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Field Evaluation of *Bacillus thuringiensis*, Insect Growth Regulators, Chemical Pesticide Against *Helicoverpa armigera* (Huber) (Lepidoptera: Noctuidae) and Their Compatibility For Integrated Pest Management

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Abstract

A large number of *Bacillus thuringiensis* (Bt) isolates separated from samples collected from different economic environments in Pakistan were characterized for Crystal Protein gene (*cry*) composition and pesticidal activity against devastating polyphagous pest, *Helicoverpa armigera*. Isolates harboring combinations *cry*2A genes were found me efficacious against the target pest. One isolate designated as PR17.4 (CEMB Bt) exhibited high levels of toxicity again *H. armigera* in lab biotoxicity assays and was therefore, chosen as a potential candidate in integrated Pest Management (1PM) strategies to control this notorious pest of valuable crops. Field efficacy of home grown cells CEMB Bt, PR17.4, Agr (50 WP), was compared with a commercial BT formulation of Novartis, growth regulators, Match (50 EC), Insegar (25 W and a popular chemical pesticide, Curacron (500 EC) to investigate their effectiveness to control *H. armigera* in potato crop Insegar was not significantly effective against the target pest. Locally isolated CEMB Bt was found to be as effective the commercial Bt and chemical control agent of Novartis. A synergistic effect was also observed among, Match/Agree CEMB Bt/Match, Match/Curacron and Agree/Curacron combinations. These studies suggest that CEMB Bt alone or combination with other biological or chemical based pesticides can be safely recommended for pest management strategy against *H. armigera* with no obvious harmful effects on its predators as is the case with chemical insecticides.

Introduction

Helicoverpa (Heliothis) *armigera* (Huber) is a polyphagous pest of agronomically important agricultural cash crops (Rehman, 1940; Pearson, 1958; Bilapate, 1984; Reed and Pawar, 1982; Zalucki *et al*., 1986). It is known as gram caterpillar or pod borer, cotton American bollworm, corn earworm and tomato fruit worm (Hsu *et al*., 1965; Metcalf *et al*., 1951; Lea, 1982; Pearson, 1958). It attacks a variety of agricultural crops, being a major pest on pulses, sunflower, potato, tomato, pea, wheat, maize tobacco, Lucerne and other crops (Ahmad, 1989). This species is widely distributed from the Pacific to Australia, throughout Southeast and South Asia from the middle east and southern Europe to Africa (Pearson, 1958; CAB, 1968). Damage is frequently localized on the nitrogen-rich reproductive plant parts and thus influences yield directly. Farmers increasingly rely on synthetic insecticides to manage this pest in different crops (Shanower *et al*., 1998). This has increased the risk of environmental contamination and loss of biodiversity and has contributed to the development of insect resistant *H. armigera* populations (Forrester *et al*., 1993; McCaffery *et al*., 1988; McCaffery *et al*., 1991; Armes *et al*., 1992; Xia, 1993; Ahmad *et al*., 1995, 1997), resulting in a urgent need for alternative insect control strategies. Besides causing resistance in pests, chemical pesticides are expensive to farmers, have adverse effects on the environment and cause health hazards (Balk and Koeman, 1984). Moreover, pesticides cause destruction of natural enemy complexes and hence disrupt the natural balance that often exists between pests and their natural enem (Ehler *et al*., 1973; Eveleens *et al*., 1973).

Considerable effort has been directed toward effective, and economically acceptable alternatives. Some new technologies have been implemented while others are some emerging. The major trend, however has been toward to use of biotechnology to replace traditional pesticides with less hazardous chemicals or biologically based products are nontoxic. In particular, much research has been direct toward the so called insect growth regulators such juvenile hormone analogues (JHA) and chitin synthe inhibitors (CSI). Lufenuron is a novel, highly active chitin synthesis inhibitor that disrupts moulting in several inects species (Schenker and Moyses, 1994). Nevertheless, there is little information available about the effectiveness of the compound against crop pests.

