

The efficacy of *Beauveria bassiana*, jasmonic acid and chlorantraniliprole on larval populations of *Helicoverpa armigera* in chickpea crop ecosystems

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Abstract

BACKGROUND: A robust integrated pest management (IPM) programme is needed to reduce the use of insecticides in controlling *Helicoverpa armigera*. Therefore, a 2 year field study was conducted to evaluate the use of alternative control measures (biochemical use) for *H. armigera* relative to exclusively using chemical insecticides. The entomopathogenic fungus *Beauveria bassiana*, jasmonic acid and the insecticide chlorantraniliprole were each applied twice during the chickpea growing season.

RESULTS: All three applied materials (either alone or combined) significantly ($P \leq 0.05$) reduced the larval population of *H. armigera* and pod infestation. Effects increased with time, and the maximum difference was observed 7 days after the second application in each year. The lowest numbers of larvae per plant and pod infestation were in the *B. bassiana* 3.21×10^6 + chlorantraniliprole treatment in both 2009/2010 and 2010/2011 year. The reduction in the larval population and pod infestation increased chickpea yield and the highest yield in both seasons, and the maximum yield was obtained in the *B. bassiana* 3.21×10^6 + chlorantraniliprole treatment. The populations of natural enemies were highest in the jasmonic acid treatment.

CONCLUSION: The results suggest that *B. bassiana*, jasmonic acid and chlorantraniliprole may be useful components for the *H. armigera* IPM strategy.

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Keywords: integrated pest management; chickpea pod borer; ecotoxicology; biochemical insecticide; *Helicoverpa armigera*

1 INTRODUCTION

Chickpea pod borer (*Helicoverpa armigera* H.) is a serious and devastating insect pest of chickpea plants (*Cicer arietinum*), affecting both quality and yield.¹ A single *H. armigera* larva is capable of destroying upwards of 30 pods before it reaches maturity.² A number of strategies have been developed to control *H. armigera*, including the wide use of synthetic insecticides. Widespread use of synthetic insecticides has resulted in *H. armigera* populations developing resistance, and they also adversely affect natural enemies.³ Therefore, a robust integrated pest management (IPM) programme is needed to reduce the use of insecticides in controlling *H. armigera*.

Entomopathogenic fungi are natural pathogens of insects that can function as microbial insecticides and are often included as components of IPM programmes.⁴ Most entomopathogenic fungi belong to the new division Hyphomycetes, i.e. Deuteromycota, which includes the important genera and species *Beauveria bassiana* (Balsamo) and Vuillemin (Ascomycota: Hypomycetes).^{5–8} *B. bassiana* is a well-known, naturally occurring and environmentally safe biological control agent.⁹ After contacting a larval or

pupal insect, the fungal spores penetrate the chitinous integument and other visceral organs of the body.^{5,10} Once penetration occurs, the fungal spores produce hyphal bodies which degrade the insect's fat and gut tissue, resulting in destruction of the malphagian tubules.

Jasmonic acid (JA) is a plant hormone that influences both stress responses and development.^{11,12} Specifically, JA has a major

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role in regulating elements of plant growth such as leaf senescence, seed germination, seedling growth, root growth, photosynthesis, fruit development and ripening.^{12,13} When plants are attacked by insects, they respond by releasing JA, which leads to a decrease in preference, performance and abundance of many common herbivores. This is partly accomplished with a reduction in the digestibility of plant tissues.^{14,15} Owing to these numerous effects, JA is often used as a component in IPM regimes.

Chlorantraniliprole is a recently developed insecticide with a novel mode of action as an activator of insect ryanodine receptors, which causes rapid muscle dysfunction and paralysis.^{16–18} Chlorantraniliprole application to plants has been shown to reduce the amount of feeding damage from pest insects while purportedly presenting minimal risk to non-target arthropods, such as parasitoids, predators and pollinators.^{17,19} These properties make chlorantraniliprole a promising component of IPM programmes.^{20,21}

In this 2 year study, we assessed the efficacy of *B. bassiana* (Balsamo), JA and chlorantraniliprole on *H. armigera* in chickpea crops. The objectives of this study were: (1) to determine the efficacy of *B. bassiana*, JA and chlorantraniliprole against *H. armigera*; (2) to quantify the population dynamics of *H. armigera* and its natural enemies in chickpea crops treated with these agrochemicals; (3) to develop a strategy for minimising the use of hazardous insecticides and reduce associated costs.

