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

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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 $1.4 \times 10^6$ and $1.0 \times 10^7$ 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.

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
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Compatibility of insecticides with entomopathogenic fungi for bio-intensive management of pink mealybug in grapes 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 Scarsetti, 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′-TCTGAGGGTAACCGGCG-3′ and ITS4 5′-TCCTCGGATTATGCATGC-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 × 103, 1 × 104, 1 × 105 and 1 × 106 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).
In vitro insecticide compatibility

Insecticides

The insecticides were selected based on their use in grapes. All the shortlisted 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%): \[ T = \frac{20 \times VG + 80 \times SP}{100} \]. 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

| 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     | Inhibitors of Acetyl COA Carboxylase (Tetronic and Tetramic acid derivatives) | 0.70 |
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GenBank-NCBI with accession number MH793596.

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). LC50 value of mealybug treated with *Metarhizium brunneum* was lower as compared to LC50 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 cent spore 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 2. Toxicity of entomopathogenic fungi against *Maconellicoccus hirsutus*

| Treatment          | LC50 (Conidia/ml) | 95% Fiducial Limits (Conidia/ml) | Slope (± SE) |
|--------------------|--------------------|----------------------------------|--------------|
|                    | Lower              | Upper                            |              |
| *Metarhizium brunneum* | 1.4 × 10^6         | 8.5 × 10^4                       | 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) |
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 abcd| 10.26                             | 21.8 ± 0.66 ij      | 27.82                              | 61.7 ± 0.84 cd          | 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 bc      | 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 bc      | 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 abcd efg| 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     | 91.80                              | 18.9 ± 0.271        | 37.33                              | 10.4 ± 0.471            | 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     | 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 abcd| 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 ± 1.21 b          | 0.00                                 |

AFR= Field Recommendation; SE= Standard Error of Mean; 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
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

| 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 cf | 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                    |
|                     | 0.5 FR| 85.25 ± 1.31 | 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 FR  | 0 ± 0 i       | 100                      | 7.7 ± 0.40 I         | 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 bcede| 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 cd ef| 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 ± 0.30 hi       | 22.13                    | 21.2 ± 0.711             | 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                     |

*FR= Field Recommendation; SE= Standard Error of Mean; Different letter within the treatments denote significant differences in the same column.

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.
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.

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 FR          | 9.86 ± 0.15j        | VT                  | 5.72 ± 0.46no      | VT                  |
|                  | 0.5FR         | 21.85 ± 0.44i       | VT                  | 23.09 ± 0.53m      | VT                  |
|                  | 2FR           | 8.68 ± 0.68j        | VT                  | 4.23 ± 0.17o       | VT                  |
| Imidaclorid      | 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 ± 1.40fg     | 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 ± 1.11h       | 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 ± 0.97fg      | 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 ± 0.71t      | 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                  |

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

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 conidioate 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 (Pachamuthu 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 entomopathogens. Tolfenpyrad can kill fungi by germination, vegetative growth and conidia formation of *M. brunneum*. The pathogenicity is 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.

**ACKNOWLEDGMENT**

The authors are thankful to the Director, ICAR-National Research Centre for Grapes, Pune, Maharashtra, India, for valuable suggestions and support during the study.

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