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Research Article

Compatibility of insecticides with *Metarhizium brunneum* (Petch) and *Beauveria bassiana* (Bals.) for bio-intensive management of pink mealybug, *Maconellicoccus hirsutus* (Green) in grapes

DEEPENDRA SINGH YADAV*, YOGITA RANADE, SAGAR MHASKE and SHASHIKANT GHULE
ICAR-National Research Centre for Grapes, Manjari Farm PO, Solapur Road, Pune -412307, Maharashtra, India
**Corresponding author E-mail*: deependra.yadav@icar.gov.in

ABSTRACT: Grape (*Vitis vinifera* Linnaeus) is a high-value crop and important as a valuable export commodity for India. Pink mealybug, *Maconellicoccus hirsutus* (Green) is one of the most important pests infesting grapes. Two entomopathogenic fungi were isolated from the field infected insects and were identified as *Metarhizium brunneum* (Petch) and *Beauveria bassiana* (Bals.). The pathogenicity study showed that both the fungi were capable of infecting *M. hirsutus*. LC_{50} values 1.4×10^6 and 1.0×10^7 conidia per ml was recorded for *M. brunneum* and *B. bassiana,* respectively. Evaluation of compatibility of these fungi with insecticides is important to develop bio-intensive management strategy for mealybugs. The compatibility of seven insecticides (emamectin benzoate, tolfenpyrad, imidacloprid, clothianidin, buprofezin, fipronil, spirotetramat) with these entomopathogens was evaluated under laboratory conditions. Compatibility studies based on sporulation, germination and vegetative growth of fungi showed that imidacloprid and emamectin benzoate were most compatible and tolfenpyrad and spirotetramat were highly incompatible with both the entomopathogens.

KEYWORDS: Bioassay, compatibility, entomopathogenic fungus, insecticide, mealybug

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INTRODUCTION

Tropical viticulture is tremendously affected by different types of pests and diseases, control of which largely depend on pesticides. Extensive use of chemical insecticides for control of pests not only affects the naturally occurring microbial flora in soil and plants, but also causes imbalance in the ecosystem leading to higher pest incidences. Increase in cost, environment and human safety, development of insecticide resistance in pests has raised the concern for development of new pest management tactics (Orr, 2009). Further, indiscriminate use of pesticides lead to increase in pesticide residues in harvested produce.

Pink mealybug, *Maconellicoccus hirsutus* (Green) is an important mealybug species infesting grapes in India (Yadav and Amala, 2013). *M. hirsutus* initially feeds on the phloem sap from the trunk, cordons and shoots. It migrates to bunches during veraison stage when sugar accumulation in the grape bunches is rapid. The entomopathogenic fungi produces profuse quantity of honeydew leading to sooty and sticky bunches which considerably reduces the quality and marketability of the fruits (Yadav and Amala, 2013). As its infestation is maximum in grapes nearing harvest, insecticides cannot be applied due to pesticide residue risks in the final produce. Therefore, an Integrated Pest Management (IPM) strategy is needed which can manage mealybugs without leading to pesticide residues.

IPM strategies are broadly developed to minimise the substantial use of chemical pesticides by combining biological control agents. These biological agents reduce the pest population significantly. Entomopathogenic fungi, *Metarhizium* and *Beauveria* are largely studied biocontrol agents and have the ability of being a potential ingredient in IPM programme (Chandler *et al*., 2011). Mass multiplication and production of commercial formulation of these entomopathogens is comparatively simple (Tajick Ghanbary *et al*., 2009). Studies on colonisation of these fungi in different plant parts (Greenfield *et al.*, 2016) and plant tissue localisation (Behie *et al*., 2015) manifest their importance as potent biological agent for plant growth and insect control. Both *Metarhizium* and *Beauveria* act by degrading cuticle of the insect and produces mycolytic enzyme to control the pest (Hatting *et al*., 2004; Wang *et al*., 2004). Species belonging

to genus *Metarhizium* are well known to target large range of host insects (Tiago *et al*., 2014). *Beauveria bassiana* (Bals.) has been shown to have wide host range and is found to be non-pathogenic to natural enemies and beneficial soil microbes (Thungrabeab and Tongma, 2007).

These potential fungi can be included in the IPM programme only if they are compatible with the pesticides that are used. The IPM may get adversely affected if incompatible pesticides, which may curb the vegetative growth and development of fungi, are used (Akbar *et al*., 2012). There are reports of study of compatibility of different fungicides, insecticides and weedicides with different biological control agents (Pelizzaand Scorsetti, 2015; Depieri *et al*., 2005; Faraji *et al*., 2016). Complexity and inconsistency of IPM programmes are high and therefore need much more study to understand and formulate such programmes (Midthassel *et al.*, 2016).

In this study, insects with natural fungal infection were collected from the field. The fungi were isolated on specific growth medium and were subsequently identified. Pathogenicity of the isolated fungi against *M. Hirsutus* was evaluated. Compatibility of different insecticides used in viticulture was also evaluated on isolated two entomopathogenic fungi, *M. brunneum* and *B. bassiana* under laboratory conditions. These studies will help in preparation of strategies for bio-intensive management of *M. hirsutus* in grapes.

MATERIALS AND METHODS

Fungus culture

Metarhizium brunneum used in the study was taken from previously available culture collection of this institute, which was obtained from field infected lepidopteran larva (Nashik, Maharashtra). Combined gene sequence of EF (EF-1α exon + 5'EF-1α intron region), beta-tubulin and RPB2 sequences of *M. brunneum* were submitted to GenBank-NCBI with accession numbers MH711929, MH711930 and MH711931, respectively. *Beauveria bassiana* was isolated from field infected mealy bug in vineyards as per the procedure described by Jaber *et al.* (2016) and stored on Potato Dextrose Agar (PDA) slants at 4°C, until further use.

