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Combined effect of entomopathogens with biorationals against Lepidopteran insect pests of greengram

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ABSTRACT : Field efficacy of compatible combination of *Beauveria bassiana* and *Bacillus thuringiensis* with two botanicals, an insect growth regulator (IGR) and a chemical insecticide was tested against *Spodoptera litura*, *Spilarctia obliqua* and *Maruca vitrata* infesting green gram. *Bacillus thuringiensis* in combination with other insecticides was found more effective to combat the lepidopteran's population than *B. bassiana*. Study revealed that *B. bassiana* and *B. thuringiensis* in combination with chlorantraniliprole and azadirachtin showed highest reduction in the population of these lepidopterans. However, combination of *B. thuringiensis* with chlorantraniliprole and azadirachtin showed more effective than combination of *B. bassiana* with chlorantraniliprole and azadirachtin. The combinations like *B. thuringiensis*+chlorantraniliprole 18.5SC, *B. thuringiensis* + azadirachtin 10000ppm, *B. bassiana* + chlorantraniliprole 18.5SC and *B. bassiana* + azadirachtin 10000 ppm were found more effective against *S. litura*, *S. obliqua* and *M. vitrata*.

Key words: *Beauveria bassiana*, *Bacillus thuringiensis*, green gram, *Maruca vitrata*, *Spodoptera litura*, *Spilarctia obliqua*

For successful pest management use of chemical insecticides proved as boon to agriculture but some unexpected ill effects of chemicals drawing attention of entomologists towards the alternative pest control techniques. The new compound of biological origin sounds best alternatives towards pest control. After this microbial control came in existence which includes use of pathogenic microorganisms to control pest's population and often referred as microbial control agents (MCA). This was assumed a vowing and sound strategy along with good potential to control insect pests, with minimizing the antagonistic effects of chemical insecticides on human health and environment. The utmost common microbial agents are viruses, bacteria, nematodes, fungi, and protozoans and benefited all over the world in addition to great success. Most demanded among all of them is entomopathogenic fungal biocontrol agents owing to easy delivery, improving formulation, enormous number of pathogenic strains are known, and easy engineering techniques (Butt, 2002; Goettel *et al.*, 2005). Among them *Beauveria* spp. has been the most widely studied fungal entomopathogen while in case of bacterial pest control agents *Bacillus* spp. are most investigated one. *Beauveria bassiana*, most rigorously

investigated fungal entomopathogens and over thousands of isolates have been collected from diverse places of the world and help to prove its importance as biocontrol, industrial, and pharmaceutical microbial agent (Rehner *et al.*, 2011). Being a renowned pathogen of broad host range arthropods and able to infect near about 700 species of hosts, together with various species in acari and insects (Inglis *et al.*, 2001; Zimmermann, 2007), the *B. bassiana* as biocontrol agent is harvested in further course of time by many workers. *Bacillus thuringiensis* is gram-positive bacteria manufacturing numerous toxins which a widespread range of insects, counting lepidopteran, dipteran together with coleopteran larvae, is accredited chiefly towards crystal proteins (Cry) formed all through the process of sporulation (Palma *et al.*, 2014). Divergent type of Cry toxins may well interact synergistically and thus upsurge the mortality rate of targeted insects, provided that the mixtures comprise toxins of minimal or no similarity along with dissimilar action's mechanism. Since then Cry toxins have the highest insecticidal activity. By this mean the biopreparation activity spectrum of target pests can be extended (Fernandez-Luna *et al.*, 2010). Alike views were communicated by Tabashnik *et*

al. (2013) but here many other types of interaction among the *B. thuringiensis* protein other than synergism may be happen like insects can develop resistant to one type of toxin (Qian *et al.*, 2015).

The basic concept of IPM is that all techniques should be compatible to each other. IPM emphasizes the production of a healthy crop with the least possible disturbance to agro-ecosystems and promotes natural pest control mechanisms (Flint *et al.*, 2012). Hence, the understanding of the insecticidal effects on entomopathogens is necessary to allow their combined use in the multifaceted integrated pest management (Purwar and Sachan, 2006; Skinner *et al.*, 2014). Interaction of entomopathogens and chemical insecticides should be studied. This study was conducted with objective to evaluate the combination of *Bacillus thuringiensis* and *Beauveria bassiana* with biorationals and chemical pesticides against lepidopteran insect pests of green gram.

MATERIALS AND METHODS

The present study was carried out during *kharif* season of 2019-20 on green gram at Norman E. Borlaug Crop Research Centre (NEBCRC) of G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) located in *tarai* region at the foot hills of Himalayas (Shivalik range) at 29° N latitude, 79.3 ° E longitude and at an altitude of 243.84 m above mean sea level.