2 MATERIALS AND METHODS

2.1 Experimental site and field trials

This research work was conducted in the research area of the Department of Agricultural Entomology, University of Agriculture Faisalabad, Pakistan. A randomised complete block design was used in both the 2009/2010 and 2010/2011 seasons to evaluate the effects of various treatments on *H. armigera* larval populations, pod infestation, natural enemies and chickpea yield. Chickpea seeds (variety Noor-91) were sown on the 1 November 2009 and 4 November 2010 with row-to-row and plant-to-plant distances of 30 and 15 cm respectively. The water regime was managed throughout the chickpea growing season and the crop was kept weed free by hand weeding throughout the crop stand. Urea, diammonium phosphate and potassium sulphate were applied at rates of 20, 100 and 60 kg ha⁻¹ respectively. Three parallel blocks as three replicates, each comprising seven plots, were established in both 2009/2010 and 2010/2011 cropping seasons. Each plot size was 2 × 3.5 m and was separated from other plots by 0.5 m broad strips.

2.2 Pesticide applications

Details of all insecticide applications performed for this study are provided in Table 1. JA was dissolved in distilled water to obtain the desired concentration. The desired amount of *B. bassiana* conidia was put into distilled water to initiate their activity. All the treatments were applied to each plot according to a randomised complete block design. Two applications were performed using a backpack sprayer (Jacto-PJH). The first spray was applied at the first *H. armigera* appearance (about 1 month following crop emergence), while a second spray was applied 15 days after the first application. Timing was identical in both seasons. All applications were applied with a volume of 200 L ha⁻¹ and while walking at normal speed.

Table 1. Treatments applied in the study

Treatment code	Name of treatment	Formulation/active ingredient application rate ha ⁻¹
T ₁	<i>B. bassiana</i> (AgriLife Ltd, India)	3.21 × 10 ⁴ conidia mL ⁻¹
T ₂	<i>B. bassiana</i>	3.21 × 10 ⁶ conidia mL ⁻¹
T ₃	Chlorantraniliprole (DuPont, Pakistan)	100 mL ha ⁻¹ (active ingredient: 18.4% + other ingredients 81.6%)
T ₄	Jasmonic acid (Sigma-Aldrich, USA)	1.2 mM
T ₅	<i>B. bassiana</i> + chlorantraniliprole	3.21 × 10 ⁴ conidia mL ⁻¹ + 100 mL
T ₆	<i>B. bassiana</i> + chlorantraniliprole	3.21 × 10 ⁶ conidia mL ⁻¹ + 100 mL
T ₇	Control	–

2.3 Sampling

The larval population was estimated from the numbers of alive and dead larvae counted in seven randomly selected plants per plot. Sampling was conducted 1 day prior to each application, and then 1, 2, 3 and 7 days post-application (DPA). Additionally, pods were monitored for infestation by counting the total number and damaged pods (with a bore hole) from seven randomly selected plants in each treatment at 1, 2, 3 and 7 days after each spray application.

2.4 Data analyses

Pod infestation ratio was calculated as follows:

$$\text{Pod infestation (\%)} = \frac{A}{B} \times 100$$

where *A* is the number of damaged pods per plant and *B* is the total pods per plant (damaged + undamaged). The population of natural enemies (green lacewings, mites, spiders and beetles) was monitored from seven randomly selected plants in each treatment plot at 1, 2, 3 and 7 days after the second application of treatments. The crop was harvested from each plot separately. Chickpea yield from each treatment plot was recorded, converted to kg ha⁻¹ and analysed without transformation. The individual plot yield was observed to check the effectiveness of treatments.

The cost benefit ratio (CBR) was calculated as follows:

$$\text{Cost benefit ratio} = \frac{B_t / (1 + i)^n}{C_t / (1 + i)^n}$$

where *B_t* is the benefit, *C_t* is the cost (including the cost of pesticides and the cost of application), *n* is the number of seasons and *i* is the interest rate.²² To determine the most economically efficient treatment, the percentage increase in yield of each treatment over the control was calculated by the formula

$$\frac{T - C}{C} \times 100$$

where *T* is the yield of the treatment plot and *C* is the yield of the control plot.

