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Evaluation of various aqua suspension formulations of *Beauveria bassiana* (Balsamo) Vuillemin against *Helicoverpa armigera*

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ABSTRACT :

Studies on laboratory evaluation of nine aqua suspension formulations of entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin comprising adjuvants *viz.* 1) B.b.+GLY+CMC, 2) B.b.+GLY+HO, 3) B.b.+SFO+CMC 4) B.b.+SFO+HO 5) B.b.+GLY+BA, 6) B.b.+GLY+BA+TW, 7) B.b.+SFO, 8) B.b.+GNO and 9) Control (*B.b.* alone) were carried out against the II and III instar larvae of *H.armigera.* The data on larval mortality was recorded at 5, 7 and 10 days after treatment (DAT). At 10 DAT all the formulations were significantly superior to control (*B.b.* alone) for the lethal effect. The formulation *B.b.*+ SFO + CMC registered significantly highest larval mortality of 90.00 and 83.33 per cent of II and III instar larvae of *H.armigera*, respectively. However, it was at par to the formulation *B.b.*+ GLY + HO recording 86.67 and 80.00 per cent mortality of II and III instar larvae of *H.armigera*, respectively. The control (*B. b. alone*) recorded lowest (63.33 and 53.33 %) mortality of the caterpillar.

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INTRODUCTION

Microbial control is the biological suppression of insect pests employing microbial world. It has advantage of higher host specificity, virulence, safety to natural enemies, ease in mass production, multiple benefit in bioefficacy due to accelerating and spreading epizootics in pests, shelf-life and compatibility with other methods. More than 750 species of entomopathogenic fungi, representing 100 genera are currently known (Hajek and St. Leger, 1994). The entomopathogenic fungi causing diseases to the insects and are practically more significant as they are epizootic in nature. Also they have the advantage of ease of production and contact action which allow direct penetration of the host cuticle without ingestion.

Bassi (1835) was the first to demonstrate that entomopathogenic fungus; *B. bassiana* could cause an

infectious disease in silkworm and suggested the concept that, an infectious micro-organism might be used to control insect pests. Steinhaus (1965) reported that *B. bassiana* causes mycosis in 175 host insects from order Lepidoptera, Coleoptera and Hemiptera.

B. bassiana is cosmopolitan fungus useful for the control of various insect pests of different crops. Narayanan (1988) reported 60-100 per cent mortality of *H. armigera* by *B. bassiana*. Devaprasad *et al*. (1990); Gopalakrishnan and Narayanan (1990); Hassani (2000); Parmar (2001); Udar (2002) and Vimaladevi and Hari (2009) also reported the pathogenicity of *B. bassiana* to *H. armigera* larvae. Devaprasad *et al*. (1989) and Ramkumar (1998) reported the infectivity of *B. bassiana* to *Spodoptera litura*. This fungus also found useful for the control of various sucking pests of important field crops. Aphids, *Aphis craccivora* Koch, *A. gossypii* and *Rhapalosiphum maidis* were found to be attacked by *B. bassiana* causing 16-80 per cent mortality (Nirmala *et al.*, 2006).

Efficiency of entomopathogens in the field depends upon virulence towards target pest, coverage and persistence on target site. However, major constraints for successful use of such bio-agents are their difficulties in use of pure cultures, survival on crop after application due to short shelf life, loosing virulence by ultra violet (UV) rays and dependability on the prevailing environmental conditions are the problems reported by Kaur *et al*. (1999). The foregoing problem can largely be overcome by developing suitable WP, suspension, granules etc. formulations. The performance and shelf-life can be improved by adding suitable adjuvants leading to growth, development and viability of the fungus that may act as nutrient, adhesive, UV protectants or wetting agents etc. Chocking of nozzles, hesitation of customers and exporters to purchase fruits and vegetables with WP spots resulted in strong demand for aqua suspension formulation of entomopathogens including fungi. Presently, crude suspensions of the fungi with short shelf-life of around one to two months are marketed. More aged preparations loss their viability due to submerging the floating fungi. For developing aqua suspension formulation, basic research on standardization of bioactive ingredient and suitable adjuvants is necessary before formulating the object. Hence, considering the eco-friendly properties of *B. bassiana*, there is a need to develop a viable liquid formulation of *B. bassiana* for pest management. Hence, the present study was undertaken with an object to determine the effectiveness of aqua suspension formulations of *B. bassiana* against II and III instar larvae of *Helicoverpa armigera*.