Fungus identification

Colony growth, appearance and coloration on PDA plates were important traits analysed. To identify the conidia of isolated fungi, the sporulating cultures were stained with lactophenol cotton blue stain and examined under light microscope (*Leica microscope DM2500)*. Isolate was named B1 and was identified by Internal Transcribed Spacer (ITS) gene region sequencing. The fungal culture was inoculated in 200 ml Potato Dextrose Broth (PDB) medium and incubated at 25 ± 1°C for 72 hours. The mycelium was collected by filtering the content through Whatman filter paper No.44 and was further rinsed thrice with sterile distilled water. Deoxyribonucleic acid (DNA) was extracted using DNeasy Plant Mini Kit (Qiagen, GMBH, Germany) following manufacturer's protocol. The primer used was ITS 1 5′-TCTGTAGGTGAACCTGCGG-3′ and ITS4 5′-TCCTCCGCTTATTGATATGC-3′. Polymerase chain reaction (PCR) amplification was performed in 50 µl reaction mixture and PCR was run on a ABI GeneAmp® PCR System 9700. Amplification of ITS region was performed as described earlier (Sawant *et al*., 2017). The PCR product was purified and used directly for sequencing in both directions (Sci Genome Labs, Kochi India). The respective gene region sequences were compared against type cultures at the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) using BLAST search. Isolates with highest hits were retrieved and used for phylogenetic analysis.

Inoculums preparation

The fungal cultures were grown in roux bottle containing 200 ml sterile PDB medium. One disc of 10mm was cut from 4 day old culture on PDA plate and was inoculated in PDB. The bottles were incubated at 25 ± 1°C and 12h photophase. After 15 days of incubation, the fungal mat was blended and filtered through muslin cloth and diluted with Sterile Distilled Water (SDW). Two to three drops of 0.1 per cent Tween 80 were added. The conidia count was adjusted using haemocytometer and was used for further laboratory bioassay.

Bioassay

Maconellicoccus hirsutus was reared in laboratory on pumpkin were used for bioassay. Healthy tender grapevine shoots were detached from insecticide unsprayed plants and washed thoroughly in laboratory with sterile distilled water and air dried for 30minutes. These shoots were then placed in glass Petri plates containing moistened filter paper to maintain humidity. Mealybugs were transferred to these shoots carefully. These mealybugs were sprayed with the suspension of 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia per ml of tested fungi using hand automizer. Untreated control Petri plates were sprayed with 0.1 per cent Tween 80 in water. Each treatment consisted of 10 replicates. Each replicate consisted of 5 adult mealybugs. These insects were monitored on daily basis and mortality was recorded for seven days. The fungal growth was confirmed by staining the conidia on the dead insect with lacto phenol blue and observing under light microscope at 400x magnification. The fungus was re-isolated from these infected insects on PDA plates. The viability of fungi was maintained time to time through strain passage by infecting fresh active mealybugs. Corrected per cent mortality was calculated using Abbott's formula (Abbott, 1925).

Table 1. Insecticides used in the study

Trade Name	Active Ingredient	Formulation	Mode of action	Recommended dose g or ml per litre
Proclaim	Emamectin benzoate	5% SG	Glutamate-Gated Chloride Channel (GLU-CL) Allosteric Modulators (Avermectins)	0.22
Keefun	Tolfenpyrad	15% EC	Mitochondrial Complex I Electron Transport Inhibitors (METI acaricides and insecticides)	1.66
Confidor	Imidacloprid	17.8% SL	Nicotinic Acetylcholine Receptor (NACHR) Competitive Modulators (Neonicotinoid)	0.30
Dantatsu	Clothianidin	50% WDG	Nicotinic Acetylcholine Receptor (NACHR) Competitive Modulators (Neonicotinoid)	0.12
Applaud	Buprofezin	25% SC	Inhibitors of Chitin Biosynthesis	1.25
Regent	Fipronil	5% SC	GABA-Gated Chloride Channel Blockers (Phenylpyrazoles)	1.00
Movento	Spirotetramat	240% SC	Inhibiotrs of Acetyl COA Carboxylase (Tetronic and Tetramic acid derivatives)	0.70

In vitro insecticide compatibility

Insecticides

The insecticides were selected based on their use in grapes. All the short listed insecticides had label claim for use either in grapes or in the process for the same (Table 1). The insecticide concentrations evaluated were, Field Recommendation (1FR), 50 per cent more of average Field Recommendation (1.5 FR), 50 per cent less of Field Recommendation (0.5 FR) and twice the concentration recommendation for field (2 FR).

Conidia germination

Sporulating cultures of fungi were obtained from 15 days old cultures maintained on PDA at 25 ± 1°C. Conidia germination was studied using slide culture technique (Silva *et al*., 2013) on water agar.

Assessment of vegetative growth

Sterile molten PDA was amended with streptomycin (0.5g/l) and insecticide concentration to be tested at 45 ± 5°C and poured into petri plates (Neves *et al*., 2001). Plate without insecticide served as untreated control. After solidification of agar, a 5mm fungal disc from 3-4 day old culture growth was placed at centre of each plate. Five replications were maintained for each test. Plates were incubated at 25 ± 1°C and 12h photophase. After 10 days of incubation the colony diameter was measured in millimetre (mm). Experiment was repeated twice.

Conidia production

After vegetative growth measurement, the central 5 colony discs from each test were drawn for conidia production analysis. Each disc was placed in test tube containing 10ml of sterile distilled water with Tween 80 (0.02 per cent). The tubes were vortexed for 30 seconds to extract the conidia from the disc. Number of conidia per ml were counted using haemocytometer.

Compatibility study

Toxicity of each insecticide was calculated using formula proposed by Alves *et al*., (1998). In this formula calculation of vegetative growth (VG) and sporulation (SP) is made in relation to control (100%): T = [20 (VG) + 80 (SP)]/100. Where T = 0 to 30 (very toxic); 31 to 45 (toxic); 46 to 60 (moderately toxic); >60 (compatible).

Statistical analysis

Lethal concentration (LC_{50}) of was calculated by using Probit analysis (Finney, 1971). Bioassay and compatibility data were analysed in Completely Randomised Design (CRD) with Analysis of Variance (ANOVA) using SAS (ver. 9.3; SAS Institute Inc., Cary, North Carolina, USA). Means were compared by the Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

Fungus identification

Isolate B1 showed flat white growth of the colony on PDA with whitish to creamish sporulation. Conidiophores bearing rounded conidia were observed. The isolate showed 99 per cent similarity with *Beauveria bassiana* (NR_111594) type culture. In phylogenetic analysis, this isolate formed clade with *B. bassiana* (NR_111594) and *B. bassiana* (GU734762) supported with 81 per cent bootstrap value. Thus, B1 was identified as *B. bassiana* (Fig. 1). Sequence was submitted to

GenBank-NCBI with accession number MH793596.