Data were tested for normality using the Shapiro–Wilk's test.^{23,24} Statistically significant differences among the treatments were identified by ANOVA and Tukey's *post hoc* tests. All the statistical

Table 2. Numbers of *H. armigera* larvae per plant (mean \pm SD) after first and second spray applications in 2009/2010

Treatments ^a	First application ^b		Second application ^b	
	Pretreatments	7 DPA	Pretreatments	7 DPA
T ₁	2.42 \pm 0.16 a	1.73 \pm 0.08 b	2.04 \pm 0.12 b	1.28 \pm 0.07 b
T ₂	2.38 \pm 0.28 a	1.47 \pm 0.12 bc	1.90 \pm 0.09 b	1.14 \pm 0.08 b
T ₃	2.95 \pm 0.12 a	1.02 \pm 0.07 cd	1.66 \pm 0.20 b	0.52 \pm 0.12 c
T ₄	2.57 \pm 0.32 a	1.95 \pm 0.13 b	2.23 \pm 0.12 ab	1.57 \pm 0.08 b
T ₅	2.80 \pm 0.20 a	0.92 \pm 0.12 cd	1.61 \pm 0.25 b	0.38 \pm 0.13 c
T ₆	2.59 \pm 0.31 a	0.80 \pm 0.12 d	1.52 \pm 0.31 b	0.28 \pm 0.18 c
T ₇	2.28 \pm 0.29 a	3.04 \pm 0.17 a	3.14 \pm 0.08 a	3.85 \pm 0.08 a

^a T₁ = *B. bassiana* 3.21 \times 10⁴ conidia mL⁻¹; T₂ = *B. bassiana* 3.21 \times 10⁶ conidia mL⁻¹; T₃ = chlorantraniliprole 100 mL ha⁻¹; T₄ = JA 1.2 mM; T₅ = *B. bassiana* 3.21 \times 10⁴ conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₆ = *B. bassiana* 3.21 \times 10⁶ conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₇ = control.
^b Letters following means indicate statistical differences within a column ($P \leq 0.05$).

Table 3. Numbers of *H. armigera* larvae per plant (mean \pm SD) after first and second spray applications in 2010/2011

Treatments ^a	First application ^b		Second application ^b	
	Pretreatments	7 DPA	Pretreatments	7 DPA
T ₁	3.46 \pm 0.13 ab	2.52 \pm 0.20 bc	2.87 \pm 0.66 ab	1.61 \pm 0.12 bc
T ₂	3.38 \pm 0.12 ab	2.36 \pm 0.14 bc	2.71 \pm 0.57 ab	1.42 \pm 0.08 bc
T ₃	3.92 \pm 0.36 a	1.61 \pm 0.20 cd	2.52 \pm 0.26 ab	0.96 \pm 0.16 c
T ₄	3.54 \pm 0.15 ab	2.80 \pm 0.21 b	3.19 \pm 0.17 ab	2.37 \pm 0.05 c
T ₅	3.29 \pm 0.15 ab	1.40 \pm 0.13 d	2.21 \pm 0.12 b	0.71 \pm 0.14 c
T ₆	3.24 \pm 0.31 b	1.29 \pm 0.12 d	2.05 \pm 0.34 b	0.42 \pm 0.24 c
T ₇	3.23 \pm 0.17 ab	4.34 \pm 0.26 a	4.57 \pm 0.43 a	5.23 \pm 0.56 a

^a T₁ = *B. bassiana* 3.21 \times 10⁴ conidia mL⁻¹; T₂ = *B. bassiana* 3.21 \times 10⁶ conidia mL⁻¹; T₃ = chlorantraniliprole 100 mL ha⁻¹; T₄ = JA 1.2 mM; T₅ = *B. bassiana* 3.21 \times 10⁴ conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₆ = *B. bassiana* 3.21 \times 10⁶ conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₇ = control.
^b Letters following means indicate statistical differences within a column ($P \leq 0.05$).

analyses were performed using SPSS for Windows, v.16.0 (SPSS Inc., Chicago, IL).

