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Evaluation of different formulations of entomopathogenic fungi against bhendi aphid, *Aphis gossypii* (Glover) under laboratory conditions

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ABSTRACT: Oil and granular formulations of three entomopathogenic fungi viz., *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticilium lecanii* were evaluated against the bhendi aphid, *Aphis gossypii.* Results showed that the dose 1×10^8 conidia/ml of *M. anisopliae* + glycerol + sunflower oil combination exhibited highest per cent mortality (73.33%) at 10 DAT in oil formulation compare to *B. bassiana*,+ glycerol + sunflower (60.00%) followed by *V. lecanii* + glycerol + sunflower (53.33%) respectively. Wherein granular formulation the same *M. anisopliae* at 10 g dose demonstrated highest mortality (53.33%) at 10 DAT respectively.

Keywords: Entomopathogen, okra, aphid, *A. gossypii*, oil and granular formulation

INTRODUCTION

Okra (*Abelmoschus esculentus*) or Bhendi is an important vegetable crop in tropical countries and India ranks first in production. Aphid, *Aphis gossypii* (Glover), a polyphagous species, is a one of the most important sucking pests on bhendi (Liang *et al.,* 2019). In general, these insects cause damage by sucking the plant sap and producing honeydew on fruits and leaves which lead to sooty mould production that directly affect photosynthesis resulting in severe yield loss and reduced market value (Carletto *et al.,* 2010). The key method for controlling of *A. gossypii* primarily relies on chemical insecticides such as organophosphates, carbamate, pyrethroids, and neonicotinoids. But the frequent and extreme utilization of these chemicals result in development of resistance, resurgence and residual effects.Thus, leads the researchers to find an alternative method of pest management practices and which must be effective, environmentally safe and economically feasible and entomopathogenic fungi (EPF) are a viable strategy for insect control. The pathogenicity of three entomopathogenic fungi viz., *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metsch.) Sorokin and *Verticillium lecanii*(Zimm.) was proven against sucking insect pests by many researchers (Selvaraj*et al.,* 2012; Sayed, 2019). This study is attempted to evaluate the pathogenicity of oil and granular formulations of three different isolates of EPF, *B. bassiana*, *M. anisopliae* and *V. lecanii* against bhendi aphid, *A. gossypii*.

MATERIALS AND METHODS

The aphids were collected from bhendi ecosystem of horticultural farm at the Faculty of Agriculture, Annamalai University, Tamil Nadu, India in 2018-19. The field collected aphids were released on potted bhendi plants (30-40 days) covered with mesh sleeves in at 23±2 °C and with 16:8h daily photoperiod for several generations. Plants were changed every 2 weeks by healthy plants. To maintain same age group of population adult aphids were transferred onto separate pot by using fine camel hair brush. The adults were then collected for bioassay and the newborn nymphs were left to develop on plant until use (Mohammed *et al.,* 2018). Potato Dextrose Agar (PDA) with conidial concentration of 1×10^8cfu/ml was prepared. Adjuvant like ten ml of glycerol and one ml of sunflower and mustard oils were added at different combinations to the broth medium containing culture of each separate isolates of *B. bassiana*, *M. anisopliae* and *V. lecanii* (Boruah *et al.,* 2015). Shelled broomcorn millet (Panivaragu) grains were used as solid substrate to prepare granular cultures of *B. bassiana*, *M. anisopliae* and *V. lecanii*. The following procedure was described (Feng and Liang, 2003; Hua and Feng, 2003) the millet grains (15g per 100ml flask) were soaked in water for 30 min at 80°C. Then after rinsing to remove the excess water and dust, the grains were autoclaved for 15 min at 121°C and cooled to room temperature. Then each flask of the autoclaved grains was inoculated with half a plate colony homogenized in 3ml Potato dextrose broth supplemented with 0.5% (v/v) mustard oil. After plugging with vent stoppers, all flasks were incubated for up to 24 days at 15°C and Light: Dark 12: 12. No agitation measures for aeration were taken during the incubation period.