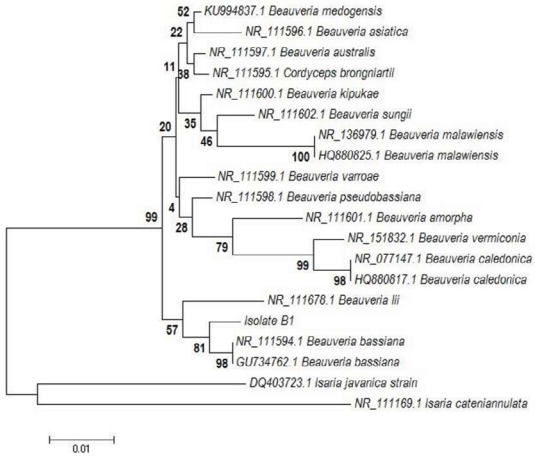

Fig. 1. Phylogenetic tree based on ITS sequences of *Beauveria bassiana* using neighbour-joining (NJ) with Kimura-2-parameter distance model. Numbers above the nodes indicated bootstrap values based on 1000 replicates. The scale below the phylogram indicates degree of dissimilarity.

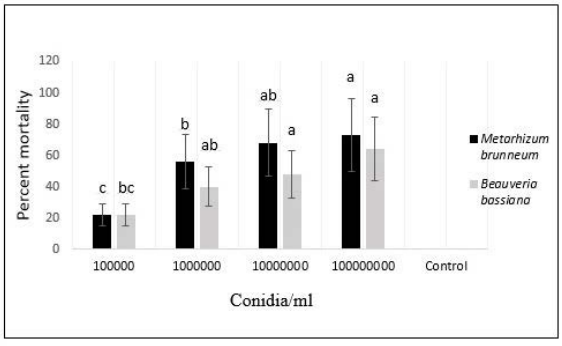

Fig. 2. Per cent mortality of pink mealybug, *Maconellicoccus hirsutus* 7 days after exposure to *Metarhizium brunneum* and B. bassiana at different conidia concentrations. Bars indicate standard error. Lower case letters represent statistically significant difference of larvae at different concentration of conidia. Means followed by same letter do not differ significantly from each other for respective fungus (Tukeys test: $\alpha = 0.05$).

Table 2. Toxicity of entomopathogenic fungi against *Maconellicoccus hirsutus*

Treatment	LC_{50} (Conidia/ml)	95% Fiducial Limits (Conidia/ml)		Slope(± SE)
		Lower	Upper	
Metarhizium brunneum	1.4×10^6	8.5×10^5	$2.4x10^6$	0.51 (0.12)
Beauveria bassiana	1.0×10^7	5.6×10^6	1.7×10^7	0.49 (0.13)

Pathogenicity to mealybug

The mealybugs were exposed to five different treatments including untreated control for both the fungi and mortality was recorded. The mortality of test fungi varied with species of fungi (Fig. 2). LC_{50} value of mealybug treated with *Metarhizium brunneum* was lower as compared to LC_{50} value for *B. bassiana* (Table 2). The mortality in test was significantly higher than the water control. Under moist condition, fungal mycelium growth followed by conidia formation on infected mealybug was seen. The recovered spores of *M. brunneum* and *B. bassiana* were elongated and rounded, respectively.

***In vitro* insecticide compatibility**

Spore germination

The effect of insecticides on germination of spores of *B. bassiana* is depicted in Table 3. Among the 7 insecticides at 4 different concentrations and control (*f=213.16, df=28, p<0.0001*), fipronil inhibited the spore germination significantly. Emamectin benzoate, imidacloprid, buprofezin and spirotetramat showed maximum conidia germination at half the Field Recommended concentrations (0.5FR), followed by clothianidin. At Field Recommended (1FR) dose, emamectin benzoate and spirotetramat showed more than 85 per centspore germination, followed by imidacloprid, buprofezin, clothianidin and tolfenpyrad. At field recommended dose imidacloprid, emamectin benzoate, clothianidin, buprofezin, and fipronil showed maximum conidia germination of *M. brunneum* (*f=237.08, df=28, p<0.0001*) (Table 4). Imidacloprid and fipronil showed effective germination of the spores at double the pesticide concentrations (2FR). Good per cent germination was recorded for all the insecticides at 0.5FR concentrations, however, significant difference in spore germination was seen for tolfenpyrad, which inhibited the conidia germination of *M. brunneum* strongly at all concentrations.

Mycelial growth and spore production

Tolfenpyrad and spirotetramat significantly inhibited the vegetative growth (*f=310.85, df=28*, *p<0.0001*) of *B. bassiana* followed by fipronil. Mycelial growth was maximum with imidacloprid at all the tested concentrations. Clothianidin was also found to support mycelium growth at 1FR, 1.5FR and 0.5FR concentrations. Except for imidacloprid, all the insecticides inhibited the growth of fungi at double the recommended concentration. After 10 days of incubation when central colony discs were taken from each treatment, significant reduction in conidia production was seen by tolfenpyrad, spirotetramat and fipronil. For treatment with imidacloprid, sporulation was higher as compared to control in 1FR and 0.5FR (*f=755.53, df=28*, *p<0.0001*). *M. brunneum* showed higher measurement of vegetative growth

Table 3. Per cent germination (average ± SE), mycelial growth (average ± SE) and conidia production (average ± SE) of strain *Beauveria bassiana* after 10 days, grown on growth media amended with insecticides