3 RESULTS

3.1 *H. armigera* population

In 2009/2010, the largest population of larval *H. armigera* was observed in the control plot, and the population increased throughout the cropping season (Table 2). Application of all treatments significantly ($P \leq 0.05$) reduced larval population size with time (at 1, 2, 3 and 7 DPA) (supporting information Table S1) as compared with the control. Reduction in the larval population after the second spray application was more pronounced ($F = 33.58$, $P \leq 0.001$) relative to the first application ($F = 14.24$, $P \leq 0.001$). The *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment was most effective in reducing per plant larval populations. The average larval population per plant in the *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment was 0.80 and 0.28 at 7 DPA after the first and second spray applications respectively. This corresponds to 69.11 and 81.58% reductions at 7 DPA relative to the pretreatment population size, and 73.68 and 92.73% less than the control plots.

Similarly to 2009/2010, the larval population in 2010/2011 was highest in the control plot and the application of treatments significantly reduced the number of larval *H. armigera* (Table 3, supporting information Table S2). The highest reduction in larval *H. armigera* was observed in the *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment following both the first ($F = 13.54$, $P \leq 0.001$) and second ($F = 57.56$, $P \leq 0.001$) spray applications. At 7 DPA, the application of *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole reduced the per plant larval populations by up to 60.19 and 79.51% relative to pretreatments, and 70.28 and 91.97% as compared with the control plots.

3.2 Pod infestation

In 2009/2010, pod infestation was significantly higher in the control plots after both the first and second spray applications ($P \leq 0.001$) (Fig. 1). The application of treatments significantly ($P \leq 0.001$) reduced pod infestation at 1, 2, 3 and 7 DPA (supporting information Table S3). The greatest reduction in pod

infestation (1.96%) was found in the *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment plot ($F = 5.93$, $P \leq 0.001$) following the second spray application ($F = 28.28$, $P \leq 0.001$) at 7 DPA.

In 2010/2011, the maximum percentage of pod infestation (32.99%) was observed in control plots 7 days after the first spray. This increased to a maximum of 46.76% after the second spray application (Fig. 1). All the treatments significantly ($P \leq 0.05$) reduced pod infestation relative to the control plots, and the reduction increased over time (supporting information Table S4). The greatest reduction in pod infestation (8.47%) following the first spray was found in the *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment ($F = 7.45$, $P \leq 0.001$). At 7 DPA following the second spray ($F = 38.25$, $P \leq 0.001$), pod infestation further decreased to the overall minimum (2.86%).

3.3 Natural enemies

In the control plots, the population of natural enemies continuously increased over time (Fig. 2). Natural enemies were significantly ($P \leq 0.05$) affected by the *B. bassiana* and *B. bassiana* + chlorantraniliprole treatments. However, JA did not show any toxic effect on natural enemy populations.

In 2009/2010, green lacewings were found to be particularly susceptible to the *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment (0.02 per plant) at 7 DPA after the second application (Fig. 2). The number of lacewings per plant continuously increased in the JA treatment plots and reached a maximum (1.71) at 7 DPA after the second spray application. The maximum numbers of mites (3.03), spiders (2.57) and beetles (2.33) per plant were also found in the JA treatment plots at 7 DPA.

There were also significant ($P \leq 0.05$) differences in natural enemies in the 2010/2011 season (Fig. 3). The *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment resulted in the greatest effect on natural enemies, with the fewest lacewings (0.05), mites (0.71), spiders (0.71) and beetles (0.24) observed at 7 DPA following the second spray in the *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment. In contrast, the number of lacewings (maximum 2.43), mites (3.76), spiders (3.38) and beetles (2.62) increased over time in the JA treatment, reaching a maximum at 7 days following the second spray application.

