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Sarwan Kumar
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Jaswinder Kaur
Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India

Correspondence
Sarwan Kumar
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India

Efficacy of *Beauveria bassiana* and *Bacillus thuringiensis* as ecosafe alternatives to chemical insecticides against sunflower capitulum borer, *Helicoverpa armigera* (Hübner)

Sarwan Kumar and Jaswinder Kaur

Abstract

During spring 2012, three biopesticides including DOR *Beauveria bassiana* SC (200, 250 and 300 mg l^{-1}) (developed by ICAR-Directorate of Oilseeds Research, Hyderabad, India), commercial *B. bassiana* formulation Daman 1 WP (5g l^{-1}) and DOR Bt-5 (*Bacillus thuringiensis* formulation) (2.5 g l^{-1}) along with synthetic insecticide profenophos 50 EC (0.05%) were evaluated under field conditions against sunflower capitulum/ head borer, *Helicoverpa armigera* (Hübner). All the biopesticides were effective in suppressing the larval population of *H. armigera* and were safe to natural enemies found in sunflower agro-ecosystem. The seed yield was also significantly higher in all the treatments as compared to control. It was concluded from the study that DOR *B. bassiana* @ 200 mg l^{-1} and DOR Bt-5 @ 2.5 g l^{-1} are the potential candidates that can be used for early season suppression of *H. armigera* on sunflower and are safe to natural enemies as well. These entomopathogens may be useful components of *H. armigera* Integrated Pest Management (IPM) strategy.

Keywords: Biopesticides, capitulum borer, Entomopathogenic fungus, Head borer

1. Introduction

Sunflower (*Helianthus annus* L.) is an important oilseed crop in India after groundnut, mustard and soybean. The crop has gained much popularity due to some characters such as short duration, photo-insensitivity and wider adaptability to different agro-climatic conditions [10] and has great potential for diversification of major cropping systems in the country. In southern states of the country (Karnataka, Maharashtra, Andhra Pradesh and Gujarat), it is principally grown as monsoon, late monsoon and *rabi* crop, while in northern states (Punjab, Haryana, Uttar Pradesh and Rajasthan), it is grown as spring season crop. During the year 2013-14, it was cultivated on an area of 6.91 lakh ha in the country with production and productivity of 5.47 lakh tones and 791 kg ha^{-1}, respectively [18].

The crop is attacked by a number of insect-pests and as many as 251 insect species are known to attack this crop the world over [21]. In India, major insect-pests associated with this crop are capitulum borer *Helicoverpa armigera* (Hübner), green semilooper *Thysanoplusia orichalcea* Fab., Bihar hairy caterpillar *Spilosoma obliqua* (Walker), tobacco caterpillar *Spodoptera litura* (Fab.), cutworm *Agrotis* sp. and green leaf hopper *Amrasca biguttula biguttula* [4]. The loss in seed yield due to these pests is estimated to be around 24.9 per cent [2]. Among these, capitulum/head borer (*H. armigera*) is the most important one inflicting annual crop damage in India worth US $ 1 billion [15]. It is a major pest of many economically important crops in India [11, 14] and remains active throughout the year on one or the other crop. A wide host range [7, 23, 31], high fecundity [22], a capacity to migrate [6] and the ability to develop high resistance to insecticides [3] have enabled this insect to attain key pest status among various major crop pests. The problem of this pest is further magnified due to its high preference for fruiting bodies, voracious feeding habit [12], high mobility and overlapping generations with ability to enter facultative pupal diapause both in winter and summer seasons [13].

The present methods of pest management largely rely on the use of toxic synthetic insecticides which have their own adverse effects such as development of insecticide resistance, pest resurgence, secondary pest outbreaks besides environmental pollution and residues in oil and cake. This necessitates the development of alternate pest management strategies.

Further, sunflower is highly cross pollinated crop and therefore, it needs the help of honey bees for pollination. Thus, there is a need for the use of safer insecticides which are least toxic to honey bees and natural enemies of the pest. Biopesticides hold a promise in this context. They are an important group of pesticides that can reduce pesticide risks and are relatively safe to natural enemies and other non-target organisms compared to conventional pesticides and have no residue problems. Entomopathogenic fungi are one such group of biopesticides that can function as microbial insecticides. Most of the 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) [1, 5, 16, 24, 30]. *B. bassiana* is a well known, naturally occurring and environmentally safe biological control agent [19]. After coming in contact with larval or pupal stage of the insect, fungus penetrates the integument and reaches the visceral organs of the body [1, 20]. After penetration, fungal hyphae degrade insect's fat and gut tissue resulting in destruction of Malpighian tubules and ultimately lead to death. Similarly, *Bacillus thuringiensis* is an entomopathogenic bacterium that is toxic to a broad range of insects. The bacterium relies on insecticidal crystal proteins known as *Cry* and *Cyt* toxins to kill their insect larval hosts [29]. Thus, keeping in view the pathogenicity of these biocontrol agents, these were evaluated against *H. armigera* larvae on sunflower during spring 2012. The objective of the study was to determine the field efficacy of *B. bassiana* and *B. thuringiensis* formulations against *H. armigera* in comparison to chemical insecticide profenophos.