Treatment	Conc.	Germination %	(%) Reduction/ increase over Control	Colony Diameter (mm)	(%) Reduction/ increase over Control	Conidia Number ($Xx10^4$)	(%) Reduction/ increase over Control
Emamectin benzoate	1.5 FR	85.25 ± 3.54 abcdef	10.26	21.8 ± 0.66 ij	27.82	61.7 ± 0.84 ed	29.75
	1 FR	90.5 ± 3.79 abc	4.60	26.0 ± 0.29 efg	13.85	64.5 ± 0.82 cd	26.69
	0.5FR	93.75 ± 2.92 ab	0.92	27.4 ± 0.45 cde	9.27	65.6 ± 1.05 cd	26.80
	2FR	29.5 ± 1.65 jk	68.77	20.8 ± 0.24 jk	31.09	54.6 ± 3.09 f	38.29
Tolfenpyrad	1.5 FR	34.5 ± 1.84 j	63.44	13 ± 0.14 n	56.93	0.00 m	100
	1 FR	66.25 ± 2.68 i	30.08	16.2 ± 0.32 m	46.42	0.00 m	100
	0.5FR	73.75 ± 1.88 ghi	22.15	19.1 ± 0.54 kl	36.73	7.3 ± 0.33 l	91.62
	2FR	29.5 ± 1.65 jk	68.77	11.4 ± 0.22 n	62.27	0.00 m	100.00
Imidacloprid	1.5 FR	77.5 ± 1.44 fgh	18.18	28.4 ± 0.16 bcd	5.87	65.4 ± 1.84 cd	24.48
	1 FR	83.5 ± 1.32 bcdefg	11.74	29.3 ± 0.21 ab	2.92	131.1 ± 2.73 a	-49.01
	0.5FR	89.5 ± 0.28 abcd	5.42	29.3 ± 0.26 ab	2.92	131.2 ± 2.77 a	-50.00
	2FR	66.75 ± 1.10 i	29.46	27 ± 0.49 de	10.61	48.6 ± 2.41 gf	45.29
Clothianidin	1.5 FR	78.75 ± 0.75 efg	16.81	27.7 ± 0.21 bcde	8.32	34.2 ± 0.87 i	61.08
	1 FR	79.25 ± 0.50 defg	16.29	29.2 ± 0.2 abc	3.27	50.5 ± 0.77 f	42.25
	0.5FR	82.5 ± 1.44 cdefg	12.77	29.5 ± 0.16 ab	2.21	66 ± 1.22 cd	24.99
	2FR	67 ± 2.34 i	29.20	24.6 ± 0.16 fgh	18.15	29.6 ± 1.19 ij	65.96
Buprofezin	1.5 FR	80.5 ± 0.28 cdefg	14.95	24.2 ± 0.13 gh	19.80	41.8 ± 0.72 gh	52.47
	1 FR	82.75 ± 1.10 cdefg	12.52	24.6 ± 0.26 fgh	18.57	54.6 ± 1.03 ef	37.55
	0.5FR	87 ± 1.73 abcdef	8.04	26.2 ± 0.24 ef	13.29	71.4 ± 1.20 cb	18.64
	2FR	64.75 ± 1.97 i	31.52	23.6 ± 0.4 hi	21.75	35.8 ± 1.05 ij	59.67
Fipronil	1.5 FR	7.75 ± 0.47 l	91.80	18.9 ± 0.27 l	37.33	10.4 ± 0.47 l	88.27
	1 FR	19.75 ± 0.85 k	79.18	20.8 ± 0.41 jk	31.04	21.1 ± 1.32 k	75.93
	0.5FR	20 ± 0.00 k	78.87	24 ± 0.44 h	20.60	24.5 ± 1.50 jk	71.38
	2FR	3.5 ± 1.19 l	96.24	18.1 ± 0.48 l	40.02	0.00 m	100.00
Spirotetramat	1.5 FR	80.75 ± 3.66 cdefg	14.50	12.4 ± 0.26 n	58.97	0.00 m	100.00
	1 FR	86.5 ± 2.17 abcdef	8.59	16.1 ± 0.31 m	46.69	0.00 m	100.00
	0.5FR	89 ± 0.40 abcde	5.94	19.8 ± 0.32 kl	34.45	7.4 ± 0.42 l	91.60
	2FR	67.5 ± 1.55 hi	28.75	12.3 ± 0.21 n	59.22	0.00 m	100.00
Control	-	94.75 ± 1.84 a	0.00	30.3 ± 0.6 a	0.00	76.60 ± 1.21b	0.00

[a]FR= Field Recommendation; SE= Standard Error of Mean; [b]Different letter within the treatments denote significant differences in the same column

and sporulation with imidacloprid when compared to control in 1FR and 0.5FR. The mycelium growth was significantly higher than control when treated with buprofezin at 1.5FR, 1FR and 0.5FR concentration (*f=315.28, df=28*, *p<0.0001*). Tolfenpyrad and spirotetramat reduced the conidia production of *M. brunneum* significantly (*f=459.63, df=28*, *p<0.0001*).

Compatibility

Compatibility of fungi tested against different insecticides calculated as per the formula proposed by

Table 4. Per cent germination (average ± SE), mycelial growth (average ± SE) and conidia production (average ± SE) of strain *Maconellicoccus brunneum* after 10 days, grown on growth media amended with insecticides