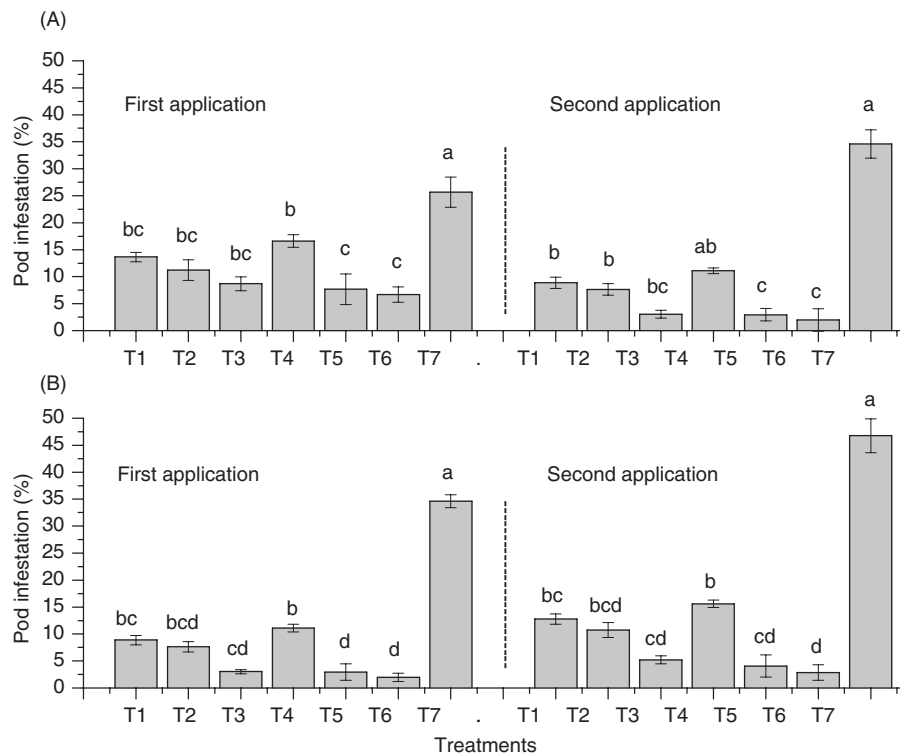


Figure 1. Pod infestation (%) at 7 DPA in 2009/2010 (A) and 2010/2011 (B) after first and second application of treatments. T₁ = *B. bassiana* 3.21×10^4 conidia mL⁻¹; T₂ = *B. bassiana* 3.21×10^6 conidia mL⁻¹; T₃ = chlorantraniliprole 100 mL ha⁻¹; T₄ = JA 1.2 mM; T₅ = *B. bassiana* 3.21×10^4 conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₆ = *B. bassiana* 3.21×10^6 conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₇ = control. Error bars denote standard deviation ($n = 3$). Means of treatments bearing different letters are statistically different ($P \leq 0.05$).

