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# Evaluation of entomopathogenic fungus for the management of pink mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae) on pineapple in Kerala

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**Abstract**
Three species of entomopathogenic fungi *viz.*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* was evaluated at three different concentrations ($1\times10^7$, $1\times10^8$ and $1\times10^9$ spores $ml^{-1}$) were effective on *D. brevipes* under laboratory conditions. Highest Spore concentration of all the entomopathegenic fungi had resulted in higher mortality of mealybug. *L. lecanii* @ $1\times10^9$ spores $ml^{-1}$ concentration had resulted in 66.67 percent mortality. Similarly, *B. bassiana* and *M. anisopliae* at $1\times10^9$ spores $ml^{-1}$ concentration recorded mortality of 60 and 40 percent, respectively. In pot culture studies, the best performing concentration of entomopathegenic fungi from the laboratory assay was evaluated along with a botanical insecticide and a standard check (quinalphos 25EC @ 0.05%). Ten days after the first treatment application, highest reduction was observed in quinalphos (96.73%) followed azadirachtin (87.75%). Among the three EPF tested, *M. anisopliae* caused the maximum reduction of (59.29%) of mealybug. Similarly, second spray of *M. anisopliae* and *B. bassiana* recorded maximum reduction of 72.72 and 70.98 percent, which were statistically on par with each other. After the third spray, *L. lecanii* resulted in highest reduction (90.04%) of mealybugs which was on par with the reduction obtained by quinalphos (95.20%) application.

**Keywords:** Pineapple mealybug, *Dysmicoccus brevipes*, bioassay, entomopathogenic fungi, pot culture

## 1. Introduction
Pineapple (*Ananas comosus* (L.) Merr.) is a tropical fruit plant belonging to the family Bromeliaceae. It is originated from regions of Southern Brazil and Paraguay. Fruits are either consumed fresh or used for making juice and an excellent source of potassium (109 mg/100g), calcium (12mg/100g), magnesium (12mg/100g), vitamin C (47.8mg/100g), carbohydrate (13.12 g/100g) and sugar (9.85 g/100g). Pineapple fruit is the only source for naturally available bromelain which is used for healing cancer, wounds and inflammation as well as in enhancing the immune system. In addition to this, pineapple leaves are used in textile industries for fibre production.

Pineapple is one of the most important fruit crop grown in India, covering an area of 1,09,900 ha with an annual production of 1736.70 metric tons. Assam has the largest area under pineapple (16.54 thousand ha), followed by Manipur (13.70 thousand ha) and Arunachal Pradesh (12.78 thousand ha). West Bengal ranks first in production (316 metric tons) followed by Assam (288.60 metric tons) and Tripura (162.26 metric tons). Kew, Giant Kew, Queen and Mauritius are the popular varieties cultivated in India.

Kerala with an area of 8.54 thousand ha and 72.86 metric tons production contributes about 4.2 percent of the pineapple production in India. The major pineapple growing areas in Kerala are Moovatupuzha, Kothamangalam in Ernakulam, Thoduphuza and Elamdesam in Idukki district and parts of Kottayam district [1].

Many insects are known to attack pineapple, but only a few are considered as major pests, such as pineapple mealybug, *Dysmicoccus brevipes* (Cockerell), scale insects [*Diaspis bromeliae* (Kerner), *Parasaissetia nigra* (Nietner) and *Melanaspis bromeliae* Dekle] and thrips [*Thrips tabaci* (Linderman), *Frankliniella schultzei* (Trybom) and *Halothrips ananasi* Costa Lima]. Among these, infestation by pink pineapple mealybug, *Dysmicoccus brevipes* often leads to complete devastation of the crop, as the insect also acts as vector of Pineapple Mealybug Wilt Diseases (PMWD). It is reported as a serious pest of pineapple in Kerala [2].

Two species of pineapple mealybugs are known to occur *ie.*, the pink strain (*D. brevipes*) and the grey strain (*D. neobrevipes*). Pink strain is mostly found on the root, crown and lower stem of pineapple plants and reproduces parthenogenetically. The grey strain, on the other hand, mostly seen on upper parts of the plant, such as leaf whorls and the developing fruits and reproduces sexually [3].