Formulations of *Bacillus thuringiensis* Berliner have been both used for more than two decades as biological insectics (Hofte and Whiteley, 1989); during this period, use insect growth regulators has been reported (Schaefer *et al*., 1987). Although the use of insect pathogens, inects regulators and chemical pesticide alone and in combination is not a new idea, the development of microbial production has been limited in the past (Federici and Maddox, 1996) *Bacillus thuringiensis*, commonly known as Bt, is a gram positive bacterium that occurs naturally in the soil around the world (Martin and Travers, 1989; Meadows, 1993). Few decades, it has been known that some strains of Bt certain insects and the toxic substance responsible for insects death is a protein called 5-endotoxin (Angus, 1954;

Table 1: Insect control agents evaluated for sensitivity to *H. armigera*

Common Name	Formulation	Trade Name	Chemical Name
Curacron	500 EC	Profenos	0-(4-bromo-2-chloropheny1)0-ethyl-S-n-propylPhospho-rothiote.
Match	50 EC	Lufeburon	N-[[2-5-dichloro-4-(1 ,1,2,3,3,3-hexafluoro-benzamide).
Insegar	25 WP	Fenoxycarb	Ethyl[2-(4-phenoxyphenoxy)ethyl]Carbamate)
Agree	50 WP	*B. Thuringiensis*	*Bacillus thuringiensis kurstaki*
CEMB Bt	5 µg/mg active toxin	*B. Thuringiensis*	*Local Bacillus thuringiensis kurstaki*

Table 2: An outline about the Insect control agents, their application doses and H. armigera egg and larval population density in the experimental plots before treatments

Plot No.	Insect control agent	Dose rate (g/h)	Number of eggs	Number of larvae
1	Control	0	61	14
2	Match	400	49	26
3	Match	600	66	26
4	Insegar	300	69	29
5	Insegar	400	63	24
6	Agree	1000	63	49
T	Agree	1500	61	33
8	CEMB Bt	1000	83	4
9	CEMB Bt	1500	91	18
10	Agree and Match 600 and	300	33	14
11	CEMB Bt and match 600 and	300	40	16
12	Agree and curacron 600 and	1000	68	13
13	Match and curacron 300 and	1000	64	19
14	Curacron	2000	50	30

Table 3: Efficacy of different treatments on the young and old larval population of *H. armigera* (n = 15)

Treatments	Dose (9/h)	7DAA(1) Mean ± SE	Standard deviation	7DAA(2) Mean ± SE	Standard deviation	7DAA(3) Mean ± SE	Standard deviation
Young Larval							
Check	0	0.62 ± 0.07a	0.27	0.46 ± 0.08a	0.33	0.53 ± 0.07a	0.26
Match	400	Ob	0	Ob	0	Ob	0
Match	600	Oh	0	Ob	0	Ob	0
Insegar	300	0.14 ± 0.68b	0.26	0.18 ± 0.08	0.31	0.18 ± 0.08b	0.31
Insegar	400	0.04 ± 0.03b	0.12	Ob	0	Oh	0
Agree	1000	0.04 ± 0.04b	0.17	Oh	0	Ob	0
Agree	1500	0.12 ± 0.07b	0.30	0.46 ± 0.04b	0.17	Oh	0
CEMB-Bt	1000	0.02 ± 0.01b	0.06	Oh	0	Oh	0
CEMB-Bt	1500	Ob	0	Ob	0	Ob	0
Agree/Match	600x300	Ob	0	Oh	0	Ob	0
CEMB-Bt/Match	600x300	0.02 ± 0.02b	0.07	0	Ob	0	Ob
Agree/Curacron	600x100	0.03 ± 0.02b	0.08	Ob	0	Ob	0
Match/Curacron	300x1000	0.07 ± 0.05b	0.20	Ob	0	Ob	0
Curacron	2000	0.01 ± 0.01b	0.07	0.04 ± 0.04b	0.17	Ob	0
Old larval							
Check	0	0.65 ± 0.07	0.03	0.62 ± 0.04	0.17	2.12 ± 4.17a	1.61
Match	400	Ob	0	Ob	0	Ob	0
Match	600	Ob	0	0.04 ± 0.04b	0.17	Ob	0
Insegar	300	0.09 ± 0.06b	0.24	0.18 ± 0.08b	0.31	0.23 ± 0.08b	0.33
Insegar	400	0.27 ± 0.09b	0.35	0.04 ± 0.04b	.17	0.b	0
Agree	1000	0.13 ± 0.07b	0.28	0.04 ± 0.04b	0.17		0
Agree	1500	0.09 ± 0.06b	0.24	Ob	0	0.b	0
CEMB-Bt	1000.	Ob	0	Ob	0	0.b	0