MATERIAL AND METHODS

The present investigation was carried out at Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra State, India during year 2009-2011.

Fungus culture :

The pure fungus culture of *B. bassiana* was available at Biocontrol Research Laboratory, Department of Entomology., Mahatma Phule Krishi Vidyapeeth, Ahmednagar, Rahuri.

Medium :

The medium used for multiplication and growth of the fungus was potato dextrose broth.

Maintenance of culture :

Sabouarauds dextrose agar (SDAY) used by Pandey and Kanaujia (2005) and potato dextrose agar (PDA) suggested by Nirmala *et al.* (2005) was utilized to maintain the culture.

Aqua suspension formulations of *B.bassiana*:

The highly promising 8 formulations with glycerol (2%) + carboxymethyl cellulose (0.5%), glycerol (2%) + honey (1%), sunflower oil (1%) + carboxymethyl cellulose (0.5%), sunflower oil (1%) + honey (1%), glycerol (2%)+ boric acid(2%), glycerol (2%) + boric acid (2%) + tween-80 (0.5%), sunflower oil (1%), groundnut oil (1%) and control without adjuvants (*B.bassiana* alone) of *B.bassiana* were tested for their bioeffficacy against II and III instar larvae of *H. armigera.*

Bioefficacy of aqua suspension formulations of *B. bassiana*:

The laboratory experiment was conducted with promising 8 formulations of *B.bassiana* as mentioned above and control (*B. bassiana* alone) in CRD with three replications against II and III and instar larvae of *H. armigera*. Ten II and III instar larvae were released in

plastic container (50 ml capacity) with over night soaked 4 g seeds per container as food for initial 3 days.Then each larva was transferred to separate formalin 2 per cent and UV sterilized plastic vial (6 x 4 cm) to avoid growth of other micro-organisms. One ml of each of the preparations was mixed in 999 ml of water and sprayed on the gram seeds using hand atomizer (sprayer) and allowed to dry for 15 minutes. Four soaked gram seeds were provided/vial as food. The vials were changed at 2 days interval. The data on larval mortality was recorded at 5, 7 and 10 days after treatment (DAT) and were transefered to arcsin transformation (Gomez and Gomez, 1984). The experimental data were then subjected to statistical analysis.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Bioefficacy against II instar larvae of *H. armigera*:

The data presented in Table 1 and Fig. 1 revealed that the larval mortality among the treatments was 26.27 to 53.33, 46.67 to 73.33 and 63.33 to 90.00 per cent at 5, 7 and 10 DAT, respectively. The differences in mortality