Pink mealybug, *D. brevipes* consists of two races, parthenogenetic race and bisexual race. Parthenogenetic race differ from the bisexual race being commonly found on upper parts of the plants and by producing green coloured spots on the infested pineapple leaves. Bisexual race was first observed on pineapple in Brazil, Dominican Republic, Martinique, Malaysia, Madagascar and Ivory Coast [4]. Both pink strain and grey strain mealybugs are responsible for transmiting PMWD in Hawaii. But the grey strains are restricted only to tropical America and Hawaii. Apart from pineapple, crops like coffee, banana, caladium, canna, citrus, eggplant, sugarcane and palms are reported as alternate hosts of pineapple mealybugs [5].

Since, *D. brevipes* is a highly polyphagous pest and a major constrain to pineapple cultivation, management of this mealybug is very important. Use of synthetic insecticides for the management of mealybugs results in residual toxicity in fruits and may cause human health hazards, besides eliminating the natural enemies which play a crucial role in bringing down the population of mealybugs. Therefore, an effective and ecologically sound management practice of pineapple mealybugs has to be developed.

## 2. Materials and methods

The present study was conducted between September 2014 and June 2016. The objective of the investigation was to formulate eco-friendly measures for the management of the mealybug.

### 2.1. Mass culturing of pink pineapple mealybug, *Dysmicoccus brevipes*

To carry out bioassay and pot culture experiments, mealybugs were reared on pumpkin in the All India Network Project on Agricultural Ornithology laboratory, College of Horticulture, Kerala Agricultural University, Vellanikkara. Pumpkin fruits were thoroughly washed with water and surface treated with bavistin (0.1%). The treated pumpkins were shade dried and kept in rearing cages. If any Damage or wounds found on pumpkin fruits, were sealed by applying wax. Nymphs and adult female of *D. brevipes* collected from pineapple field were released on pumpkins for mass multiplication.

### 2.2. Preparation of spore concentration of entomopathogenic fungi

Cultures of the three entomopathogenic fungi *viz.*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillim lecanii* were obtained from AICRP on BCCP & W, Kerala Agricultural University, Vellanikkara were ground in wearing blender and made into liquid spore suspension. The suspension was filtered through double layered muslin cloth to remove the mycelial mat. For uniform distribution of fungal spores, 5 ml of Tween 80® (0.02%) was added to the spore suspension and filtered through a clean muslin cloth. The spore count in the fungal suspension was assessed by using a haemocytometer and was estimated using the formula [6].

$$\text{Number of spore/ml} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

Where,

X = Number of spores counted from small squares of haemocytometer

Y = Number of small squares counted in haemocytometer

400 = Total number of small squares in haemocytometer

10 = Depth factor

1000 = Conversion factor from $mm^3$ to $cm^3$

D = Dilution factor

Based on the number of spores, all the cultures were adjusted to $1\times10^9$ spores $ml^{-1}$ from which the lower concentrations *viz.*, $1\times10^8$ and $1\times10^7$ were prepared by serial dilution method for bioassay studies.

### 2.3 Laboratory bioassay

Spore suspensions of each of three fungi *viz.*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* at three different concentrations of ($1\times10^7$, $1\times10^8$ and $1\times10^9$ spores $ml^{-1}$) were used for the bioassay (Table 1). It was carried out in the laboratory by dipping method of inoculation [7]. Twenty second instar nymphs of *D. brevipes* were released on pineapple leaf bits of size eight centimetre length and allowed to settle on the leaf bits to prevent them from escaping. After 24 h of releasing the nymphs, the leaf bits along with the nymphs were dipped in the 200 ml of different spore concentrations of entomopathogenic fungi for 10 seconds. Later, the leaf bits were transferred to Petri dish (9 cm diameter) lined with moist cotton. To ensure constant humidity, all the Petri dishes containing treated nymphs were placed in a plastic container and covered with a white muslin cloth. Mortality count was taken on third, fifth and seventh day after the treatment. Cadavers of dead mealybugs were kept in humid chamber for two days to observe the hyphal growth and were observed under the microscope for mycelial growth.

#### 2.3.1 Statistical analysis

Percent mortality data was corrected with the control mortality by using Abbott's formula [8]. Percent mortality was calculated and subjected to ANOVA test and the means were separated by Duncan's Multiple Range Test (DMRT).