Treatment	Conc.	Germination%	% Reduction over Control	Colony Diameter (mm)	% Reduction over Control	Conidia Number ($Xx10^4$)	% Reduction over Control
Emamectin benzoate	1.5 FR	64.5 ± 1.84 ef	29.04	24.5 ± 0.34 fg	13.29	48.4 ± 1.08 ij	49.98
	1 FR	85.5 ± 0.95 ab	5.97	25.6 ± 0.48 efg	9.26	64.8 ± 1.39 fg	32.79
	0.5FR	85.25 ± 1.31 ab	6.24	25.9 ± 0.50 def	8.24	76.3 ± 0.74 de	21.25
	2FR	15.5 ± 2.10 h	82.88	21.3 ± 0.45i	24.58	42.6 ± 0.54 ij	56.10
Tolfenpyrad	1.5 FR	0 ± 0 i	100	7.7 ± 0.40 l	72.29	0 n	100
	1 FR	0 ± 0 i	100	14.7 ± 0.82 k	47.93	1.7 ± 0.51 n	98.36
	0.5FR	7.5 ± 0.5 hi	94.74	19.2 ± 0.44 j	31.95	7.9 ± 0.70 mn	91.85
	2FR	0 ± 0 i	100	5.5 ± 0.22 m	80.56	0 n	100.00
Imidacloprid	1.5 FR	83.5 ± 3.37 ab	8.15	27.6 ± 0.22 bcd	2.24	89 ± 5.04 c	7.40
	1 FR	89 ± 2.73 a	2.10	28.3 ± 0.21 abc	-0.20	122.1 ± 4.46 a	-26.79
	0.5FR	89 ± 2.67 a	2.24	28.7 ± 0.15 ab	-1.62	127.3 ± 2.41 a	-31.68
	2FR	84.75 ± 3.03 ab	6.72	27.7 ± 0.26 bcd	1.94	70.1 ± 0.69 ef	27.61
Clothianidin	1.5 FR	75 ± 1.77 bcde	17.58	26.1 ± 0.18 def	7.59	51.5 ± 0.58 hi	46.75
	1 FR	84.5 ± 1.84 ab	7.02	26.2 ± 0.25 def	7.18	63.8 ± 0.32 fg	34.12
	0.5FR	85.5 ± 1.84 ab	5.90	26.8 ± 0.13 cde	5.10	69.6 ± 2.21 fe	27.83
	2FR	71.25 ± 0.47 cdef	21.64	25.6 ± 0.40 efg	9.34	38.8 ± 1.16 jk	59.87
Buprofezin	1.5 FR	76.5 ± 1.75 bcd	15.92	28.7 ± 0.30 ab	-1.57	40.5 ± 0.95 jk	58.18
	1 FR	83.25 ± 2.01 ab	8.55	29.2 ± 0.13 ab	-3.36	77.3 ± 1.11 de	20.20
	0.5FR	85.25 ± 2.49 ab	6.34	29.8 ± 0.13 a	-5.47	84.1 ± 1.33 cd	13.41
	2FR	67.75 ± 2.46 def	25.43	28.1 ± 0.10 abc	0.47	38.4 ± 1.03 jk	60.28
Fipronil	1.5 FR	69.75 ± 2.01 def	23.21	23.8 ± 0.25 gh	15.69	31.8 ± 0.71 k	67.08
	1 FR	77.75 ± 1.10 bcd	14.46	26.8 ± 0.36 cde	5.14	59.5 ± 0.98 gh	38.53
	0.5FR	80.5 ± 2.87 abc	11.61	28.1 ± 0.18 abc	0.49	68.2 ± 0.67 efg	29.55
	2FR	61.75 ± 1.79 f	32.18	22 ± 0.30 hi	22.13	21.2 ± 0.711 l	78.02
Spirotetramat	1.5 FR	40.75 ± 0.75 g	55.18	21.9 ± 0.46 i	22.43	14.6 ± 0.30 lm	84.89
	1 FR	65.5 ± 2.62 ef	28.05	23.9 ± 0.23 g	15.39	14.6 ± 0.30 lm	84.89
	0.5FR	69 ± 0.40 def	24.14	24.8 ± 0.20 fg	12.21	41.2 ± 0.87 jk	57.62
	2FR	32.5 ± 3.17 g	64.21	19.3 ± 0.40 j	31.71	12 ± 0.63 lm	87.51
Control	-	91 ± 1.35 a	0.00	28.3 ± 0.42 abc	0.00	99.2 ± 5.59 b	0.00

[a]FR= Field Recommendation; SE= Standard Error of Mean; [b]Different letter within the treatments denote significant differences in the same column

Alves *et al*. (1998) is presented in Table 5. Emamectin benzoate was highly compatible with *B. bassiana* in contrary to tolfenpyrad and spirotetramat which were highly toxic. Imidacloprid, clothianidin and buprofezin were compatible at 0.5FR and 1FR dose. Although *B. bassiana* was compatible with emamectin benzoate at all the concentrations tested, the T value for imidacloprid was significantly higher (*f=254.61, df=27, p<0.0001*). *M. Brunneum* was highly compatible with imidacloprid followed by emamectin benzoate, clothianidin, buprofezin and fipronil. The T values for imidacloprid and buprofezin at 0.5FR were significantly higher (*f=484.04, df=27, p<0.0001*). Tolfenpyrad inhibited the growth of *M. brunneum* and was found to be very toxic.

Under suitable environmental conditions fungi infect insect naturally and are important factor for their death (Sandhu *et al*., 2012). Two important entomopathogens, *M. brunneum* and

Table 5. Toxicity value and compatibility classification

Insecticides	Concentration	*B. bassiana*		*M. brunneum*	
		T Values (± SE)	Classification	T Values (± SE)	Classification
Emamectin benzoate	1.5 FR	79.14 ± 1.78c	C	51.37 ± 1.53jk	MT
	1 FR	84.08 ± 1.21cb	C	63.60 ± 1.14efgh	C
	0.5FR	86.99 ± 1.87b	C	72.08 ± 1.63e	C
	2FR	70.64 ± 2.33d	C	49.51 ± 4.98jk	MT
Tolfenpyrad	1.5 FR	9.86 ± 0.15j	VT	5.72 ± 0.46no	VT
	1 FR	12.32 ± 0.44j	VT	13.14 ± 0.52n	VT
	0.5FR	21.85 ± 0.44i	VT	23.09 ± 0.53m	VT
	2FR	8.68 ± 0.68j	VT	4.23 ± 0.17o	VT
Imidacloprid	1.5 FR	56.21 ± 1.20e	MT	71.82 ± 2.69ef	C
	1 FR	94.11 ± 1.44a	C	92.40 ± 2.66ab	C
	0.5FR	94.15 ± 1.35a	C	95.79 ± 1.31a	C
	2FR	45.80 ± 1.53fg	MT	60.73 ± 0.51ghi	C
Clothianidin	1.5 FR	48.82 ± 0.89fg	MT	63.30 ± 1.40fgh	C
	1 FR	64.25 ± 0.69d	C	81.58 ± 2.23c	C
	0.5FR	78.15 ± 1.06c	C	85.91 ± 2.59bc	C
	2FR	42.67 ± 1.11gh	T	54.92 ± 0.87hij	MT
Buprofezin	1.5 FR	52.79 ± 0.70ef	MT	55.59 ± 1.51hij	MT
	1 FR	64.11 ± 1.13d	C	88.86 ± 2.06abc	C
	0.5FR	79.67 ± 1.14c	C	95.13 ± 0.95a	C
	2FR	47.22 ± 0.97fgh	MT	53.08 ± 0.67ijk	MT
Fipronil	1.5 FR	26.35 ± 1.02i	VT	45.28 ± 0.71k	MT
	1 FR	41.58 ± 2.43h	T	72.25 ± 0.93de	C
	0.5FR	49.92 ± 3.44ef	MT	80.97 ± 0.70dc	C
	2FR	10.97 ± 0.47j	VT	34.48 ± 0.77l	T
Spirotetramat	1.5 FR	8.23 ± 0.11j	VT	33.82 ± 0.79l	T
	1 FR	8.23 ± 0.11j	VT	56.50 ± 1.05hij	MT
	0.5FR	24.71 ± 0.90i	VT	67.17 ± 0.75efg	C
	2FR	8.17 ± 0.09j	VT	28.77 ± 0.76lm	VT