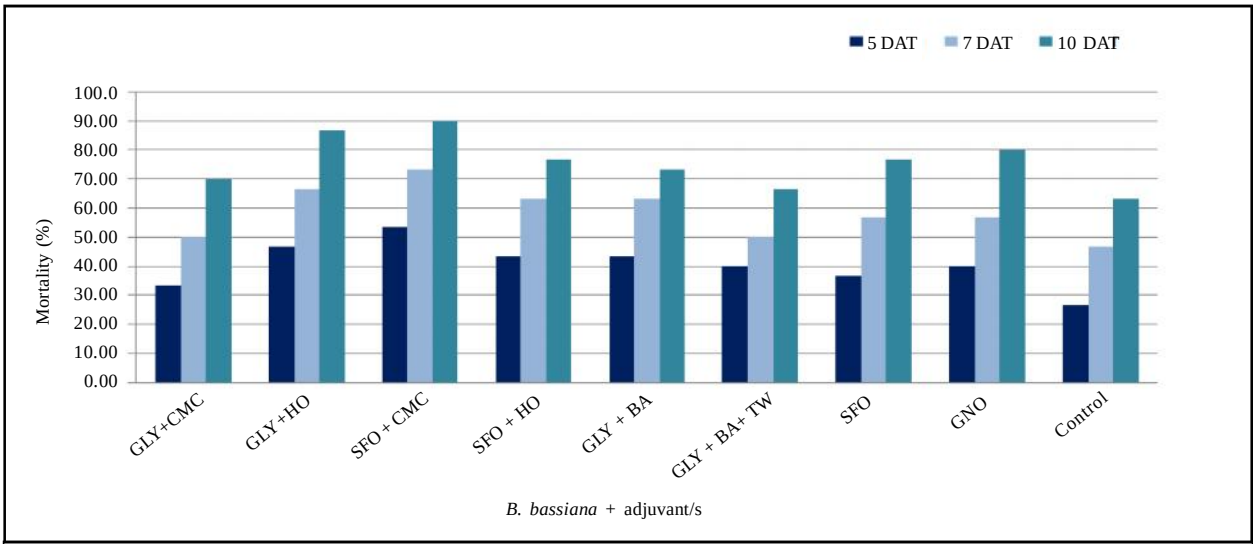

Fig. 1 : Influence of promising formulation of *B. bassiana* (AS) on mortality of II instar larvae of *H. armigera*

Table 1 : Effect of advanced test formulations of *B. bassiana* (AS) on mortality of II instar larvae of *H. armigera*

Tr. No	Treatments**	Conc. (%) of adjuvants	Larval mortality (%)		
			5 DAT	7 DAT	10 DAT
T_1	*B.b.* + GLY+CMC	2.0+0.5	33.33 (35.00)*	50.00 (45.00)	70.00 (56.78)
T_2	*B.b.* + GLY+HO	2.0+1.0	46.67 (43.07)	66.67 (54.78)	86.67 (68.85)
T_3	*B.b.* + SFO+CMC	1.0+0.5	53.33 (46.92)	73.33 (59.00)	90.00 (71.56)
T_4	*B.b.* + SFO+HO	1.0+1.0	43.33 (41.15)	63.33 (52.77)	76.67 (61.22)
T_5	*B.b.* + GLY+BA	2.0+2.0	43.33 (41.15)	63.33 (52.77)	73.33 (59.00)
T_6	*B.b.* + GLY+BA+TW	2.0+2.0+0.5	40.00 (39.14)	50.00 (45.00)	66.67 (54.78)
T_7	*B.b.* + SFO	1.0	36.67 (37.22)	56.67 (48.84)	76.67 (61.22)
T_8	*B.b.* + GNO	1.0	40.00 (39.14)	56.67 (48.84)	80.00 (63.43)
T_9	Control (*B.b.* alone)	-	26.67 (30.99)	46.67 (43.07)	63.33 (52.77)
T_{10}	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E. ±		2.21	2.45	1.82
	C.D. (P=0.05)		6.58	7.29	5.43

***** Figures in the parentheses indicate arcsin transformed values

* * *B.b.*= *Beauveria bassiana*, DAT = Days after treatment, TW = Tween 80, CMC = Carboxymethyl cellulose, GLY = Glycerol, SFO = Sunflower oil, HO = Honey, GNO = Groundnut oil, BA = Boric acid

among the treatments were significant and all the treatments were significantly superior to untreated control, water spray (0.00%).

At 5 DAT the formulation T_3- *B.b.*+ SFO + CMC registered significantly highest mortality of 53.33 per cent. However, it was at par with that in formulations T_2- *B.b.*+ GLY + HO, T_4- *B.b.*+ SFO + HO and T_5- *B.b.*+ GLY + BA (43.33 to 46.67%). The next promising treatments in their order of effectiveness were the formulation T_6- *B.b.*+ GLY + BA + TW (40.00 %), T_8- *B.b.*+ GNO (40.00 %), T_7- *B.b.*+ SFO (36.67 %) and T_1- *B.b.*+ GLY + CMC (33.33 %). In control (*B.b.* alone) lowest (26.67 %) mortality was observed. The trend of effectiveness at 7 DAT was more or less similar to that was observed at 5 DAT.