**Table 1:** Entomopathogenic fungi sporeload tested against *D. brevipes* under laboratory conditions

| S. No | Treatments | Concentration |
|---|---|---|
| 1 | T1: *Metarhizium anisopliae* | $1x10^7$spores $ml^{-1}$ |
| 2 | T2: *M. Anisopliae* | $1x10^8$ spores $ml^{-1}$ |
| 3 | T3: *M. Anisopliae* | $1x10^9$ spores $ml^{-1}$ |
| 4 | T4: *Beauveria bassiana* | $1x10^7$spores $ml^{-1}$ |
| 5 | T5: *Beauveria bassiana* | $1x10^8$ spores $ml^{-1}$ |
| 6 | T6: *Beauveria bassiana* | $1x10^9$ spores $ml^{-1}$ |
| 7 | T7: *Lecanicillium lecanii* | $1x10^7$spores $ml^{-1}$ |
| 8 | T8: *Lecanicillium lecanii* | $1x10^8$ spores $ml^{-1}$ |
| 9 | T9: *Lecanicillium lecanii* | $1x10^9$ spores $ml^{-1}$ |
| 10 | T10: Control (Distilled water) | |

### 2.4 Evaluation of entomopathogenic fungi under pot culture experiment

To conduct pot culture experiment of Entomopathogenic fungi (EPF), pineapple (cv. Mauritius) slips were procured from Pineapple Research Station, Kerala Agricultural University, Vellanikkara. Pineapple slips were planted in polythene bags and allowed for establishing. The experiment

was laid out in Completely Randomized Design (CRD) with six treatments and four replications (eight plants per replication). Best concentration of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillim lecanii* were selected based on laboratory evaluation (Table 2). Nymphs of 0 to 24 h old were released at the rate of 100 nymphs per pineapple slips planted in polythene bags using the paper strip method. Paper strips were left on the pumpkins for 24 h and once the nymphs were found crawling over the stripes and settled over it, strips along with crawlers were slowly removed and kept in the plastic jar without much disturbance and later transferred to pineapple slips. One month after the release of nymphs, pre-count of mealybug numbers was taken prior to entomopathogenic fungi treatments. The treatments were applied using as pneumatic hand sprayer during the evening hours.

**Table 2:** Evaluation of the entomopathogenic fungi on *Dysmicoccus brevipes* in pot culture experiment

| Treatments | Frequency of application |
|---|---|
| T1: *Lecanicillium lecanii* @ $1x10^8$ spores $ml^{-1}$ | Three sprays at 10 days interval |
| T2: *Metarhizium anisopliae* @ $1x10^8$ spores $ml^{-1}$ | Three sprays at 10 days interval |
| T3: *Beauveria bassiana* @ $1x10^8$ spores $ml^{-1}$ | Three sprays at 10 days interval |
| T4: Quinalphos 25 EC @ 0.05% | Single spray |
| T5: Azadirachtin 1% @ 0.005% | Three sprays at 10 days interval |
| T6: Control | |

### 2.4.1 Observation

The population of mealybugs were recorded before and after the application of each treatment by destructive sampling method. Count of the mealybugs per each plant was taken at ten days interval upto $40^{th}$ day after the first treatment. The dead mealybugs were collected and placed in a Petri dish lined with a moist filter paper and was observed for the mycelial growth.

### 2.4.2 Statistical analysis

The mean population of mealybugs of both pre and post count was analysed by Analysis of Covarience. Percent reduction in number of mealybugs over control was analysed by ANOVA and means were separated by Duncan's Multiple Range Test (DMRT).

## 3. Results

### 3.1 Laboratory bioassay

Nymphs infected with the entomopathogenic fungus were hard and mummified. After 24 h of the death of the nymphs, germination of the conidia and penetration of hyphae through the integuments of nymphs were observed. Later, entire body surface was covered with mycelial growth.