The aphids (30 numbers in each treatment) were released in petri plate containing bhendi leaves placed

on blotting papers. Then insects were treated with the formulations like oil and granular of all the fungi and a water spray as control by using a hand automizer on bhendi leaves containing these insects. The mortality on each insect was recorded from third day to tenth day after spray. The data on per cent mortality were analysed by using statistical tool and data were displayed in tables. Each treatment was replicated three times.

RESULTS AND DISCUSSION

The oil formulation (1×10^8 conidia/ml) of *B. bassiana* had shown pathogenicity against *A. gossypii*. The highest per cent mortality (60.00%) was observed in the combination of *B.bassiana* + glycerol + sunflower oil at 10 DAT followed by mustard oil (40.00%) whereas, the lowest per cent mortality (20.00%) was found with *B.bassiana* applied alone. *M. anisopliae* treated with glycerol + sunflower oil the fungi shown increased percent mortality (73.33%) compared to all other oil were used in the experiment. The dead insects were observed from 3rdDATwith 33.33 per cent mortality at initial stage itself and further gradually increased up to 10th DAT. Wherein *M. anisopliae* + glycerol + mustard combination the result shown the mortality of 53.33 per cent and when *M. anisopliae* treated alone it only produced 33.33 per cent mortality respectively. Among the three EPF, *V. lecanii* has demonstrated the lowest insecticidal activity compared to all the formulations. The highest per cent mortality was observed with the mixture of *V. lecanii*+ glycerol + sunflower oil at the range of 53.33 per cent insecticidal activity. The lowest mortality was observed in *V. lecanii* (alone) with 6.66 per cent after 5 DAT, whereas no mortality observed in first 3 DAT (Table 1).

Table 1. *In vitro* evaluation of oil based bio-formulation of different EPF against *A.gossypii*

Treatment	Dosage	Per cent mortality of aphids			
		3 DAT	5 DAT	7 DAT	10 DAT
B.bassiana (alone)	1×10^8 conidia/ml	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20 (26.56)
B.bassiana+ *G*	1×10^8 conidia/ml	6.66 (9.04)	13.33 (17.80)	20 (26.56)	33.33 (35.01)
B.bassiana + G + S	1×10^8 conidia/ml	20 (26.56)	33.33 (35.01)	40.00 (38.85)	60.00 (51.14)
B.bassiana + M + G	1×10^8 conidia/ml	13.33 (17.80)	20 (26.56)	33.33 (35.01)	40.00 (38.85)
M. anisopliae(alone)	1×10^8 conidia/ml	6.66 (9.04)	13.33 (17.80)	20 (26.56)	33.33 (35.01)
M. anisopliae+ *G*	1×10^8 conidia/ml	13.33 (17.80)	20 (26.56)	20 (26.56)	40.00 (38.85)
M. anisopliae + G + S	1×10^8 conidia/ml	33.33 (35.01)	40.00 (38.85)	60.00 (51.14)	73.33 (59.21)
M. anisopliae + M + G	1×10^8 conidia/ml	20 (26.56)	33.33 (35.01)	46.66 (43.07)	53.33 (46.92)
V.lecanii(alone)	1×10^8 conidia/ml	00.00 (0.286)	6.66 (9.04)	6.66 (9.04)	13.33 (17.80)
V.lecanii+ *G*	1×10^8 conidia/ml	6.66 (9.04)	13.33 (17.80)	20 (26.56)	33.33 (35.01)
V.lecanii + G + S	1×10^8 conidia/ml	20 (26.56)	33.33 (35.01)	46.66 (43.07)	53.33 (43.07)
V.lecanii + M + G	1×10^8 conidia/ml	13.33 (17.80)	20 (26.56)	33.33 (35.01)	40.00 (38.85)
Untreated control (Water spray)	-	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)
C. D (p= 0.05)	-	9.096	5.570	6.086	7.522
SE (d)	-	19.283	11.810	12.901	15.946

Each value is mean of three replications. Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.
G = Glycerol; **S** = Sunflower oil; **M** = Mustard oil ; **DAT** = Days after treatment

Table 2. *In vitro*evaluation of granular formulation of different EPF against *A.gossypii*