[a]T = 0 to 30 (very toxic); 31 to 45 (toxic); 46 to 60 (moderately toxic); >60 (compatible); [b]Values followed by the same letter in the same column are not significantly different

B. Bassiana were tested for pathogenicity against mealybug, *M. hirsutus*. Both the fungi are well-known biocontrol agents. Taxonomic revision of large species complex of *M. anisopliae* has resulted in nine terminal taxa one of which is *M. brunneum* (Bischoff *et al*., 2009). *M. brunneum* is not only an entomopathogen but is also known to benefit plant by providing Fe nutrition (Sánchez–Rodríguez *et al*., 2015). Infectivity of isolated fungi showed that both the entomopathogens were pathogenic to grape mealybug, *M. hirsutus*. Virulence of *M. brunneum* and *B. bassiana* against mealybug has been reported earlier (Chartier *et al*., 2016). Amala *et al.* (2014) while working on mealybug in grapes, obtained similar results.

The toxicity effect of insecticide, *i.e*., from antagonism to synergism on *M. brunneum* and *B. bassiana* varied between the two fungi. Pesticides may alter the sporulation, germination and vegetative growth of fungi. Spore germination may be a more important criterion of compatibility between insecticide and entomopathogen than mycelial growth (Anderson and Roberts, 1983). In pest management with entomopathogenic fungi, conidial germination is very important factor as initiation of epizootics is conditioned by the capacity of these conidiato germinate on the host (Alizadeh *et al*., 2007). In the present study, fipronil and tolfenpyrad showed maximum inhibition of conidial germination in *B. bassiana* and *M. anisopliae*, respectively. Therefore, their mixing should

be avoided and spray timings should not coincide in IPM programme. Emamectin benzoate and spirotetramat did not show any significant germination reduction of *B. bassiana* at field recommended dose. Similarly, emamectin benzoate, imidacloprid, clothianidin and buprofezin were compatible with respect to conidial germination of *M. brunneum* at field recommended dose. However, at concentrations higher than field dose, the germination was inhibited significantly by most of the tested insecticides. The inhibition of conidial germination may be attributed to effect on substrate recognition process (Boucias and Pendland, 1988), inhibition of germination initiation trigger (St. Leger *et al*., 1991) or ion accumulation on the surface of the cellular membrane causing metabolic blockage (Ghini and Kimati, 2000).

Even though the insecticides do not affect the conidial germination, the presence of high concentration of insecticide during the growth phase may have deleterious effect on mycelium growth and spore formation (Pachamuthe *et al*., 1999). When evaluated for effect on colony area and sporulation, imidacloprid was found to be highly compatible with both *B. bassiana* and *M. brunneum*. Work of Niassy *et al*. (2012) showed that there was no deleterious effect of imidacloprid on vegetative growth and conidia production. James and Elzen (2001) reported that there was no direct effect of imidacloprid on *B. bassiana*. Russel *et al*. (2010) reported non-significant effect of imidacloprid on *M. brunneum.* The fungi may metabolize the insecticide which would result in release of compounds that can be used as secondary nutrients by the fungi (Moino Jr and Alves, 1998). Paula *et al.* (2011) while working with imidacloprid and *M. brunneum*, showed similar results. Emamectin benzoate, clothianidin and buprofezin were also found to be compatible with both the tested fungi at field recommended dose. Compatibility of emamectin benzoate with *B. bassiana* was reported by Khorasiya *et al*. (2018). At lower concentrations, buprofezin was found to be compatible with *M. brunneum*. Similar results were shown by Jin *et al*. (2011) in control of nymphs of rice plant-hoppers. Tolfenpyrad inhibited germination, vegetative growth and conidia formation of both the entomopathogens. Tolfenpyrad can kill fungi by acting on complex I NADH oxido-reductase of respiratory process (FRAC, 2018). Spirotetramat was also toxic to both the entomopathogenic fungi. Spirotetramat kills insects by inhibiting acetyl CoA carboxylase, which affects the process of lipid synthesis (IRAC, 2018). Acetyl CoA carboxylase catalysed lipid synthesis is also present in fungi, therefore, this can be attributed as possible reason for incompatibility.

Laboratory bioassays are representative about the possibility of activity in field conditions. There are the possibilities that the same phenomena may occur in field as well. The compatibility of same chemical *in vitro* may differ from that in field conditions. Ambethgar *et al*. (2009) studied successful co-application of entomopathogens with selective insecticide against different pest earlier. In tropical regions, where vineyards tremendously encounter different pests and diseases, management becomes very costly and time consuming. Such compatibility studies would help in proper planning of cost effective, combined spray schedules and would enhance the management of pests.

The North American Plant Protection Organisation has specified that IPM is the basis for pest management and biocontrol is the basis for IPM (Dorworth, 1997). These fungi are found everywhere and are largely studied biocontrol agents, which makes them potential component in integrated pest management programmes. In this study, we are reporting pathogenicity of isolated two entomopathogenic fungi from field infected dead insects. The pathogenicity study showed that both the fungi were capable of infecting pink mealybug, *M. hirsutus* infesting grapes. Our study on compatibility of different insecticides with these fungi would help in formulating bio-intensive IPM programme for the management of mealybug in vineyards. Further research on performance of these entomopathogens in field, individually and as a part of integrated pest management technology, is required.