After three days of treatment application, significant difference was observed in the mortality of the nymphs in all the treatments (Table 3). The highest mortality of 15 percent was observed in *L. lecanii* @ $1x10^9$ spores $ml^{-1}$ while, a mortality of 11.67 and 13.33 percent was recorded at $1x10^7$ and $1x10^8$ spores $ml^{-1}$, respectively. This was followed by *B. bassiana* where mortality of varied from 11.67 percent at $1x10^7$ spores $ml^{-1}$ to 13.33 percent at $1x10^9$ spores $ml^{-1}$. However, *M. anisopliae* $1x10^9$ spores $ml^{-1}$ resulted in least mortality of 8.33 percent followed by 6.67 percent mortality was observed at $1x10^7$ and $1x10^8$ spores $ml^{-1}$. Similar trend was observed after five days after treatment application, where *L. lecanii* treatment caused mortality of 36.67, 33.33 and 26.67 percent was observed at $1x10^9$, $1x10^8$ and $1x10^7$ spores $ml^{-1}$, respectively. This was followed by *B. bassiana* where $1x10^9$ spores $ml^{-1}$ gave a mortality of 31.67 percent while, both $1x10^8$ and $1x10^7$ spores $ml^{-1}$ concentrations of *B. bassiana* recorded mortality of 28.33 percent, respectively. Mortality recorded in *M. anisopliae* varied from 15 percent at $1x10^7$ spores $ml^{-1}$ to 20 percent at $1x10^9$ spores $ml^{-1}$ (Table 3).

On seventh day after treatment highest mortality was observed in *L. lecanii* treatment @ $1x10^9$ spores $ml^{-1}$ (66.67%) followed by 56.67 percent at $1x10^8$ spores $ml^{-1}$ and 43.33 percent at $1x10^7$ spores $ml^{-1}$. Similarly, in *B. bassiana* treatment, a maximum reduction in nymphs was observed with 60 percent at $10^9$ spores $ml^{-1}$ which was on par with the mortality recorded by *L. lecanii* @ $10^9$ spores $ml^{-1}$. About 53.33 and 46.67 percent reduction of nymphs was noticed at $1x10^8$ and $1x10^7$ spores $ml^{-1}$ of *B. bassiana*, respectively. Least mortality of the nymphs was recorded with *M. anisopliae* treatment where 40 percent mortality was recorded at $1x10^9$ spores $ml^{-1}$ and 33.33 and 26.67 percent mortality at $1x10^8$ and $1x10^7$ spores $ml^{-1}$, respectively (Table 3).

Among the three different entomopathogenic fungi treated, maximum mortality of nymphs was recorded in *L. lecanii* $1x10^9$ spores $ml^{-1}$ which was on par with the *B. bassiana* $1x10^9$ spores $ml^{-1}$. Least of mortality was recorded in *M. anisopliae* treatment (40%) at $1x10^9$ spores $ml^{-1}$ and was significantly different with mortality recorded in *L. lecanii* and *B. bassiana* at $1x10^9$ spores $ml^{-1}$ treatments. The mortality recorded by higher spore concentrations of the three entomopathogenenic was found to be statistically on par with the succeeding lower spore concentrations. Hence, $1x10^8$ spores $ml^{-1}$ was used to test the efficacy with pot culture.

**Table 3:** Effects of entomopathogenic fungi on *Dysmicoccus brevipes* under laboratory conditions

| Treatments | Mortality of nymphs of *D. brevipes* (%) | | |
|---|---|---|---|
| | 3 DAT | 5 DAT | 7 DAT |
| T1: *M. anisopliae* ($10^7$ spores $ml^{-1}$) | $6.67^{cd}$ (14.76) | $15.00^{d}$ (22.29) | $26.67^{e}$ (30.78) |
| T2: *M. anisopliae* ($10^8$ spores $ml^{-1}$) | $6.67^{cd}$ (14.79) | $16.67^{cd}$ (24.05) | $33.33^{de}$ (35.29) |
| T3: *M. anisopliae* ($10^9$ spores $ml^{-1}$) | $8.33^{bc}$ (16.59) | $20.00^{bcd}$ (26.07) | $40.00^{cde}$ (39.15) |
| T4: *B. bassiana* ($10^7$ spores $ml^{-1}$) | $11.67^{abc}$ (19.88) | $28.33^{ab}$ (32.02) | $46.67^{bcd}$ (43.08) |
| T5: *B. bassiana* ($10^8$ spores $ml^{-1}$) | $10.00^{abc}$ (18.05) | $28.33^{abc}$ (32.02) | $53.33^{abc}$ (47.01) |
| T6: *B. bassiana* ($10^9$ spores $ml^{-1}$) | $13.33^{ab}$ (21.34) | $31.67^{a}$ (34.16) | $60.00^{ab}$ (50.86) |
| T7: *L. lecanii* ($10^7$ spores $ml^{-1}$) | $11.67^{abc}$ (19.88) | $26.67^{abc}$ (31.07) | $43.33^{bcde}$ (41.15) |
| T8: *L. lecanii* ($10^8$ spores $ml^{-1}$) | $13.33^{ab}$ (21.14) | $33.33^{a}$ (35.17) | $56.67^{abc}$ (48.93) |

