Pest Management in Horticultural Ecosystems
Vol. 25, No.2 pp 224-228 (2019)

Pathogenicity and formulation of three entomopathogenic fungi for the management of bhendi leafhopper, *Amrasca biguttula biguttula* (Ishida)

M. SENTHILKUMAR., M .PAZHANISAMY, and V. SATHYASEELAN

Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, India

E-mail: senthilvasum2014@yahoo.co.in

ABSTRACT: The *In vitro* evaluation of three mycoinsecticides resulted that all tested fungi (*Beauveria bassiana*, *Metarhizium anisopliae* and *Verticilium lecanii*) produced toxic effect against *A.b. biguttula.* Among the three different formulation (crude, oil and granular) tested, oil formulation of *M. anisopliae* + coconut oil was effective compared to both crude and granular formulations.

Keywords: Entomopathogenic fungi - *A.biguttula biguttula*, crude, oil & granular formulation

INTRODUCTION

Bhendi crop is prone to attack by more than 72 insect species and leafhopper, *Amrasca biguttula biguttula*, is one of the important pests. The excessive use of insecticides has caused the development of resistance and resurgence in sucking insects (Rohini *et al.,* 2012). Considering these drawbacks, alternative options are being explored. To overcome these problems recommendation of entomopathogenic fungi would be better option because they have ability to infect the sucking insects under natural conditions (Harischandra Naik and Shekharappa, 2009). In general, more than 60 biocontrol agents along with 38 species Entomopathogenic fungi were used for management of insect pests around the world (Faria and Wraight 2007). The mycoinsecticidal activty of three different isolates of entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metsch.) Sorokin and *Verticillium lecanii* (Zimm.) already have been reported to be effective against sucking insect pests by (Saranya *et al.,* 2010; Sayed, 2019). This experiment was conducted to test the pathogenicity and effect of different formulations of three different entomopathogenic fungi such as, *B. bassiana*, *M. anisopliae* and *V. lecanii* against bhendi leafhopper (*Amrasca biguttula biguttula*).

MATERIALS AND METHODS

Culturing of test insects

Adults of *A. b. biguttula* was collected from bhendi field in the horticultural farm of Annamalai university, Faculty of Agriculture, Tamil Nadu, India by using sweep net. Then which were established on 30 days okra plants covered with cages (1.0 m height x 1.5 m long). Plants were changed every 2 weeks by healthy plants. To maintain same age group, adult leafhoppers were transferred onto separate pot by using aspirator. The adults were then collected for bioassay and the newborn nymphs were left to develop on plant until use (Al-Hamadany and Al-Karboli, 2017).

Preparation of crude formulation

For preparing crude formulation 250 ml of SDAY broth was taken in 500 ml round bottom flask and autoclaved at 121 ^{o}C for 20 minutes. After cooling, 1.0 ml of spore suspension (10^{8} spores ml^{-1}) of each fungal isolate was inoculated into a separate conical flask and incubated at room temperature for a week. After sporulation, the fungus along with broth was ground in a mixer and filtered through double layered muslin cloth. The suspension was shaken thoroughly with 0.25 ml of Tween 80® in order to disperse the spores in solution. The conidial suspension was vortexes for 5 min to produce a homogenous conidial suspension and utilized for field evaluation immediately (Saranya *et al.,* 2013).

Potato Dextrose Agar (PDA) with conidial concentration of 1×10^{8} cfu/ml was prepared. Adjuvant like ten ml of glycerol and one ml of oils like sunflower, mustard and coconut oil were added at different combination to the broth medium containing culture of each separate isolates of *B. bassiana*, *M. anisopliae* and *V. lecanii* (Ummidi and Vadlamani, 2014; 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)

Table 1. Efficacy of different formulation of *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* against *A.b.biguttula* in bhendi