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REFERENCES

Abbott WS. 1925. A method for computing the effectiveness of an insecticide. *J Econ Entomol.* **18**: 265-267. https://doi.org/10.1093/jee/18.2.265a

Akbar S, Freed S, Hameed A, Gul HT, Akmal M, Malik MN, Khan MB. 2012. Compatibility of *Metarhizium anisopliae* with different insecticides and fungicides. *Afr J Microbiol Res.* **6**: 3956-3962.

Alves SB, Moino A Jr., Almeida JEM.1998. Produtosfitossanitários e entomopatógenos, pp. 217-238. In: Alves SB (Ed.) *Controle microbiano de insetos*. São Paulo, Fealq. 1163 pp.

Amala U, Chinniah C, Indu S, Muthukrishnan N, Muthiah C. 2014. Bio-efficacy and lethal reproductive effects of three entomopathogenic fungi against pink mealybug,

Maconellicoccus hirsutus (Green) infesting grapes. *Green Farming* **5**: 697-700.

Ambethgar V, Swamiappan M, Rabindra RJ, Rabindran R. 2009. Biological compatibility of *Beauveria bassiana* (Balsamo) Vuillemin isolate with different insecticides and neem formulations commonly used in rice pest management. *J Biol Control* **23**: 11-15.

Anderson TE, Roberts DW. 1983. Compatibility of *Beauveria bassiana* isolate with insecticide formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. *J Econ Entomol.* **76**: 1437-1441. https://doi.org/10.1093/jee/76.6.1437

Alizadeh A, Samih MA, Khezri M, Saberi R. 2007. Compatibility of *Beauveria bassiana* (Bals.) Vuill. with several pesticides.*Int J Agriic Biol.* **9**: 31-34.

Behie SW, Jones SJ, Bidochka MJ. 2015. Plant tissue localization of the endophytic insect pathogenic fungi *Beauveria and Metarhizium*. *Fungal Ecol*. **13**: 112-119. https://doi.org/10.1016/j.funeco.2014.08.001

Bischoff JF, Rehner SA, Humber RA.2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. *Mycologia* **101**: 512-530. https://doi.org/10.3852/07-202 PMid:19623931

Boucias DG, Pendland JC, Latge JP. 1988. Nonspecific factors involved in attachment of entomopathogenic deuteromycetes to host insect cuticle. *Appl Environ Microbiol*. **54**: 1795-1805. PMid:16347689 PMCid:PMC202748

Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP. 2011. The development, regulation and use of biopesticides for integrated pest management. *Philos Trans R Soc London Ser B*. **366**: 1987-1998. https://doi.org/10.1098/rstb.2010.0390 PMid:21624919 PMCid:PMC3130386

Chartier Fitzgerald V, Hill MP, Moore SD, Dames JF. 2016. Screening of entomopathogenic fungi against citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae). *Afr Entomol.* **24**: 343-351. https://doi.org/10.4001/003.024.0343

Depieri RA, Martinez SS, Ayres O, Menezes JR. 2005. Compatibility of the fungus *Beauveria bassiana* (Bals.) Vuill. (Deuteromycetes) with extracts of neem seeds and leaves and the emulsible oil. *Neotrop Entomol*. **34**: 601-606. https://doi.org/10.1590/S1519-566X2005000400010

Dorworth CE. 1997. Two models for the development of fungal biological control agents as instruments of ecological management, pp. 211-224. In: Chapela IH and Palm ME (Eds.). *Mycology and sustainable development: expanding concepts, vanishing borders.* Parkway, Boone, NC, USA.

Faraji S, Shadmehri AD, Mehrvar A. 2016. Compatibility of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* with some pesticides. *J Entomol Soc Iran* **36**: 137-146.

Finney DJ. 1971. *Probit Analysis*. 3rd ed. Cambridge University Press, NewY ork, N.Y. 333 pp.

FRAC. 2018. FRAC Code List 2018: Fungicides sorted by mode of action(including FRAC Code numbering). Fungicicide Resistance Action Committee (FRAC). Available from: http://www.phi-base.org/images/fracCodeList.pdf

Ghini R, Kimati H. 2000. Resistência de fungos a fungicidas. Jaguariúna, EMBRAPA Meio Ambiente, 78p. PMCid:PMC1729535

Greenfield M, Jimenez MIG, Ortiz V, Vega FE, Kramer M, Parsa S. 2016. *Beauveria bassiana and Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. *Biol Control* **95**: 40-48. https://doi.org/10.1016/j.biocontrol.2016.01.002 PMid:27103778 PMCid:PMC4825668

Hatting J, Wraight S, Miller RM. 2004. Efficacy of *Beauveria bassiana* (Hyphomycetes) for control of Russian wheat aphid (Homoptera: Aphididae) on resistant wheat under field conditions. *Biocontrol Sci Tech*. **144**: 459–473. https://doi.org/10.1080/09583150410001683501

IRAC. 2018. IRAC Mode of Action Classification Scheme. Issued, May 2018. Version 8.4. Insecticide Resistance Action Committee (IRAC) International MoA Working

Group. Available from: https://www.irac-online.org/modes-of-action/

Jaber S, Mercier A, Knio K, Brun S, Kambris Z. 2016. Isolation of fungi from dead arthropods and identification of a new mosquito natural pathogen. *Parasites Vectors* **9**: 491. https://doi.org/10.1186/s13071-016-1763-3 PMid:27595597 PMCid:PMC5012000

James RR, Elzen GW. 2001. Antagonism between *Beauveria bassiana* and imidacloprid when combined for *Bemisia argentifolii* (Homoptera: Aleyrodidae) control. *J Econ Entomol.* **94**: 357-361. https://doi.org/10.1603/0022-0493-94.2.357

Jin SF, Feng MG, Ying SH, Mu WJ, Chen JQ. 2011. Evaluation of alternative rice planthopper control by the combined action of oil formulated *Metarhizium anisopliae* and low-rate Buprofezin. *Pest Manag Sci*. **67**: 36-43. https://doi.org/10.1002/ps.2026 PMid:20949549