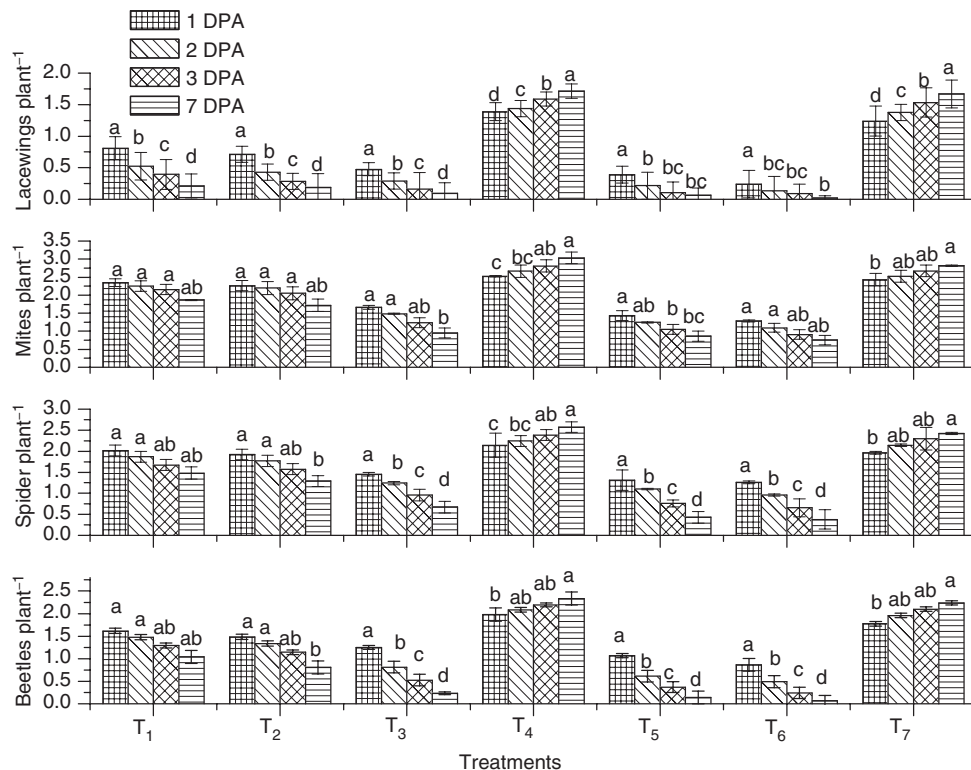


Figure 2. Natural enemies after the second spray application in 2009/2010. T₁ = *B. bassiana* 3.21×10^4 conidia mL⁻¹; T₂ = *B. bassiana* 3.21×10^6 conidia mL⁻¹; T₃ = chlorantraniliprole 100 mL ha⁻¹; T₄ = JA 1.2 mM; T₅ = *B. bassiana* 3.21×10^4 conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₆ = *B. bassiana* 3.21×10^6 conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₇ = control. Error bars denote standard deviation ($n = 3$). Means of treatments bearing different letters are statistically different ($P \leq 0.05$).

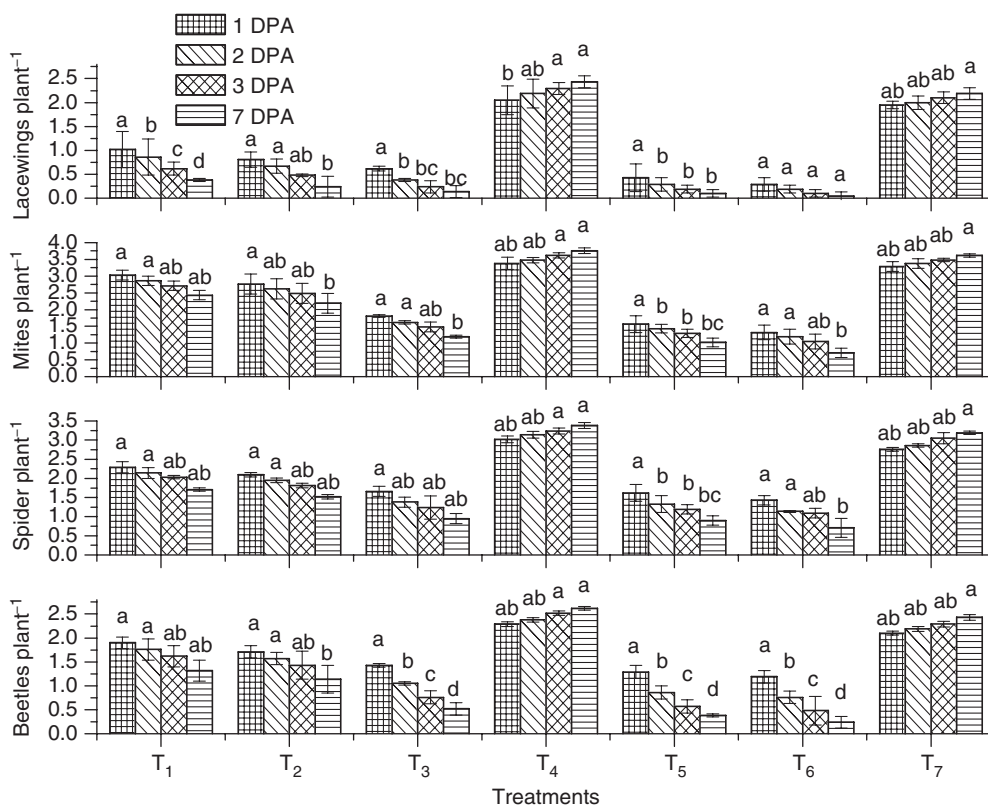


Figure 3. Natural enemies after the second spray application in 2010/2011. T₁ = *B. bassiana* 3.21 × 10⁴ conidia mL⁻¹; T₂ = *B. bassiana* 3.21 × 10⁶ conidia mL⁻¹; T₃ = chlorantraniliprole 100 mL ha⁻¹; T₄ = JA 1.2 mM; T₅ = *B. bassiana* 3.21 × 10⁴ conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₆ = *B. bassiana* 3.21 × 10⁶ conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₇ = control. Error bars denote standard deviation (n = 3). Means of treatments bearing different letters are statistically different (P ≤ 0.05).