2. Materials and Methods

2.1 Experimental Layout

The study on evaluation of bioefficacy of *Beauveria bassiana* and *Bacillus thuringiensis* formulations was conducted at Research Farm of Oilseeds Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30.9°N and 75.85°E, 244 m above msl), India during spring 2012. The sunflower hybrid PSH 569 (available from Senior Scientist-cum-Incharge (Oilseeds), Punjab Agricultural University, Ludhiana, India) was sown in a randomized complete block design with seven treatments and three replications with a plot size of 4 x 3 m. The recommended package of practices for raising a good crop was followed except spray of insecticides. Natural infestation was allowed to take place and the different treatments were applied at the time of pest appearance.

2.2 Treatments

The different treatments included DOR *B. bassiana* SC formulation (available from ICAR-Directorate of Oilseeds Research, Hyderabad, India) @ (T_1) 200, (T_2) 250 and (T_3) 300 mg litre^{-1} of water, (T_4) commercial formulation of *B. bassiana* (Daman 1% WP) @ 5.0 g litre^{-1}, (T_5) profenophos 50 EC @ 0.05 %, (T_6) DOR Bt-5 formulation (available from ICAR-Directorate of Oilseeds Research, Hyderabad, India) @ 2.5 g litre^{-1} and (T_7) untreated control. The treatments were imposed when eggs and early instar larvae of head borer were reported in the field.

2.3 Observations

Data on the number of head borer larvae and natural enemies (predators and parasitoids) per plant were recorded before, 3 and 7 days after treatments. For this 10 plants from middle six rows of each plot were selected at random and data on number of head borer larvae and natural enemies were recorded by observing the whole plant. Yield data were recorded at harvest of the crop.

3. Data Analysis

Data on the number of head borer larvae, natural enemies and yield were subjected to analysis of variance (ANOVA) using the statistical software OPSTAT [17]. For the data on head borer density and natural enemies' population, $\sqrt{n+1}$ transformation was used to stabilize variances. Means among the treatments were separated at 5 per cent level of significance.

4. Results and Discussion

After three days of application, the treatments T_5: profenophos (0.05%), T_6: DOR Bt-5 formulation and T_4: commercial *B. bassiana* formulation (Daman 1% WP) resulted in significantly lower larval population (0.07, 0.47 and 0.60 larvae plant^{-1}) than T_7: the untreated control (Table. 1), while it did not differ significantly in the all the three treatments involving DOR *B. bassiana* formulation.

However, after seven days, all the treatments resulted in significant reduction in head borer population over control. The minimum population of 0.00 larvae plant^{-1} was recorded in T_5: profenophos (0.05%). It was followed by T_6: DOR Bt-5 formulation (2.5 g l^{-1}), T_4: Daman 1% WP, DOR *Beauveria bassiana* SC formulation @ (T_3) 300 mg l^{-1}, (T_2) 250 mg l^{-1} and (T_1) 200 mg l^{-1}.

No apparent adverse effect of different treatments was observed on the natural enemies (Table. 2). Although, the population of natural enemies in the treatment involving chemical insecticide was the lowest among different treatments, but statistically the differences were non-significant.

All the treatments resulted in significant increase in yield over the control. The maximum seed yield of 1765.19 kg ha^{-1} was recorded in the treatment (T_5) profenophos (0.05%) followed by (T_6) DOR Bt-5 (1620.37 kg ha^{-1}), (T_4) Daman 1% WP (1431.85 kg ha^{-1}), (T_3) DOR *B. bassiana* @ 300 mg l^{-1} (1340.74), (T_2) DOR *B. bassiana* @ 250 mg l^{-1} (1257.04 kg ha^{-1}) and (T_1) DOR *B. bassiana* @ 200 mg l^{-1} (1212.22 kg ha^{-1}). Mane *et al.* [15] studied the efficacy of different biopesticides against *H. armigera* and reported that *B. bassiana* (250 g l^{-1}) and DOR Bt-5 (2.5 g l^{-1}) were effective in reducing the larval population on sunflower. Similarly, Jagadish *et al.* [8] also reported DOR *B. bassiana* (@ 200, 250 and 300 mg l^{-1}) and DOR Bt (2.5 g l^{-1}) to be highly effective in reducing the larval population of *H. armigera* on sunflower. Almost similar results were obtained by Jayewar and Sonkamble [9] who reported DOR *B. bassiana* (250 mg l^{-1}) and DOR Bt-5 (2.5 g l^{-1}) to be effective against *H. armigera* for up to 7 days and safe to natural enemies. These treatments also resulted in significantly high seed yield than untreated control.