CEMB-Bt	1500	Ob	0	Ob	0	0.b	0
Agree/Match	600x300	0.02 -±0.02b	0.07	Ob	0	0.b	0
CEMB-Bt/Match	600x300	Ob	0	Ob	0	0.b	0
Agree/Curacron	600x100	Ob	0	Ob	0	0.b	0
Match/Curacron	300x1000 Ob	Ob	Ob	0	0.b	0.b	
Curacron	2000	0.04±0.04b	0.04	Ob	0	0.b	0

Mean in the same column bearing different common letter are significantly different ($P < 0.001$)

Hannay, 1953). When certain susceptible insects ingest either the bacterium or the protein produced by the bacterium, their digestive system is disrupted, resulting in their eventual death (Mathavan *et al*., 1989). Mode of action of Bt δ-endotoxins consist of ingestion, solubilization, proteolytic activation in some cases, receptor binding, membrane insertion, ion channel formation and cell lysis (Karim and Riazuddin, 1997). Bt proteins were the first to be used as insect control agent (Husz, 1928), highly toxic to insect pest and Bt δ-endotoxins are generally safe to vertebrate, beneficial arthropods (Franz *et al*., 1980; Hassan, 1983; Hassan *et al*., 1983; Oatman *et al*., 1983; Flexner *et al*., 1986; McDonald *et al*., 1990).

Here, we report the field efficacy of synthetic and biologically based pesticides (*Bacillus thuringiensis* and growth regulators) for control of *Helicoverpa armigera* on potato crop. This study shows the results of insect control agents for control of *Helicoverpa armigera*.

Materials and Methods

Insect control agents: Curacron (500 EC), profenofos (Table 1) belongs to a WHO class 111 organophosphate is commercially available in 500 g/l packages. Match (50 EC), Lufenuron (Table 1), 50 g/l is an insect growth inhibitor (IGI). Insegar (25 WP), Fenoxycarb (Table 1) is an insect growth regulator (IGR) for use against lepidopteran and scale insects. The active ingredients of Agree (50 WP) (Table 1), *Bacillus thuringiensis* var. *Kurstaki*, is δ-endotoxin that is a stomach poison. Local Bacillus thuringiensis isolate PR17.4 (CEMB Bt) was isolated and used for these studies (Khan *et al*., 1995; Rubi, 1994). Commercial preparations of Curacron, Match, Insegar and Agree were generous gifts from Novartis, Pakistan (Table 1).

Determination of protein concentration: An equal amount 150 mg) of both Agree and CEMB Bt dry formulations were taken and dry powder was resuspended in 5 ml of alkalic buffer (50 mM Sodium carbonate, 10 mM dithiothreitol, pH 9.5) and incubated at 37°C for 4-5 h with continuous shaking. Protein concentration of both solubilized proteins of Bt formulations was measured using the method of Bradford (Bradford, 1976) to use equal amounts of active ingredients (δ-endotoxin) for plot treatments.

Study area: The present study was conducted in an area near the Pakistan/India border. A typical traditional potato growing area, Mouza Karwarr is 20 km east of Lahore (Provincial capital of Punjab, Pakistan). The mean monthly temperatures in the area range from 18°C in October to 10°C in December (data from Pakistan Meteorological Office, Lahore). The study was conducted in the month November-December, 1993, when the natural infestation was above economic injury level in the potato plots. In the experimental plots, the potato crop was fully grown and covered most of the area on the furrows. During the past several years, potato has been traditionally, the main short duration crop between rice and wheat crops.

Insecticide treatments: An area of 3450 m^2. Each plot had 10 rows that were 30 m long. The potato crop was sown during October, 1993. A pretreatment sampling was carried out on November 15, 1993. A pretreatment sampling was carried out on November 15, 1993 to determine the natural infestation level of *Helicoverpa armigera* in the potato plots. Each plant was naturally infested with an average of 1.51 larvae and 12 eggs per potato plant.

