B.t.* var. *kurstaki* spore-δ-endotoxin as an active ingredient having 32,000 (IU/mg potency) was applied @ 1.6 Kg/ha^{-1} (51.2 BIU/ha^{-1}) with and without adjuvants. The adjuvants used in the experiment were potassium carbonate (K_2CO_3 @ 0.37 Kg/ha^{-1}), powdered milk (2.5%), and molasses (7.0%). The Karate® 2.5 EC (cypermethrin *a.i.*, Imperial Chemical industry (ICI) product) @ 0.5 L (12.5 g a.i.)$/ha^{-1}$ was used as chemical insecticide check treatment. The control plot was sprayed with water.

Bt field test chickpea (year 1)

Chickpea variety ICC11514XILC482 was sown on 25 November in a randomized complete block design with 4 replications, 4 meter row length, 30 cm row to row and 10 cm plant to plant distance with 4 rows per plot. No irrigation and no fertilizer was used.

Treatments were applied on 08 April (1st treatment), 17 April (2nd treatment) and 25 April (3rd treatment) in 1994. All the treatments were applied as per our larval infestation observation. The quantities of insecticides (with and without adjuvants) were determined for 4.8 m^2 plot (4 replicates). Quantity of water was calculated to apply high volume spray @ 250.0 L/ha^{-1} to give full coverage to the plots. When plants attained more than 90.0% maturity, 15 plants were randomly pulled out for data records. The data were recorded on the number of undamaged pod, number of damaged pods, undamaged pod %age and grain yield/15 plants. The grain $yield/ha^{-1}$ was also calculated for comparison with the control plot yield. The data were subjected to analysis of variance and Duncan's Multiple Range Test using MSTAT-C programme (Version 1.42) to indicate the significant differences among the treatments.

Bt field test on chickpea (year 2)

The experiment was sown on 30 November using chickpea variety ICC11514XILC482 in randomized complete block design with four replications. The plot size and the treatments details were the same as that used for the *Bt* field test on chickpea (year one).

The treatments were applied on 10 April (1st treatment), 18 April (2nd treatment) and 27 April (3rd treatment) as per our observation of larval infestation. The experiment was harvested on 20 May after pulling out 15/plants randomly from each replicate for data records and analysis on the same parameters as mentioned for the *Bt* field test on chickpea (year 1)

Bt field test on chickpea (year 3)

The experiment was initiated with chickpea variety ICC11514XILC482 sown on 16 November in randomized complete block design with four replications. Plot, treatments, and application technology was the same as mentioned for the field trial of year one. Treatments were applied on 09 April, 17 April and 24 April according to larval infestation. The experiment was terminated after pulling out 15 plants randomly from each of the replicates on 20 May at 90% maturity of the experimental plants. Data records and analysis were done on the same parameters as mentioned in the previous field experiments.

RESULTS AND DISCUSSION

Table I showed that the number of undamaged pods and percentage of undamaged pods in all the treatments of DiPel 2X® @1.6 Kg (51.2 BIU) + adjuvants and chemical insecticide (Karate®12.5g cypermethrin a.i./0.5 liter product) were significantly different ($p<0.05$) from the control. Grain yield/15 plants was the highest (83.0 g) in Karate® treatment. The grain yields in treatment of DiPel 2X® + adjuvant and Karate® was significantly different from the control. Calculated grain yield in Karate and DiPel 2X® + pottasium carbonate (K_2CO_3) treatments were 1822.0 Kg/Ha^{-1} (the highest) and 1535.0 Kg/Ha^{-1}, respectively. The grain yield was noted to be 2.0 to 2.5 times higher in all the DiPel 2X® + adjuvants treatments as compared to the grain yield (591.0 Kg/Ha^{-1}) of control. The overall results of the trial indicated that there was non-significant increase in grain yield due to incorporation of adjuvants; however, the use of commercial *Bt* preparation (DiPel 2X®) caused significant increase in grain yield (Table I).