Preparation of biorationals and insecticidal solution

In autumn season young and mature leaves of *Melia azedarach* were plucked and washed in distilled and sterilized water. Two hundred fifty grams of leaves were weighed and soaked in 500 ml of distilled and

sterilized water for 24 hours aseptically. Then soaked leaves were crushed with the help of mortar and pestle that turned into the form of paste, water was added to paste and volume was made up to 1 litre and left for two hours and finally filtered through double layer sterilized muslin cloth and whatmann no.42 filter paper in different conical flasks aseptically. The aliquot was used for further treatment and stored inside refrigerator in less than 4°C. This aliquot again filtered through syringe filter 0.2 micron (Axiva®) before use (Hammad *et al.*, 2000). The spore density of entomopathogens was calculated by using haemocytometer and solution made of recommended spore density i.e. 2×10^8 and 4.1×10^7 spores/ml for *B. bassiana* and *B. thuringiensis*, respectively by serial dilution. Insecticide solutions (Table 1) were made by filtering insecticide formulation through syringe filter 0.2 micron (Axiva®) in order to avoid contamination. Filtered insecticides used to make solution by adding half of the recommended concentration in distilled water. Sublethal doses of insecticides were sprayed in field immediately after the application of fungal and bacterial solutions.

Observation on Lepidopteran insect pests

Observations on tobacco caterpillar (*Spodoptera litura*) and Bihar hairy caterpillar (*Spilarctia obliqua*) were recorded by counting number of larvae collected on one-meter long of polythene sheet by tapping the plant three times from all three replications. While incidence of spotted pod borer population was determined by counting the number of larvae on three randomly selected plants since presence of webs. Data was taken prior to spray and then taken on 1DAS (Day after spray), 3DAS, 5DAS, 7DAS and 10DAS.

Table 1: Insecticide used during experiment

Type of insecticides	Recommended insecticides	Recommended dose*
Botanical pesticides	Azadirachtin 10000 ppm	1000ppm
	Aqueous leaf extract of <i>Melia azedarach</i>	50000ppm
Insect growth regulators	Buprofezin 25% SC	300ppm
Chemical insecticides	Chlorantraniliprole 18.5 %SC	40ppm

*Directorate of Plant Protection, Quarantine and Storage (Department of Agriculture, Cooperation and Farmers Welfare) GOI.

Table 2: Field efficacy of various treatments against *S. litura* population in greengram during kharif crop season, 2019-20

Treatment details	Per cent reduction over Control					
	1DAS	3DAS	5DAS	7DAS	10DAS	Mean
<i>Bb</i> +BUPROFEZIN 25SC	17.03 (24.72)**	24.04(23.06)	30.98(28.80)	35.13(33.49)	37.27(42.11)	27.23(24.72)
<i>Bb</i> +CHLORANTRANILIPROLE 18.5SC	23.29(10.81)	47.36(24.07)	63.62(43.46)	78.06(53.51)	82.60(62.66)	50.04(10.81)
<i>Bb</i> +AZADIRACHATIN 10000 ppm	34.46(29.21)	56.34(42.52)	65.88(53.26)	71.42(60.48)	77.25(69.23)	51.77(29.21)
<i>Bb</i> +BAKAIN EXTRACT 50000 ppm	33.91(36.98)	37.86(40.30)	39.92(39.47)	40.48(47.27)	41.81(51.10)	37.41(36.98)
<i>Bt</i> +BUPROFEZIN 25SC	28.35(22.15)	36.01(32.08)	40.33(36.83)	45.86(44.02)	49.33(48.38)	36.71(22.15)
<i>Bt</i> +CHLORANTRANILIPROLE 18.5SC	17.72(0.00)	35.19(23.77)	62.84(35.48)	84.06(52.50)	91.12(66.58)	48.49(0.00)
<i>Bt</i> +AZADIRACHATIN 10000 ppm	46.05(10.81)	64.19(35.83)	75.39(48.65)	87.27(54.53)	87.60(57.67)	64.36(10.81)
<i>Bt</i> +BAKAIN EXTRACT 50000 ppm	41.91(37.32)	43.86(44.13)	46.92(50.02)	48.48(56.28)	51.81(58.78)	44.91(37.32)
C.D.(P=0.05)	NS (12.03)	19.26 (NS)	20.26 (12.07)	17.86 (12.54)	17.62 (11.40)	
SE(m)±	8.15 (3.93)	6.29 (6.35)	6.62 (3.94)	5.83 (4.09)	5.75 (3.72)	
CV	43.87 (31.64)	24.02 (33.10)	19.50 (16.26)	14.57 (14.10)	13.63 (11.30)	

** Parentheses values are angular transformed, NS- Non significant, DAS-Day after spray, *Bb*-*Beauveria bassiana*, *Bt*-*Bacillus thuringiensis*

Table 3: Field efficacy of various treatments against *Spilarctia obliqua* (Walker) population in greengram during kharif crop season, 2019-20