The first treatment of plots with *Bacillus thuringiensis* based formulations and chemical pesticides was done on the same day in the evening hours after one hour of pest scouting, Treatments were applied in a completely random design (Cochran and Cox, 1950). One plot was left untreated and served as control. The insecticide formulations which were used are as follows. Curacron (500 EC), Match (50 EC), Insegar (25 WP), AgreeB (50 WP) and CEMB Bt based on locally isolated *Bacillus thuringiensis* PR17.4. The required amount of the material (Table 2) for each plot was stirred in water taken from the irrigation channel and sprayed at a rate of 7 liters per plot with a hand operated compressed air knapsack sprayer. A total of three spray applications were made every week. Equal amounts of active ingredients based on solubilized crystal proteins were applied in the case of Agree and the Local Bt. The efficacy of the treatments was determined by examining and recording the number of young larvae (neonatal to 3rd instar larvae), older larvae (3rd instar to prepupal stage larvae) and eggs per 15 plants per plot before any treatment.

Statistical analysis: All the data were subjected to analysis of variance using (IRRISTAT 4,0-SAS Institute)(IRRI, 1997) and T-test was applied to determine the significance of difference between mean values.

Results

Ecological conditions: During this study in 1993, the weather was often cool, dry and cloudy with temperature highs ranging from 20-25°C and lows ranging from 8-12°C. No rainfall occurred during the spray applications.

Assessment of *H. armigera* mortality: Insect pest population of *H. armigera* on potato crop was considerably high in our experiments (Table 2). Average numbers of eggs and larvae (both young and older) found per plant in pre-treatment plot sampling were 1.51 larvae (young, 1.15 and older 0.36 larvae) and 12 eggs. After 1st spray application, fewer small larvae were found on potato sprayed with *B. thuringiensis* formulations than on untreated plants. No significant differences were observed among formulations containing Bt, IGR, IGI and chemical pesticide. After the 3rd spraying, however, the population of *H. armigera* (both young and older larvae) was totally eradicated from the potato field treated with formulations except in the plot sprayed with insegar (Table 3). Bt in combination with Match, greatly affected the pest population. Bt treated plots had a significantly greater density of predators than chemical pesticide treated plots at the end of the third spray application. Results as listed in Table 3 showed the difference in results of insegar treatments as compared with other insecticides. There was significant difference in rriortality between insegar and Bt treated plots.

Match (Insect growth Inhibitor): Two doses (400 and 600 g/h) of insect growth inhibitor were tested (Table 2). The rates of mortality after three successive applications were significantly different from check (Table 3). As evident from the mode of action, this pesticide interferes with the chitin synthesis of arthropods. It seems that *H. armigera* larvae are susceptible to both doses of Match and the treated plots showed the significant effect on pest population. The predator population in Match treated plots was significantly high after the second spray application; that is an indication of its being safe to non target organisms.

Insegar (Insect growth regulator): This particular insect growth regulator has a translaminar activity and can disrupt the transformation of egg to larvae and larvae to pupae. In both treated plots, insegar did not show any significant difference in mortality as compared with Match. *H. armigera* is not very susceptible to this growth regulator (Tables 3) and higher doses may be required to eradicate this pest from the plot. The predator population in the insegar treated plots was also considerably high after 3rd application that is also an indication insegar is safe to non target organisms.

Curacron (Organophosphate): The chemical pesticide most widely used to control *H. armigera* in Pakistan was used as positive control. Only one dose, 2000 g/h as recommended by the manufacturers, was used to control the target pest. Curacron eradicated significantly the population of *H. armigera* after three successive applications. There was not a single predator in the Curacron treated plot; this shows the pesticides broad spectrum effectiveness.

Agree (Commercial *Bacillus thuringiensis* formulation): Two doses of agree as recommended by the manufacturers was applied for the control of *H. armigera*. There was a significant response to both doses in terms of the eradication of the target pest. After the 2nd application, an increase in the population of predators was observed. The effectiveness of Agree was comparable with that of chemical pesticides statistically.

CEMB Bt (Local *Bacillus thuringiensis* isolate): Two doses of CEMB Bt were applied to test the effectiveness of microbial pesticide. After three applications, the pest population was significantly suppressed in the plots and was comparable with results of other plot treatments (Table 2). The predator and parasite population were enhanced after the 2nd application. That shows, locally CEMB Bt has no adverse effect on non target organisms in the field. CEMB Bt activity was significantly comparable with other applied pesticides like Curacron, Match and Agree.

Agree and Match: To understand and test the effectiveness of both Agree and Match, dose combinations (600/300 g/h) were checked. Data showed a synergistic effect of both pesticides (Table 3). A combination dose eradicated the population of target pest significantly after three applications without any adverse effect on the predators on the plots.