At 10 DAT significantly highest (90.00 %) larval mortality was recorded in the formulation T_3- *B.b.*+ SFO + CMC which was at par to the formulation with T_2- *B.b.*+ GLY + HO (86.67 %). The later was at par to the formulation containing groundnut oil (T_3) (80.00%). The

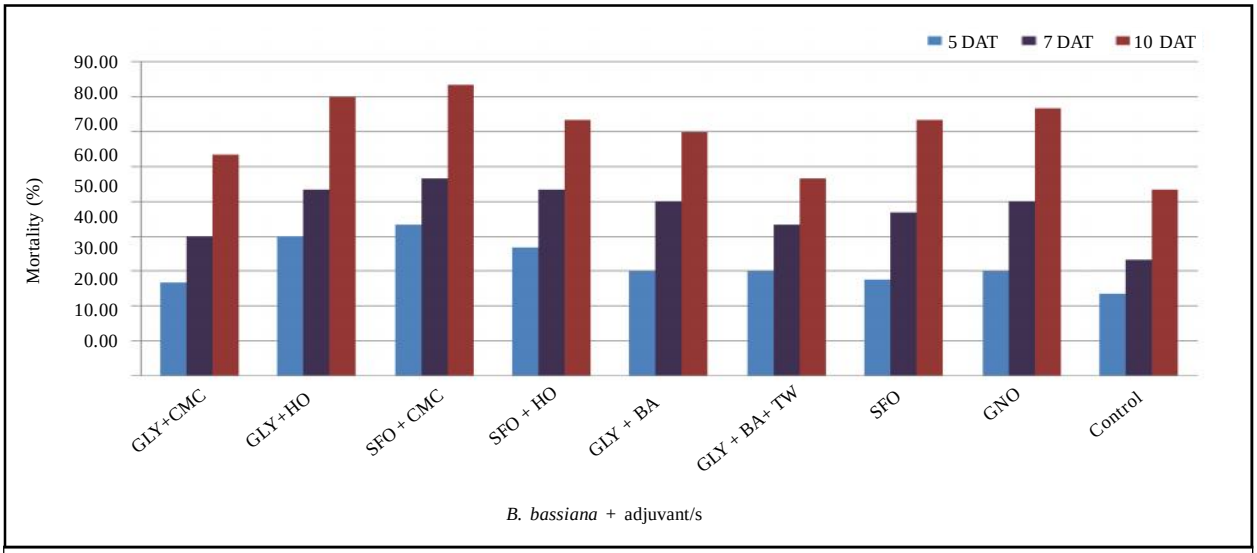

Fig. 2 : Influence of advanced test formulations of *B. bassiana* (AS) on mortality of III instar larvae of *H. armigera*

Table 2: Effect of advanced test formulations of *B. bassiana* (AS) on mortality of III instar larvae of *H. armigera*

Tr. No.	Treatments**	Conc. (%) of adjuvants	Larval mortality (%)		
			5 DAT	7 DAT	10 DAT
T_1	B.b. + GLY+CMC	2.0+0.5	26.67 (30.99)*	40.00 (39.14)	63.33 (52.77)
T_2	B.b. + GLY+HO	2.0+1.0	40.00 (39.14)	53.33 (46.92)	80.00 (63.43)
T_3	B.b. + SFO+CMC	1.0+0.5	43.33 (41.15)	56.67 (48.84)	83.33 (66.14)
T_4	B.b. + SFO+HO	1.0+1.0	36.67 (37.22)	53.33 (46.92)	73.33 (59.00)
T_5	B.b. + GLY+BA	2.0+2.0	30.00 (33.21)	50.00 (45.00)	70.00 (56.99)
T_6	B.b. + GLY+BA+TW	2.0+2.0+0.5	30.00 (33.00)	43.33 (41.15)	56.67 (48.84)
T_7	B.b. + SFO	1.0	27.50 (31.63)	46.67 (43.07)	73.33 (59.00)
T_8	B.b. + GNO	1.0	30.00 (33.21)	50.00 (45.00)	76.67 (61.22)
T_9	Control (B.b. alone)	-	23.33 (28.78)	33.33 (35.00)	53.33 (46.92)
T_{10}	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E. ±		2.83	2.42	2.28
	C.D. (P=0.05)		8.50	7.19	6.78