| | | | |
|---|---|---|---|
| T9: *L. lecanii* ($10^9$ spores $ml^{-1}$) | 15.00[a] (22.79) | 36.67[a] (37.23) | 66.67[a] (54.78) |
| T10: Control (Distilled water) | 1.67[d] (4.73) | 3.33[d] (8.83) | 3.33[f] (8.83) |
| CD(0.05) | 5.818 | 11.63 | 17.38 |

Mean values in columns followed by same alphabet(s) are not significantly different by DMRT at P=0.05
DAT- Days after Treatment
Figures in the parentheses are arc sin transformed values
Values in the columns are mean of three replications

### 3.2 Evaluation of entomopathogenic fungi (EPF) under pot culture experiment

Efficacy of entomopathogenic fungi was evaluated under pot culture experiment. The concentration of the EPF was taken based on laboratory evaluation. One month after the release of the first instar nymphs on pineapple plants, pre count was taken a day before the treatments application. *L. lecanii*, *M. anisopliae* and *B. bassiana* @ $1x10^8$ spores $ml^{-1}$ and azadirachtin (1% @ 0.005%) were sprayed three times by maintaining a standard check (quinalphos 25EC @ 0.05% - single spray). Each treatment was repeated at ten days intervals and the observation was taken after each spray up to 40 days of the first application by destructive sampling method.

The mean pre and post-treatment counts of mealybugs was taken at ten days after the treatment application. The pre-treatment count of the mealybug found to be non significant (Table 4). Ten days after the application of quinalphos there was a drastic reduction in the number of mealybugs with the reduction of 96.73 percent followed by azadirachtin which resulted in the reduction of 87.75 percent of the mealybugs and statistically this was on par with the quinalphos (Table 5). Among three different entomopathogenic fungi *viz., L. lecanii*, *B. bassiana* and *M. anisopliae* treated, *M. anisopliae* effected maximum reduction of 59.29 percent, followed by *B. bassiana* (30.13%) and *L. lecanii* (17.26%). Effects of all the entomopathogenic fungus in the reduction of mealybugs was found on par with each other.

Twenty days after the treatment, *M. anisopliae* and *B. bassiana* treatments had similar effect in lowering the number of mealybugs with a reduction of 72.72 and 70.98 percent, respectively, while in *L. lecanii* could reduce to 56.37 percent. However, all the treatments were on par with each other. Compared to the entomopathogenic fungi, quinalphos and azadirachtin treatments reduced the mealybug population significantly (96.47 and 94.43% reduction, respectively).

At 30 DAT, *L. lecanii* treatment was more effective in reducing the mealybugs (90.04%) and it was on par with the quinalphos treatment (95.20%). The effect of *B. bassiana* (76.04%), azadirachtin (75.23%) and *M. anisopliae* (74.36%), were on par with each other. The efficacy of all the entomopathogenic was found to be increased with repeated sprays and also time lapse after the treatment.

**Table 4:** Effect of entomopathogenic fungi on *Dysmicoccus brevipes* in the pot culture experiment.

| Treatments | PTC | Mean population at 10 DAT | | |
|---|---|---|---|---|
| | | †† First spray | †† Second spray | †† Third spray |
| T1- *L. lecanii* @ $1x10^8$ spores $ml^{-1}$ (three sprays at ten days interval) | 87.25 (9.17) | 46.73 (6.59) | 29.35 (5.37) | 7.52 (3.04) |
| T2- *M. anisopliae* @ $1x10^8$ spores $ml^{-1}$ (three sprays at ten days interval) | 58.75 (7.52) | 27.76 (5.23) | 24.46 (4.69) | 23.50 (4.79) |
| T3- *B. bassiana* @ $1x10^8$ spores $ml^{-1}$ (three sprays at ten days interval) | 64.50 (7.859) | 45.37 (6.75) | 19.39 (4.45) | 22.13 (4.65) |
| T4- Quinalphos 25EC @ 0.05% (single spray) | 66.75 (8.18) | *0.89 (1.44) | *12.64 (2.58) | *7.57 (1.85) |
| T5- Azadirachtin 1% @ 0.005% (three sprays at ten days interval) | 38.25 (6.20) | 14.67 (3.51) | 12.33 (2.75) | 24.36 (4.28) |
| Control | 57.12 (7.57) | 68.44 (8.27) | 69.45 (8.19) | 81.92 (9.85) |
| CD (0.05) | NS | 22.76 (1.58) | 21.38 (NS) | 28.42 (1.89) |