Treatment detail	Per cent reduction over Control					
	1DAS	3DAS	5DAS	7DAS	10DAS	MEAN
<i>Bb</i> +BUPROFEZIN 25SC	29.12 (34.74)**	33.34(35.396)	38.19(41.066)	42.95(49.269)	44.45(53.942)	34.68(42.89)
<i>Bb</i> +CHLORANTRANILIPROLE 18.5SC	36.15(25.77)	56.73(39.944)	66.25(53.995)	79.42(68.816)	80.18(77.752)	53.69(53.26)
<i>Bb</i> +AZADIRACHATIN 10000 ppm	25.68(6.684)	49.51(31.484)	64.25(44.24)	71.53(53.906)	76.32(60.974)	48.44(39.46)
<i>Bb</i> +BAKAIN EXTRACT 50000 ppm	24.21(30.216)	40.93(37.98)	42.67(41.066)	44.77(47.188)	45.27(45.257)	35.03(40.35)
<i>Bt</i> +BUPROFEZIN 25SC	43.11(30.662)	46.36(29.816)	48.25(39.835)	50.83(46.91)	53.16(49.695)	46.08(39.39)
<i>Bt</i> +CHLORANTRANILIPROLE 18.5SC	47(12.99)	63.89(33.16)	74.91(48.374)	84.95(65.662)	88.25(77.752)	63.89(47.59)
<i>Bt</i> +AZADIRACHATIN 10000 ppm	20.44(4.677)	38.46(22.091)	63.52(42.197)	82.95(48.238)	86.85(52.613)	49.62(33.97)
<i>Bt</i> +BAKAIN EXTRACT 50000 ppm	28.49(33.272)	39.2(37.388)	43.04(40.473)	48.48(50.711)	48.84(49.806)	37.76(42.33)
C.D.(P=0.05)	NS	NS	NS	18.16 (13.28)	14.00 (13.21)	
SE(m)±	10.66 (7.68)	8.04 (8.38)	6.78 (4.76)	5.93 (4.33)	4.57 (4.31)	
CV	55.73 (59.44)	28.94 (43.45)	18.31 (18.79)	14.73 (13.95)	10.39 (12.78)	

** Parentheses values are angular transformed, NS- Non significant, DAS-Day after spray., *Bb*-*Beauveria bassiana*, *Bt*-*Bacillus thuringiensis*

Table 4: Field efficacy of various treatments against *Maruca vitrata* (Geyer) population in greengram during kharif crop season, 2019-20

Treatment detail	Per cent reduction over control					
	1DAS	3DAS	5DAS	7DAS	10DAS	MEAN
<i>Bb</i> +BUPROFEZIN 25SC	47.22 (47.16)**	72.22(38.23)	72.22(63.23)	80.55(63.23)	72.22(68.23)	66.35(56.02)
<i>Bb</i> +CHLORANTRANILIPROLE 18.5SC	8.33(8.08)	26.11(9.99)	38.88(30.59)	55.55(38.49)	65.55(48.22)	33.33(27.09)
<i>Bb</i> +AZADIRACHATIN 10000 ppm	44.44(19.3)	69.44(41.73)	80.55(56.46)	91.66(68.23)	91.66(79.99)	66.97(53.17)
<i>Bb</i> +BAKAIN EXTRACT 50000 ppm	30.55(32.16)	50.00(28.23)	61.11(44.98)	72.22(51.47)	73.88(58.22)	54.13(43.02)
<i>Bt</i> +BUPROFEZIN 25SC	8.33(8.02)	16.66(9.99)	27.77(14.99)	33.33(26.74)	45.00(29.98)	22.77(17.95)
<i>Bt</i> +CHLORANTRANILIPROLE 18.5SC	52.77(31.1)	69.44(46.73)	88.88(61.74)	100.00(78.23)	100.00(90.00)	74.38(61.58)
<i>Bt</i> +AZADIRACHATIN 10000 ppm	80.55(49.39)	88.88(68.23)	88.88(78.23)	100.00(78.23)	100.00(90)	85.95(72.82)
<i>Bt</i> +BAKAIN EXTRACT 50000 ppm	33.33(19.39)	47.22(29.98)	58.33(43.49)	63.88(49.98)	73.88(53.22)	50.12(39.22)
C.D.(P=0.05)	NS	42.59 (39.21)	37.64 (31.06)	35.15 (31.72)	25.99 (24.03)	
SE(m)±	15.10 (12.38)	13.90 (11.89)	12.29 (10.14)	11.48 (10.35)	8.48 (7.84)	
CV	86.26 (79.93)	63.07 (60.31)	38.71 (35.69)	30.78 (31.57)	19.69 (20.99)	

** Parentheses values are angular transformed, NS- Non significant, DAS-Days after spray. *Bb*-*Beauveria bassiana*, *Bt*-*Bacillus thuringiensis*

et al. (2017) also found 86.14 per cent mortality of *S. litura* by the combination of *B. bassiana* + chlorantraniliprole at 10DAS as compare to individual treatment per cent mortality of 40.58 and 58.47 for *B. bassiana* (1.3x10⁶) and chlorantraniliprole (25ppm), respectively.