* Figures in the parentheses indicate arcsin transformed values

* * B.b.= *Beauveria bassiana*, DAT = Days after treatment, TW = Tween 80, CMC = Carboxymethyl cellulose, GLY = Glycerol, SFO = Sunflower oil, HO = Honey, GNO = Groundnut oil, BA = Boric acid

next promising formulations for satisfactory larval mortalities were T_4- *B.b.*+ SFO + HO (76.67 %), T_7- *B.b.*+ SFO (76.67 %), T_5- *B.b.*+ GLY + BA (73.33 %), T_1- *B.b.*+ GLY + CMC (70.00 %) and T_6- *B.b.*+ GLY + BA + TW (66.67 %). *B. bassiana* alone showed lowest kill (63.33%) of the pest.

Bioefficacy against III instar larvae of *H. armigera*:

The data on the mortality of III instar larvae of *H. armigera* was 23.33 to 43.33, 33.33 to 56.67 and 53.33 to 83.33 per cent at 5, 7 and 10 DAT, respectively (Table 2 and Fig. 2). At 5 DAT, the formulation with T_3- *B.b.*+ SFO + CMC registered significantly highest (43.33 %) larval mortality and that in formulations with T_2- *B.b.*+ GLY + HO (40.00 %), T_4- *B.b.*+ SFO + HO (36.67 %) and T_5- *B.b.*+ GLY + BA (30.00 %), T_6- *B.b.*+ GLY + BA + TW (30.00 %) and T_8- *B.b.*+ GNO (30.00 %) was on par with it. The lowest (23.33%) larval mortality was noticed in *B. bassiana* alone. The trend of larval kill at 7 DAT was more or less similar to that was observed at 5 DAT.

At 10 DAT all the formulations were significantly superior (56.67 to 83.33 %) to control (*B.b.* alone) (53.33%) for the lethal effect. The formulation T_3- *B.b.*+ SFO + CMC recorded highest (83.33 %) larval mortality and that T_2- *B.b.*+ GLY + HO (80.00 %) and T_8- *B.b.*+ GNO (76.67 %) were at par to it. The next promising formulations were T_4-*B.b.*+ SFO + HO (73.33 %), T_7- *B.b.*+ SFO (73.33 %) and T_5- *B.b.*+ GLY + BA (70.00 %) which were at par with formulation T_2- *B.b.*+ GLY + HO. The control (*B. b. alone*) recorded lowest (53.33 %) mortality of the caterpillar.

It was evident from the study on the bioefficacy of the promising formulations that the formulations with T_2- *B.b.*+ GLY (2%) + HO (1%) and T_3- *B.b.*+ SFO (1%) + CMC (0.5%) were most promising formulations which included the combinations of glycerol, honey, sunflower and carboxymethyl cellulose with mycoagent *B. bassiana*.

The above findings confirmed the effectiveness of *B. bassiana* and its oil based formulations against *H. armigera*. Nahar *et al*. (2004) reported that oil based conidia formulations of indigenous isolates of *M. anisopliae*, *B. bassiana* and *N. rileyi* were effective against *H. armigera* infesting pigeonpea. Gopalakrishnan and Narayanan (1990) found that *B. bassiana* was pathogenic to all stages of *H. armigera* infecting 60-100 and 100 % mortality, respectively, to the larval instars I-IV. Devi and Hari (2009) reported that when *B. bassiana* isolates were formulated in sunflower, groundnut and mineral oils were superior to unformulated conidia as reflected by the higher mortality of *H. armigera* larvae in laboratory bioassay and field trial on sunflower. At 9 DAT groundnut oil SC of *B. bassiana* recorded 93.3 per cent mortality of 6 day old *H. armigera* larvae. However, sunflower oil caused 86.7 per cent mortality. Observations on larval mortality from 2 to 9 DAT showed that both the *B. bassiana* isolates formulated in oils were equally effective against *H. armigera* and superior in performance to unformulated conidia.

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