Khorasiya SG, Raghavani KL, Bharadiya AM, Bhut JB. 2018. Compatibility of *Beauveria bassiana* with different insecticides. *Int J Chem Stud*. **6**: 556-558.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*. **16**: 111-120. https://doi.org/10.1007/BF01731581 PMid:7463489

Midthassel A, Leather SR, Wright DJ. 2016. Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complimentary biocontrol agents. *Bio Control* **61**: 437. https://doi.org/10.1007/s10526-016-9718-3

Moino Jr. A, Alves SB. 1998. Efeito de Imidacloprid e Fipronil sobre *Beauveria bassiana* (Bals.) Vuill. E *Metarhizium anisopliae* (Metsch.) Sorok. e no comportamento de limpeza de *Heterotermes tenuis* (Hagen). *Annu Soc Entomol Brasil* **27**: 611-619. https://doi.org/10.1590/S0301-80591998000400014

Neves PMOJ, Hirose E, Tchujo PT, Moino A Jr. 2001. Compatibility of entomopathogenic fungi with neonicotinoid insecticides. *Neotrop Entomol*. **30**: 263-268. https://doi.org/10.1590/S1519-566X2001000200009

Niassy S, Maniania NK, Subramanian S, Gitonga ML, Maranga R, Obonyo AB, Ekesi S. 2012. Compatibility of *Metarhizium anisopliae* isolate ICIPE 69 with agrochemicals used in French bean production. *Int J Pest Manage*. **58**: 131-137. https://doi.org/10.1080/09670874.2012.669078

Orr D. 2009. Biological control and integrated pest management, pp. 207-239. In: Peshin R, Dhawan AK (Eds.). *Integrated pest management: innovation development process,* Springer Science & Business Media, Dordrecht, Netherlands. https://doi.org/10.1007/978-1-4020-8992-3_9

Pachamuthe P, Kamble ST, Yuen GY. 1999. Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) strains ESC-1 to the German cockroach (Dictyoptera: Blattellidae) and its compatibility with insecticides. *J Econ Entomol.* **92**: 340-346. https://doi.org/10.1093/jee/92.2.340

Paula R, Carolino AT, Paula CO, Samuels RI. 2011. The combination of the entomopathogenic fungus *Metarhizium anisopliae* with the insecticide Imidacloprid increases virulence against the dengue vector *Aedes aegypti* (Diptera: Culicidae). *Parasites Vectors* **4**: 8. https://doi.org/10.1186/1756-3305-4-8 PMid:21266078 PMCid:PMC3037915

Pelizza SA, Scorsetti AC. 2015. Compatibility between entomopathogenic fungi and biorational insecticides in toxicity against *Ronderosia bergi* under laboratory conditions. *BioControl* **60**: 81-91. https://doi.org/10.1007/s10526-014-9606-7

Russell CW, Ugine TA, Hajek AE. 2010. Interactions between imidacloprid and *Metarhizium brunneum* on adult Asian longhorned beetles *Anoplophora glabripennis* (Motschulsky. (Coleoptera, Cerambycidae). *J Invertebr Pathol*. **105**: 305-311. https://doi.org/10.1016/j.jip.2010.08.009 PMid:20807541

Sánchez–Rodríguez AR, del Campillo MC, Quesada–Moraga E. 2015. *Beauveria bassiana*: An entomopathogenic fungus alleviates Fe chlorosis symptoms in plants grown

on calcareous substrates. *Sci Hortic*. **197**: 193-202. https://doi.org/10.1016/j.scienta.2015.09.029

Sandhu SS, Sharma AK, Beniwal V, Goel G, Batra P, Kumar A, Jaglan S, Sharma AK, Malhotra S. 2012. Myco-biocontrol of insect pests: Factors involved, mechanism, and regulation. *J Pathogens* **126819***:* 1-10.

Sawant IS, Wadkar PN, Ghule SB, Rajguru YR, Salunkhe VP, Sawant SD. 2017. Enhanced biological control of powdery mildew in vineyards by integrating a strain of *Trichoderma afroharzianum* with sulphur. *BioControl*. **114**: 133-143.

Silva RA, Quintela ED, Mascarina GM, Barrigossi JAF, Liao LM. 2013. Compatibility of conventional agrochemicals used in rice crops with the entomopathogenic fungus *Metarhizium anisopliae*. *Sci Agri*. **70**: 152-160. https://doi.org/10.1590/S0103-90162013000300003

St. Leger RJ, Bidochka MJ, Roberts DW. 1994. Germination triggers of *Metarhizium anisopliae* conidia are related to host species. *Microbiology* **140**: 1651–1660. https://doi.org/10.1099/13500872-140-7-1651

Tajick Ghanbary MA, Asgharzadeh A, Hadizadeh AR and Mohammadi Sharif MA. 2009. Quick method for *Metarhizium anisopliae* isolation from cultural soils. *Am J Agr Biol Sci*. **4**: 152-155. https://doi.org/10.3844/ajabssp.2009.152.155

Thungrabeab M, Tongma S.2007. Effect of entomopathogenic fungi, *Beauveria bassiana* (Balsam) and *Metarhizium anisopliae* (Metsch) on non-target insects. *KMITL Sci Tech J.* **7**: 8-12.

Tiago PV, de Oliveira NT, de Luna EÁ, Lima A. 2014. Biological insect control using *Metarhizium anisopliae*: morphological, molecular, and ecological aspects. *Cienc Rural* **44**: 645-651. https://doi.org/10.1590/S0103-84782014000400012

Wang CS, Skrobek A, Butt TM. 2004. Investigations on the destruxin production of the entomopathogenic fungus *Metarhizium anisopliae*. *J Invertebr Pathol*. **85**: 168-174. https://doi.org/10.1016/j.jip.2004.02.008 PMid:15109899

Yadav DS, Amala U. 2013. Insect and mite pest management, pp. 44-51. In: Adsule PG, Yadav DS, Upadhayay A, Sharma AK and Satisha J (Eds.). *Good agricultural practices for production of quality table grapes,* ICAR-National Research Centre for Grapes, Pune, Maharashtra, India.