CEMB Bt and Match: The results of a combination dose of CEMB Bt and Match also showed a significant suppression of the pest population after three applications on the plot (Table 2). Predator, parasite and non-target organism populations was also observed in the treated plot. This shows both pesticides have synergistic effect on the pest population.

Agree and Curacron: Another combination of pesticides, microbial and chemical was assessed to see the effectiveness of both control agents when applied in the field application. Both pesticides did not show any adverse effect on each other and a synergism was seen in this plot (Table 3). Furthermore, no predator/parasite or non target organism populations were observed on this plot.

Match and Curacron: This combination was also found to be very effective in eradicating the pests. Both pesticides did not show any effect on each other and synergised at very low doses. No non target organisms were found in this plot.

Discussion

We did a research to study the field efficacy and potential of insect control agents based on *Bacillus thuringiensis*, IGR, !GI, individually and in combination with each other to totally eliminate or reduce the pest population of *H. armigera* from the potato crop. The results of different formulations individually and in combinations are listed in Table 3. Microbial pesticides such as those based on *Bacillus thuringiensis* var. *Kurstaki* exhibited residual activity when applied as foliar sprays. Formulation ingredients and application timing may be able to counter their effects on the environment by offering protection from sunlight, rain, or both.

Residual activity of *Bacillus thuringiensis* has long been a source of discussion among pest management decision makers. Loss of activity to rain is a problem related to almost all pesticides, including Bt; but loss of activity caused by sunlight is a well known characteristic of Bt, other insect pathogens and certain chemical pesticides (Ignoffo and Hostetter, 1977). A limitation in the use of Bt formulation is the deactivation of crystals within a few days on foliage (Pinnock *et al*., 1971; Addison, 1993). Sunlight irradiation was shown to cause tryptophan destruction in protein crystals of Bt var. *Kurstaki* HD-1 and NR0-12 (Pozsgay *et al*., 1987).

Timing of the biopesticide application and larval behavior are crucial for the effective control of target pests (Ghidiu and Zehnder, 1993). We have observed that evening hours are the best time to spray Bt formulations; that can affect the longevity of Bt in the field for nocturnal pests like *H. armigera* in the long winter nights. We noted during this experiment thet all larval stages were feeding primarily on the upper side of the leaves of the potato crop. This is an additional advantage for foliar spray and its residual activity. This characteristic may limit the usefulness of Bt formulations, these are applied on crops of long days and short nights like cotton. Residual activity of foliar deposits of Bt and other entomopathogen formulations currently under commercial development needs improvements to gain wide scale acceptance by growers and consultants making pest management decisions.

In our study, the use of Agree, CEMB Bt, Insegar or Match did not reduce or eliminate the natural enemy population, plots treated with them had as many predators as the control plot. Bt based pesticides significantly eliminated all stages of *H. armigera* after its 3rd application, A possible explanation for the total eradication of target pests could be the synergism among outbreak of predatory arthropods with biopesticide since the biopesticide did not show any harmful effect on predatory population in our trials.

Our results seem to indicate that the use of microbial pesticides has increased the population density of predators and parasites. Microbial pesticides in conjunction with natural predators and parasites can synergies each other and even enhance the effectiveness of biopesticides in the field. It further strengthens our conclusion, that if population of natural enemies had not been deliberate reduced by the chemical applications then it is likely that greater suppression of *H. armigera* larval population has been achieved due to natural predators or parasites. The difficulty with chemical pesticides in combination with biopesticide is that natural predators and parasites are very sensitive to many conventional chemical pesticides (Bull *et al*., 1979). The development of indigenous Bt strains mass result in better control than imported strains. Thus, isolatic and identification of local strains of Bt and screening that for appropriate pesticidal properties would enhance the chances of Bt for success. The genetic diversity of Bt insecticidal genes and their activities has yet to be full exploited and, therefore, provides unique opportunities for biotechnology. The availability of a large number of divers: Bt toxins will enable better management of pest resistance broaden the host range and also allow the design of chimeric toxins - the philosophy of our Bt program CEMB. Experimental results of this study support sever important conclusions that *B. thuringiensis* base biopesticides tested alone or in combination with other biologically based pesticides were more effective and can replace or reduce the usage of chemical pesticides.

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