3.4 Crop yield and CBR

Chickpea yield differed significantly among treatments (P ≤ 0.05) in both the 2009/2010 and 2011/2012 seasons. In 2009/2010, the maximum yield (600.86 kg ha⁻¹) was obtained in the *B. bassiana* 3.21 × 10⁶ + chlorantraniliprole treatment plot with CBR = 8.43 (Table 4). The lowest yield (344.15 kg ha⁻¹) was observed in the control plots. In 2010/2011, the *B. bassiana* 3.21 × 10⁶ + chlorantraniliprole treatment showed the highest yield (617.04 kg ha⁻¹) with CBR = 7.23, and the control plots generated the lowest yield (392.91 kg ha⁻¹). In both 2009/2010 and 2010/2011, the highest economic return was obtained in the *B. bassiana* 3.21 × 10⁶ + chlorantraniliprole treatment.

4 DISCUSSION

This field-based study demonstrated that *B. bassiana*, chlorantraniliprole, JA and the combination of *B. bassiana* + chlorantraniliprole are effective in reducing populations of *H. armigera* larvae. A higher application dose of *B. bassiana* was more effective in reducing larval populations than a low application dose. The level of pod infestation, which is associated with larval population size, in the control plots increased, along with the larval populations. The application of treatments reduced pod infestation, and the combination of *B. bassiana* + chlorantraniliprole treatment was most effective compared with solo applications of *B. bassiana*, chlorantraniliprole and JA throughout the growing season in two successive years. Moreover, *B. bassiana* 3.21 × 10⁶ + chlorantraniliprole showed better results in reducing pod infestation as compared with *B.*

bassiana 3.21 × 10⁴ + chlorantraniliprole. Pod infestation was likely reduced owing to a reduction in larval populations, which corresponds to earlier studies.^{25–27} A combined application of bio- and chemical insecticides in crops can substantially reduce pod infestation through reduction in larval population.²⁷ In both years of the present study, pod damage was lowest following the combined application of *B. bassiana* and chlorantraniliprole as compared with the application of either material alone. Further, the lowest yields were detected in the control plots, which also had the largest larval populations and the greatest rates of pod infestation. However, the application of treatments increased chickpea yields in both study seasons. The combined application of *B. bassiana* and chlorantraniliprole reduced the larval population and pod infestation, and subsequently increased the yield of chickpea. The income per hectare and CBR were the highest in the plot receiving the combined application of *B. bassiana* and chlorantraniliprole, and better results were observed in *B. bassiana* 3.21 × 10⁶ + chlorantraniliprole as compared with *B. bassiana* 3.21 × 10⁴ + chlorantraniliprole. These results correspond to previous studies where the application of biopesticides led to increased yield of tomato and reduction in larval pest populations.¹⁵ The results of the present study suggest that increased chickpea yield is associated with reduction in larval population and pod infestation.²⁵ Therefore, the present results indicate that the combined use of bio- and chemical insecticides is a potential strategy to reduce larval populations and pod infestation in chickpea crops.

B. bassiana has been reported as a biological control agent against insects.^{9,28} In this study, reductions in larval population increased with time following *B. bassiana* application, with the

Table 4. Chickpea yield (mean \pm SD) and CBR in 2009/2010 and 2010/2011

Treatments ^a	Yield ^b (kg ha ⁻¹ \pm SD)	Cost (Rs) ^c	Increased yield over control (kg ha ⁻¹)	Income increase ha ⁻¹	Increased benefit	CBR
2009/2010						
T ₁	478.87 \pm 10.53 c	1640	155.72	14 014.8	12 374.8	5.55
T ₂	501.18 \pm 6.86 c	1640	169.65	15 268.5	13 523.5	6.75
T ₃	544.92 \pm 13.30 bc	2120	200.77	18 069.3	15 949.3	7.52
T ₄	413.36 \pm 10.50 d	1650	69.21	6228.9	4578.9	2.78
T ₅	588.49 \pm 6.47 ab	2360	244.34	21 990.6	19 630.6	8.32
T ₆	600.86 \pm 5.70 a	2450	256.71	23 103.9	20 653.9	8.43
T ₇	344.15 \pm 10.56 e	900	–	–	–	–
2010/2011						
T ₁	511.54 \pm 4.75 c	1640	118.63	10 676.7	9036.7	5.51
T ₂	526.94 \pm 9.12 c	1640	134.03	12 062.7	10 317.7	5.91
T ₃	570.23 \pm 12.11 b	2120	177.32	15 958.8	13 838.8	6.53
T ₄	465.63 \pm 11.79 d	1650	72.72	6544.8	4894.8	2.97
T ₅	601.02 \pm 6.16 ab	2360	208.11	18 729.9	16 369.9	6.94
T ₆	617.04 \pm 4.00 a	2450	224.13	20 171.7	17 721.7	7.23
T ₇	392.91 \pm 6.96 e	900	–	–	–	–