Bt field test on chickpea (year 2)

Table I shows that the number of undamaged pods and percentage of undamaged pod in all the DiPel 2X ®@1.6 Kg (51.2 BIU)+ adjuvants treatments were significantly higher than the control pods and accordingly the grain yield/15 plants was also significantly higher than the control. The highest calculated grain yield (1157.0 Kg/Ha^{-1}) and (1022.0 Kg/Ha^{-1}) were noted in chemical insecticide and DiPel 2X®+ Powder milk (P. milk) treatments, respectively. Application of DiPel 2X® with adjuvants significantly increased grain yield 4-6 times as compared to control plot yield (171.0 Kg/Ha^{-1}) (Table I).

Bt. field test on chickpea (year 3)

Table I shows that the maximum percentage of undamaged pods (75.1 and 59.1%) was obtained in chemical insecticide (Karate®12.5g cypermethrin a.i./0.5 liter product) and DiPel 2X® @1.6 Kg (51.2 BIU) + 7.0% molasses treatments, respectively which was significantly different from the control (20.0%). The highest grain yield/15 plants was noted in Karate® (42.1 g/15 plants) and DiPel 2X® + K_2CO_3 (31.1 g/15 plants) which was significantly different from the control yield (9.3 g/15 plants). The overall calculated grain yield obtained in chemical insecticide (Karate®) and DiPel 2X® + K_2CO_3 were 934.0 and 693.0 Kg/Ha^{-1}, respectively being 3 to 4 times higher than the control grain yield (206.0 Kg/ha^{-1}).

Bt field tests conducted over 3 successive chickpea seasons indicated consistently a significant ($p<0.05$) increase in yield between the DiPel 2X® alone/DiPel® + adjuvant treatments and control. The grain yield in all the *Bt* treatments was at least 2 to 3 times higher than control plot yield (Table I). The application of microbial insecticide (DiPel 2X®)

Table I.- **Effect of DiPel 2X® (*Bt*-based bio-pesticide) with adjuvants (Calcium carbonate K_2CO_3, powder milk and molasses) on control of chickpea pod borer during three years of chickpea season (n=3).**

Treatment Kg or Liter/ha	Undamaged pods/15 plants	Damaged Pods/15 plants	Undamaged pod (%)	Yield/15 Plants	Yield (Kg/ha-1)
Year 1 of chickpea season					
DiPel 2X ®@1.6 Kg	230. 0 ab	26.0 b	89.9 a	67.3 a	1495
DiPel 2X ®@1.6 Kg +K_2CO_3(375.0 g)	225.7 ab	26.2 b	89.5 a	69.1 a	1535
DiPel 2X ®@1.6 Kg + P. Milk (2.5%)	231.4 a	27.3 b	88.7 a	66.2 a	1470
DiPel 2X ®@1.6 Kg + Molasses (7.0%)	201.1 ab	24.9 b	88.6 a	62.7 a	1392
Karate® (12.5g a.i.)	253.9 a	12.6 c	95.4 a	83.0 a	1822
Control	91.3 b	72.3 a	55.6 b	16.6 b	591
LSD	46.35	11.84	6.23	16.10	
S.E.	15.37	3.93	2.067	5.00	
CV(%)	14.96	24.9	4.72	16.03	
Year 2 of chickpea season					
DiPel 2X®@1.6 Kg	112.0 ab	70.0 b	61.9 b	34.3 a	762
DiPel 2X®@1.6 Kg + K_2CO_3 (375.0g)	131.5 a	76.8 b	62.5 b	35.8 a	796
DiPel 2X®@1.6 + P. Milk (2.5%)	157.5 a	57.3 bc	70.7 ab	46.0 a	1022
DiPel 2X® @1.6 + Molasses (7.0%)	116.0 ab	53.8 bc	62.6 b	36.4 a	808
Karate® (12.5ga.i.)	163.0 a	42.5 c	78.7 a	52.1 a	1157
Control	26.25 b	115.5 a	17.16 c	7.7 b	171
LSD	90.39	24.16	11.71	26.35	
S.E.	29.99	8.01	3.89	8.74	
CV%	50.95	23.13	13.18	49.45	
Year 3 of chickpea season					
DiPel 2X® @1.6 Kg	128.3 b	110.5 b	53.5 b	25.3 b	562
DiPel 2X® @1.6 Kg + K_2CO_3 (375.0g)	127.0 b	96.7 b	56.5 b	31.1 b	693
DiPel 2X® @1.6 Kg + P.milk (2.5%)	145.0 b	102.8 b	58.5 b	23.0 b	511
DiPel 2X @ 1.6 Kg + Molasses (7.0%)	148.8 b	102.0 b	59.1 b	29.6 b	657
Karate® (12.5g a.i.)	207.3 a	67.50 c	75.1 a	42.1 a	935
Control	45.50 c	177.8 a	20.0 c	9.3 c	206
LSD	38.65	21.07	7.95	6.05	
S.E	12.83	6.99	2.64	2.01	
CV(%)	19.19	12.76	9.81	14.40	