Treatments	Dosage	Percent mortality of Leafhoppers					
		1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
Crude formulation							
B.bassiana	1×10^8 cfu/ml	00.00 (0.286)	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20 (26.56)	33.33 (35.01)
M. anisopliae	1×10^8 cfu /ml	00.00 (0.286)	6.66 (9.04)	6.66 (9.04)	13.33 (17.80)	13.33 (17.80)	26.66 (30.78)
V.lecanii	1×10^8 cfu /ml	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20.00 (26.56)
Oil formulation **(Sun flower)**							
B.bassiana	1×10^8 cfu /ml	6.66 (9.04)	13.33 (17.80)	33.33 (35.01)	40.00 (38.85)	53.33 (43.07)	66.66 (54.99)
M. anisopliae	1×10^8 cfu /ml	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20.00 (26.56)	26.66 (30.78)	40.00 (38.85)
V.lecanii	1×10^8 cfu /ml	00.00 (0.286)	6.66 (9.04)	6.66 (9.04)	13.33 (17.80)	20 (26.56)	33.33 (35.01)
Granular formulation							
B.bassiana	1×10^8 cfu /ml	00.00 (0.286)	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	13.33 (17.80)	26.66 (30.78)
M. anisopliae	1×10^8 cfu /ml	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20.00 (26.56)
V.lecanii	1×10^8 cfu /ml	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)
Untreated control (Water spray)	-	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)
C. D (p= 0.05)	-	**8.121**	6.422	7.353	7.442	8.501	9.096
SE (d)	-	**18.113**	12.312	14.360	14.652	18.433	19.283

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.

Table 2. Effect of different oil formulation against*A.b.biguttula* in bhendi

Treatments	Dosage	Percent mortality of Leafhoppers					
		1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	12 DAT
B.bassiana							
B.bassiana + Sunflower oil	1×10^8 cfu/ml	6.66 (9.04)	13.33 (17.80)	33.33 (35.01)	40.00 (38.85)	53.33 (43.07)	66.66 (54.99)
B.bassiana + Mustard oil	1×10^8 cfu /ml	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20.00 (26.56)	20.00 (26.56)	26.66 (30.78)
B.bassiana + Coconut oil	1×10^8 cfu /ml	00.00 (0.286)	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20.00 (26.56)	20.00 (26.56)
M. anisopliae							
M. anisopliae + Sunflower oil	1×10^8 cfu/ml	00.00 (0.286)	6.66 (9.04)	6.66 (9.04)	13.33 (17.80)	20.00 (26.56)	26.66 (30.78)
M. anisopliae + Mustard oil	1×10^8 cfu /ml	6.66 (9.04)	6.66 (9.04)	13.33 (17.80	20.00 (26.56)	26.66 (30.78)	33.33 (35.01)
M. anisopliae + Coconut oil	1×10^8 cfu /ml	6.66 (9.04)	26.66 (30.78)	33.33 (35.01)	53.33 (43.07)	66.66 (54.99)	73.33 (59.21)
V.lecanii							
V.lecanii + Sunflower oil	1×10^8 cfu/ml	00.00 (0.286)	6.66 (9.04)	6.66 (9.04)	13.33 (17.80	20.00 (26.56)	33.33 (35.01)
V.lecanii + Mustard oil	1×10^8 cfu /ml	00.00 (0.286)	6.66 (9.04)	13.33 (17.80)	20 (26.56)	33.33 (35.01)	40.00 (38.85)
V.lecanii + Coconut oil	1×10^8 cfu /ml	6.66 (9.04)	13.33 (17.80)	20.00 (26.56)	33.33 (35.01)	40.00 (38.85)	46.66 (42.70)
Untreated control (Water spray)	-	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)	00.00 (0.286)
C. D (p= 0.05)	-	**9.322**	5.302	6.452	7.310	8.004	8.498
SE (d)	-	**19.213**	10.210	12.533	14.427	18.528	19.531

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.

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 young leafhoppers (30 numbers in each treatment) were released in petriplate containing bhendi leaves placed on blotting papers. Then insects were treated with the formulations like crude, 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 insects 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

Among the different formulation evaluated (Crude, Oil and Granular) the oil formulation at the dose 1×10^8 conidia/ml of *M. anisopliae* hadshown maximum mycoinsecticidal activity against *A.b.biguttula*. The highest percent mortality (73.33%) was observed in the combination of *M. anisopliae* + coconut oil at 12 DAT. Followed by *B.bassiana* + sunflower oil produced 66.66 per cent mortality. Whereas, the lowest percent mortality (33.33%) was found in *V. lecanii* + sunflower oil respectively. In the crude formulation treatment the highest per cent mortality (33.33%) was observed in *B.bassiana* followed by 26.66 per cent mortality found in *M. anisopliae* wherein lowest dead was exhibited by *V. lecanii* at 20.00 per cent respectively.