Values in the columns are mean of four replications
Figures in the parenthesis are square root transformed values (x+0.5)
PTC- Pre-treatment Count, DAT- Days After Treatment
*- Single spray treatment
††- Values in the parenthesis are adjusted means of square root transformed values based on ANOCOVA

**Table 5:** Percent reduction of Dysmicoccus brevipes over control by entomopathogenic fungi in pot culture experiment

| Treatments | Percent reduction over control | | |
|---|---|---|---|
| | First spray | Second spray | Third spray |
| T1- *L. lecanii* @ $1x10^8$ spores $ml^{-1}$ (three sprays at ten days interval) | 17.26[d] | 56.37[c] | 90.04[a] |
| T2- *M. anisopliae* @ $1x10^8$ spores $ml^{-1}$ (three sprays at ten days interval) | 59.29[bc] | 72.72[b] | 74.36[b] |
| T3- *B. bassiana* @ $1x10^8$ spores $ml^{-1}$ (three sprays at ten days interval) | 30.13[cd] | 70.98[bc] | 76.04[b] |
| T4- Quinalphos 25EC @0.05% (single spray) | *96.73[a] | *96.47[a] | *95.20[a] |
| T5- Azadirachtin 1% @ 0.005% (three sprays at ten days interval) | 87.75[ab] | 94.44[a] | 75.23[b] |
| CD (0.05) | 34.19 | 16.17 | 12.59 |

Means in columns followed by same alphabet(s) is not different by DMRT at P=0.05
DAT- Days after Treatment,
*- Result of single spray treatment
Values in the columns are mean of four replications

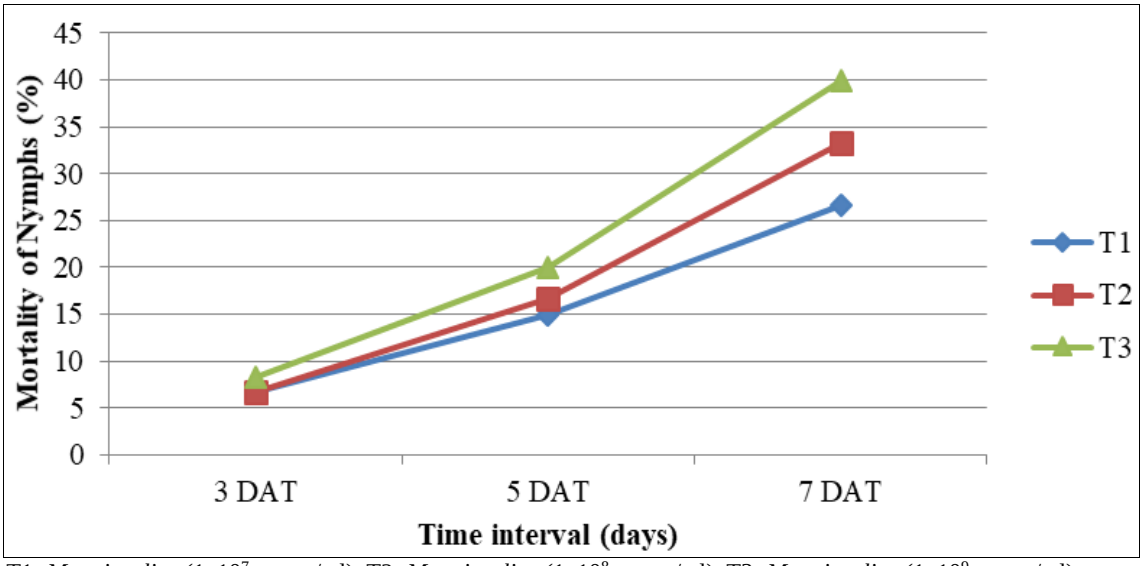
## 4. Discussion

### 4.1 Laboratory bioassay

During bioassay studies mortality of nymphs increased with the increase in period of exposure (Figure 1 to Figure 3**).** The highest mortality was recorded @ $1x10^9$ spores $ml^{-1}$ concentration in all the entomopathogenic fungi, followed by the lower concentrations *viz.*, $1x10^8$, $1x10^7$ spores $ml^{-1}$ which were on par with each other **(**Table 3**)**. Saranya *et al.* [9] also reported that the mortality of *Aphis craccivora* increased with the time. Seven days after treatment, *V. lecanii* @ $10^8$ spores $ml^{-1}$ everted 100 percent mortality, followed by *B. bassiana* and *M. anisopliae* with 96.66 and 80.76 percent mortality which was on accordance with the present finding.