Means followed by the same letters are not significantly different at $p < 0.05$

@ 1.6 kg Ha^{-1} (51.2 BIU Ha^{-1}) alone or with adjuvants costs more than the cost of chemical insecticides application in sub-tropical climate at temperature ranges between 16 to 34°C.

The idea behind the use of adjuvant (heavy organic matters other than K_2CO_3) was to increase the *Bt* persistency. There was noted an inconsistency in grain yield of chickpea in treatments of DiPel 2X + adjuvants as compared to the treatment of DiPel 2X alone (Table I).

Salama (1989) obtained highest soybean yield after treatment with *Bt* + potassium carbonate against infestation of *Spodoptra littoralis* (Boisd.). Kulkarni and Amonkar (1988a) reported reduced larval population of *H. armigera* infesting chickpea following treatment with *B.t.* but they observed no effect on chickpea yield. As far as yield of chickpea is concerned, our observations did not correspond with the findings of Kulkarni and Amonkar (1988b) for the reason that we observed significant increase in the yield of chickpea as compared to control plot yield during the three years of field trials.

There have been cases in which small–scale experimentation with microbial insecticides did not reflect their effectiveness as to large scale commercial field application (Beegle *et al.*, 1982).

Broza *et al.* (1984) reported successful control of *S. littoralis* by *Bt* application on cotton for 2 seasons. Ahmed *et al.* (1994) tests of *Bt* for 3 consecutive seasons on chickpea crop and reported that DiPel® 2X and DiPel ES® at the rate of 1.6 Kg Ha^{-1} and 1.5 liters/Ha (with and without molasses), respectively, caused significant increase in grain yield as compared to control plots and the present results also agreed with the report of Ahmed *et al.* (1994). Abbas and Young (1993) reported insignificant difference between the larval mortalities caused by Javelin WG ® (*Bt*) at 4.48 kg ha^{-1} and cyprmethrin at 0.05 kg (AI) ha^{-1} in the cotton field against *H. virescens* than *H. zea*. Puntambekar *et al.* (1997) reported that application of *Bt* var. *kurstaki* (NCIM2514) @ 10^{10} spores/ml concentration was found effective against *H. armigera* infesting pigeon pea and gave 1.5 times more grain yield as compared with control.

Bt field test results indicated that microbial insecticides can be used (with and without adjuvants) for management of *H. armigera* populations infesting chickpea and their use would reduce reliance on toxic chemical released into the agro-ecosystem of Pakistan, *e.g.* soil toxicity, phytotoxicity, air-pollution, toxicity to mammals and birds. This study further application of Bt-based biopesticide on large scale to provide an alternate tool which eco-friendly approach as part of an IIMP programmes.

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