***Bihar hairy caterpillar, Spilarctia obliqua* (Walker)**
Data on per cent reduction of larval population of *S. obliqua* (Walker) population over control varied non-significantly among various treatments one day after spray from 20.04 (*B. thuringiensis* + azadirachtin) to 47.0 (*B. thuringiensis* + chlorantraniliprole)

(Table 3 & Figure 2). At 7DAS the per cent reduction over control varied significantly and highest reduction (84.95 per cent) was observed in *B. thuringiensis* + chlorantraniliprole combination followed by *B. thuringiensis* + azadirachtin (82.95 per cent). Similar trend was observed at 10 days after spraying with significant per cent reduction over control. Based on per cent reduction over control 10 DAS *B. thuringiensis* + chlorantraniliprole (88.25) and *B. thuringiensis* + azadirachtin (86.85) were found more effective against this noxious arctiids. Overall mean data indicated that the combinations of *B. thuringiensis* with insecticides were found more effective than *B. bassiana* against bihar hairy caterpillar at pantnagar infesting green gram. Younas *et al.* (2017) found that *B. bassiana* 3.21×10^4 + chlorantraniliprole showed more reduction in pod infestation as compare to their individual treatment against *H. armigera* in chickpea. The results of Bandyopadhyay *et al.* (2014) supports the present study. They found that synergistic effect was apparent from the combinations at LC₂₅ level of Btk (0.003%) + LC₂₅ level of azadirachtin (0.004%) and LC₃₀ treatment level of Btk (0.044%) + LC₂₀ level of azadirachtin (0.031%) at 72 hours post treatments against Bihar hairy caterpillar. Dubai (2019) also found the effect of neem oil on the larvae of *Ephestia cautella* showed a significant variation with the concentrations. The combine effect of *B. thuringiensis* and neem oil reported an increase in the mortality rate of larvae when treated with both of them separately and compared with combination.

Spotted pod borer, *Maruca vitrata* (Geyer)

At one day after spray maximum per centage reduction over control was recorded in *B. thuringiensis* + azadirachtin (80.55) followed by *B. thuringiensis* + chlorantraniliprole (52.77) while minimum was observed in *B. thuringiensis* + buprofezin and *B. bassiana* + chlorantraniliprole (8.33) (Table 4 & Figure 3). Similar trend was observed at 3 and 5 day after spraying with significant per cent reduction over control. The data on per cent reduction over control varied from 27.77 (*B. bassiana* + buprofezin) to 88.88 (*B. thuringiensis* + chlorantraniliprole and *B. thuringiensis* + chlorantraniliprole) at 5 days after spraying against

spotted pod borer. At 10 days after spray cent per cent reduction over control was recorded in *B. thuringiensis* + azadirachtin and *B. thuringiensis* + chlorantraniliprole treatment. Overall mean data indicated that the combinations of insecticides with *B. thuringiensis* were found more effective than *B. bassiana* against *M. vitrata*. *B. thuringiensis* + azadirachtin was found more effective against the pod borer at 1,3,5,7 and 10DAS among all the treatments whereas *B. thuringiensis* + buprofezin. Combination of *B. thuringiensis* with chlorantraniliprole, azadirachtin and *Bb* with azadiractin were found better than other treatments against *M. vitrata*. Wakil *et al.* (2013) also reported similar results, they found synergistic effect due to combination of chlorantraniliprole with a high dose of *B. thuringiensis* offered higher mortality (100%) against 3rd instar larvae of chickpea pod borer. Similar results were obtained by Yule and Srinivasan (2014) they found that the pod damage was less in *B. bassiana* and neem based treatments as compare to control and same in case of *B. thuringiensis* when combined with cypermethrin offered more reduction in pod damage by *M. vitrata* as compared to *B. bassiana* and neem based treatments. As per present results Nouri- Ganbalani *et al.* (2016) findings signifies that both *B. thuringiensis* and azadirachtin, either singally or in combination of both have significant to control *Plodia interpunctella*.

CONCLUSION

The present study indicated that *B. thuringiensis* in combination with other insecticides was more virulent than *B. bassiana* to lepidopteran population infesting green gram. The combinations like *B. thuringiensis* + chlorantraniliprole 18.5SC, *B. thuringiensis* + azadirachtin, *B. bassiana* + chlorantraniliprole 18.5SC and *B. bassiana* + azadirachtin were found more effective against *S. litura*, *S. obliqua* and *M. vitrata* and can be used against these insect pests infesting green gram.

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