Interval (days)	Mortality at dose							
	1g	3g	5g	7g	10g	Control	C. D (p= 0.05)	SE (d)
Beauveria bassiana								
3DAT	00.00 (0.286)	00.00 (0.286)	6.66 (9.04)	6.66 (9.04)	13.33 (17.80)	00.00 (0.286)	1.14	0.5332
5DAT	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	13.33 (17.80)	20 (26.56)	00.00 (0.286)	5.06	2.389
7DAT	6.66 (9.04)	13.33 (17.80)	20 (26.56)	20 (26.56)	26.66 (30.78)	00.00 (0.286)	11.28	5.180
10DAT	13.33 (17.80)	13.33 (17.80)	20 (26.56)	26.66 (30.78)	33.33 (35.01)	00.00 (0.286)	13.81	6.339
Metarhizium anisopliae								
3DAT	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20 (26.56)	26.66 (30.78)	00.00 (0.286)	7.56	3.450
5DAT	6.66 (9.04)	13.33 (17.80)	20 (26.56)	26.66 (30.78)	33.33 (35.01)	00.00 (0.286)	9.43	6.321
7DAT	13.33 (17.80)	20 (26.56)	26.66 (30.78)	33.33 (35.01)	40.00 (38.85)	00.00 (0.286)	11.24	8.110
10DAT	20 (26.56)	26.66 (30.78)	33.33 (35.01)	40.00 (38.85)	53.33 (43.07)	00.00 (0.286)	13.43	11.22
Verticillium lecanii								
3DAT	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	6.66 (9.04)	6.66 (9.04)	00.00 (0.286)	2.32	1.501
5DAT	00.00 (0.286)	6.66 (9.04)	6.66 (9.04)	13.33 (17.80)	13.33 (17.80)	00.00 (0.286)	7.52	3.421
7DAT	6.66 (9.04)	6.66 (9.04	13.33 (17.80)	20 (26.56)	20 (26.56)	00.00 (0.286)	11.33	5.240
10DAT	6.66 (9.04)	13.33 (17.80)	20 (26.56)	20 (26.56)	26.66 (30.78)	00.00 (0.286)	12.24	6.201

Each value is mean of three replications. Figures in parentheses are arc sine transformed values. In a column means followed by a common letter are not significantly different (P=0.05) by DMRT.

The granular formulation of all three EPF produced very wide range of deviation where maximum per cent mortality (53.33%) was exhibited in *M. anisopliae* at 10g dose at 10 days after treatment. Wherein *B. Bassiana* was (33.33%) followed by *V. lecanii* (26.66%) respectively. There was zero per cent mortality with 1g dose. The *In vitro* study of these three mycoinsecticides resulted that all tested fungi (*B. bassiana*, *M. anisopliae* and *V. lecanii*) were significantly produced toxic effect against *Aphis gossypii.* Among the two different formulation (oil and granular) were tested oil formulation of *M. anisopliae* + glycerol + sunflower was resulted best insecticidal activity than granular formulation. This might be due to the reason that addition of oils and adjuvants increases infectivity of entomopathogenic fungi by enhancing conidial adherence and prolonged persistence (Visalaksy *et al.,* 2006). Somervaille*et al*. (2012) reported that adjuvants lower the surface tension of spray droplet which will help in better retention of spray droplet on the plant surface. The present study also revealed that *M. anisopliae*+ Glycerol + Sunflower oil were better in causing mortalities of *Aphis gossypii.*

The present results are in agreement with Hidalgo *et al*. (1998) who reported that rape seed oil containing 1×10^{10} conidia of *B. bassiana* gave 100% mortality of *Sitophilus zeamais* in maize. Malsame*et al*. (2002) reported that 100% mortality of whitefly, *Trialeurodes vaporariorum* (Westwood) with *Metarhizium*+ sunflower oil. Sabbour and Shadia (2007) reported that 74.2% and 60.1% mortality of broad bean beetle, *Bruchus rufimanus* (Boheman) with mustard oil and nigella oil, respectively in cowpea.

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