The granular formulation of *B.bassiana* demonstrated by 26.66 per cent mortality was the highest in that formulation followed by *M. anisopliae* exhibited 20.00 per cent mortality and lowest (13.33 %) was observed in *V. lecanii* (Table 1). *B. bassiana* along with Sunflower oil shown increased percent mortality than compare to castor, mustard and coconut oil whereas when *M. anisopliae* were applied with coconut oil shown to maximum dead per cent (73.33%) compare to all other oil were used in the experiment. The dead insects were observed from 1st DAT with 6.66 per cent mortality at initial stage and further gradually increased up to 12th DAT with 73.33 per cent mortality (Table 2). Among the tested three EPF, *M. anisopliae* was best in result when applied as oil formulation along with coconut oil followed by *B. bassiana* also shown acceptable effect (66.66%) when mixed with sunflower oil whereas very lowest insecticidal activity was demonstrated by both castor and mustard oil in all the EPF were used.

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 *A.b. biguttula.* Among the three different formulation (crude, oil and granular) were tested oil formulation of *M. anisopliae* + coconut oil was resulted best insecticidal activity than both crude and granular formulations. 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 (Meyer *et al*., 2002; 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*+ coconut oil were better in causing mortalities of *A. b.biguttula.*

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.

REFERENCES

Al-Hamadany M N and Al-Karboli H H. 2017. First Record of Okra leafhopper, *Amrasca biguttula biguttula* Ishida on Okra in Iraq. *International journal of Agricultural Technology,* **13** (3): 393-402

Boruah S, Dutta P, Puzari K C and Hazarika G N. 2015. Liquid bioformulation of *Metarhizium anisopliae* is effective for tha management of cowpea mosaic disease. *International journal of Applied Biology and Pharmaceutical Technology,* **6**(1): 178 – 185.

Faria M R and Wraight S P. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control*, **43**:237-256.

Feng M G and Liang Y.2003. Biological aspects on the cultures of the entomphthoralean fungus *Pandora delphacis* grown on broomcorn millets. *Chinese Science Bulletin*, **48:** 1816 – 1821

Harischandra Naik P R and Shekharappa. 2009. Field evaluation of different entomopathogenic fungal formulations against sucking pests of okra. *Karnataka Journal of Agricultural Science,* **22** (3): (575-578).

Hidalgo E, Moore D and Patourel G. 1998. The effect of different formulation of formulation of *B. bassiana* on *sitophilus zeamais* in stored maize. *Journal of Stored product Research,* **34(**2-3): 171 -179

Hua L and Feng M G. 2003. New use of broomcorn millets for production of granular cultures of aphid-pathogenic fungus *Pandora neoaphidis* for high sporulation potential and infectivity to *Myzus persicae*. *FEMS Microbiology letter*, **227:** 311 – 317

Malsame O, Michael K, Erich-Christian O and Heinz – Wilhelm D. 2002. Oils for increased efficacy of *M. anisopliae* to control whiteflies. *Biocontrol Science and Technol*ogy, **12**(3): 337 – 348.

Meyer U, Sermann H and Buettner C. 2002. Spore adhesion of entomopathogenic fungi to larvae of *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae). 54th International Symposium on Crop Protection, Part II, Gent, Belgiumm, pp.601 – 607

Rohini A, Prasad N V V S D and Chalam M S V. 2012. Management of major sucking pests in cotton by insecticides. *Journal of Cotton Research and Development,* **20**: 102-106.

Sabbour M M and Shadia E. 2007. Efficacy of some bioinsecticide against broad bean beetle, *Bruchus rufimanus* (Coleoptera: Bruchidae). *Research Journal of Agriculture and Biological Sciences*, **3**(2):67 – 72.

Saranya S, Ushakumari R, Jacob S and Philip B M. 2010. Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch). *Journal of Biopesticides* 3(1):138–142

Sayed S M, Ali E F and Al-Otaibi S S. 2019. Efficacy of indigenous entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, isolates against the rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae) in rose production. *Egypt Journal of Biological Pest Control*, **29**:19

Somervaille A, Gorden, B, Green V, Burgis M and Henderson R. 2012. Adjuvants – oils, surfactants and other additives for farm chemicals. *Grains Research and Development Corporation*. p.48.

Ummidi V R S and Vadlamani P. 2014. Preparation and use of oil formulations of *Beauveria bassiana* and *Metarhizium anisopliae* against *Spodoptera litura* larvae. *African Journal of Microbiology Research,* **8** (15): 1638 - 1644.

Visalakshy P N, Krishnamoorthy A and Kumar A M. 2006. Compatibility of plant oils and additives with *Paecilomyces farinosus*, a potential entomopathogenic fungus. *Journal of Food Agriculture and Environ*ment, **4**(1):333 – 335

MS Received 19 August 2019
MS Accepted 14 October 2019