Plants are susceptible to various biotic and abiotic stresses in nature and insects are one of the largest groups of biotic stresses that limit productivity of many agricultural crops. Insects from order Lepidoptera are one such diversified group of phytophagous pests, which includes *Helicoverpa armigera* – a key pest of many agricultural crops including sunflower, cotton, chickpea, pigeonpea, tomato, peas, lentil, chilli and tobacco [25]. The pest has become difficult to control due to a number of factors as listed in the introduction. The over reliance on synthetic chemical insecticides has led to many problems including development of insecticide resistance, decrease in biodiversity, environmental pollution, secondary pest outbreaks and human health hazards besides toxicity to non-target organisms. Due to the repeated control failures with the use of chemical insecticides against *H. armigera*, there is increasing interest towards alternate strategies for its management. *H. armigera* has made this situation more important because of the extent of damage it inflicts to different crops. With an increased interest for development of environment friendly, effective and economic methods for insect-pest management, alternative to chemical insecticides, insecticides from microbial control agents will take a major share of the future insecticide market. Few studies have started flourishing in isolation. Vimala Devi and Hari [26, 27] have formulated a suspension concentrate of local isolate (ITCC 4513) of *B. bassiana* using mineral oil as carrier which was found to be effective against *H. armigera* on sunflower crop with no phytotoxic effect. The formulation gave a clear suspension in water and could be sprayed with knapsack sprayer. No apparent adverse effects of the myco-insecticide were recorded on the egg parasitoid, *Trichogramma chilonis.* Similarly, Vimala Devi and Vineela [28] has developed a formulation of *Bacillus thuringiensis* var. *kurstaki* using boric acid as an adjuvant which was found to be effective against *H. armigera* both in laboratory bioassays and field trials even at the lowest dose of 1.0 ml/ litre. Thus, microbials have great potential to offer valuable versatile products against insect-pests of agricultural crops and can go far long to meet the major challenges in pest management.

Table 1: Effect of different treatments on number of head borer larvae per plant

Treatment	Head borer larvae plant^{-1}			Yield
	Pre-treatment	3 DAT†	7 DAT	(kg ha^{-1})
T_1: DOR *Beauveria bassiana* SC formulation @ 200 mg l^{-1}	1.13 (1.45)*	1.13 (1.46)	0.80 (1.34)	1212.22
T_2: DOR *B. bassiana* SC formulation @ 250 mg l^{-1}	1.00 (1.41)	1.00 (1.41)	0.73 (1.31)	1257.04
T_3: DOR *B. bassiana* SC formulation @ 300 mg l^{-1}	1.30 (1.50)	0.80 (1.34)	0.60 (1.26)	1340.74
T_4: Daman 1% WP @ 5.0 g l^{-1}	1.20 (1.48)	0.60 (1.26)	0.47 (1.21)	1431.85
T_5: Profenophos 50 EC @ 0.05%	1.07 (1.43)	0.07 (1.03)	0.00 (1.00)	1765.19
T_6: DOR Bt-5 formulation @ 2.5g l^{-1}	1.17 (1.46)	0.47 (1.21)	0.20 (1.09)	1620.37
T_7: Unsprayed control	1.13 (1.45)	1.47 (1.57)	1.73 (1.65)	975.19
CD (p=0.05)	(NS)	(0.28)	(0.31)	219.10
SEm ±	0.04	0.09	0.10	70.32
CV (%)	5.55	11.96	13.76	8.88

†DAT: Days after Treatment
* Figures in parentheses are √n+1 transformed values

Table 2: Effect of different treatments on natural enemies' population

Treatment	Number of natural enemies plant^{-1}		
	Pre-treatment	3 DAT†	7 DAT
T_1: DOR *Beauveria bassiana* SC formulation @ 200 mg l^{-1}	1.20 (1.48)*	1.33 (1.52)	1.40 (1.54)
T_2: DOR *B. bassiana* SC formulation @ 250 mg l^{-1}	1.13 (1.46)	1.13 (1.46)	1.20 (1.48)
T_3: DOR *B. bassiana* SC formulation @ 300 mg l^{-1}	1.40 (1.54)	1.13 (1.46)	1.27 (1.50)
T_4: Daman 1% WP @ 5.0g l^{-1}	1.20 (1.48)	1.33 (1.52)	1.40 (1.54)
T_5: Profenophos 50 EC @ 0.05%	1.13 (1.46)	0.53 (1.23)	0.40 (1.18)
T_6: DOR Bt-5 formulation @ 2.5g l^{-1}	1.13 (1.46)	1.00 (1.41)	1.47 (1.57)
T_7: Unsprayed control	1.07 (1.43)	1.20 (1.48)	1.40 (1.54)
CD (p=0.05)	(NS)	(NS)	(NS)
SEm ±	0.08	0.11	0.12
CV (%)	9.74	13.99	13.50

†DAT: Days after Treatment
* Figures in parentheses are √n+1 transformed values

5. Conclusion

DOR *Beauveria bassiana* @ 200 ml l^{-1} and DOR Bt-5 @ 2.5 g l^{-1} can be effectively used as initial sprays in controlling the larval population of *H. armigera* under field conditions as alternative to synthetic pesticides. Under spring season in Punjab, sunflower harbours a plethora of natural enemies and these pesticides are comparatively safe to these natural enemies. Initial sprays of these pesticides will lead to least destruction of the natural control in sunflower agro-ecosystem.

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