^a T₁ = *B. bassiana* 3.21 \times 10⁴ conidia mL⁻¹; T₂ = *B. bassiana* 3.21 \times 10⁶ conidia mL⁻¹; T₃ = chlorantraniliprole 100 mL ha⁻¹; T₄ = JA 1.2 mM; T₅ = *B. bassiana* 3.21 \times 10⁴ conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₆ = *B. bassiana* 3.21 \times 10⁶ conidia mL⁻¹ + chlorantraniliprole 100 mL ha⁻¹; T₇ = control.
^b Means bearing different letters within a column are statistically different ($P \leq 0.05$).
^c Rs: Pakistani rupees.

maximum reduction at 7 DPA. These results correspond to the results of earlier studies that found that insects infected with *B. bassiana* died within 3–5 days of hyphal penetration.⁹ El-Sinary²⁹ observed that inoculation of entomopathogenic fungi resulted in continuous larval decay until the fourth day post-infection. Typically, this resulted from damage to fat tissues. Similar results of larval reduction by entomopathogenic fungi were also observed by Quesada-Moraga *et al.*¹⁰ We found that *B. bassiana* alone could be used successfully for reduction in *H. armigera* larval populations with an increased efficacy of application dose. However, the combination of *B. bassiana* and chlorantraniliprole was especially effective for the control of *H. armigera* larvae.

Chlorantraniliprole is a new insecticide chemistry that has demonstrated efficacy against larval populations of *H. armigera* at low application rates.¹⁷ In the present study, the efficacy of chlorantraniliprole in controlling larval populations was enhanced by combined application with *B. bassiana*. JA has been demonstrated to reduce larval insect populations by increasing the difficulty of digesting plant tissues, resulting in starvation.^{14,30} JA application resulted in reduced larval populations; however, its effect was less than that of *B. bassiana* and chlorantraniliprole.

Natural enemies monitored in the present study were affected by the toxicity of treatments. Among all the applied treatments, the *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment showed the highest toxicity to natural enemies, and therefore the beneficial population per plant decreased significantly with time. However, the toxicity of *B. bassiana* to these non-target organisms was lower than that of chlorantraniliprole. Although reports suggest that the toxicity of chlorantraniliprole is selective for insects, there are still very little data on the impact of chlorantraniliprole on natural enemies of insect pests.¹⁹ The applications of JA showed the lowest toxic effects on natural enemies, and hence the maximum numbers of natural enemies per plant were observed. Among the insects examined, lacewings were observed to be

more susceptible to toxic effects of treatments. This is presumably because of their soft body. The maximum numbers of lacewings in the JA treatment imply that JA showed less toxic effects on lacewing. Similarly, limited effects of JA were also demonstrated by an increase in the population of beetles, spiders and mites. The monitoring of the natural enemy population dynamics provides a more holistic picture of the ability of biocontrol agents to control insect pest populations.³¹ Therefore, the data of the present study can also provide the basis for the development of future strategies for IPM programmes.

5 CONCLUSION

Taken as a whole, the results of this research showed that the greatest reduction in the larval population of *H. armigera* and pod infestation was achieved by the combined application of *B. bassiana* and chlorantraniliprole. The efficacy of *B. bassiana* increased with application dose. The reduction in larval populations and pod infestation increased the chickpea yield and CBR, with the highest income obtained in the *B. bassiana* 3.21 \times 10⁶ + chlorantraniliprole treatment. JA showed lowest toxicity to natural enemies. In short, the application of chlorantraniliprole and *B. bassiana* reduced the incidence of *H. armigera* in the chickpea crop, while JA showed increased populations of natural enemies, and hence this IPM strategy proved to be economical and profitable. The results of our study suggest that the use of biochemical insecticides provides adequate approaches to managing pests. Further research is needed to establish strategies for the control of insect pests in different crops.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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