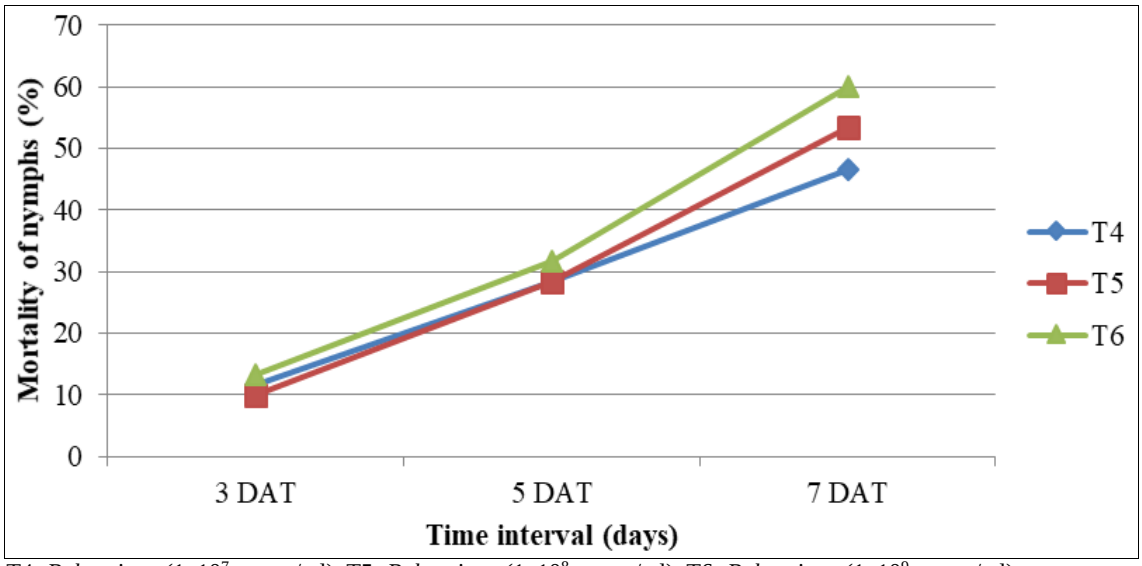
The highest mortality of mealybug was recorded (66.67%) in *L. lecanii* ($10^9$ spores $ml^{-1}$) followed by *B. bassiana* ($10^9$ spores $ml^{-1}$) with 60 percent mortality of nymphs which were on par with each other. *M. anisopliae* had resulted in 40 percent mortality. The present finding was in agreement with results of Banu *et al*. [10] where, nymphs of *Paracoccus marginatus* were more susceptible to entomopathogenic fungi than adults, leading to the mortality of 51.11, 44.45 and 44.44 percent with the spraying of *V. lecanii*, *B. bassiana* and *M. anisopliae*, respectively ($2\times10^8$ cfu/gm) @ 5g/l. It might be due to the fact that wax covering on body of the nymphs was lesser than the adults, which made the easy degradation of the cuticle by hydrolytic enzymes secreted by the fungus and hyphal penetration.

Makadia *et al*. [11] also reported that, first and second instar nymphs of *Maconellicoccus hirsutus* were more susceptible to *V. lecanii* (2g/l) than the later instars. Ten days after entomopathogenic fungal treatment, the highest mortality of 65.10 and 58.99 percent of the first and second instars nymphs of *M. hirsutus* was recorded, whereas 45.96 and 30.66 percent mortality in third instar nymphs and adult mealybugs. The present study was found to be in conformity with the finding of Halder *et al.* [12], where the percent mortality of nymphs increased with the increase in time where six days after the spraying *V. lecanii* (@ $2\times10^9$ cfu/g) resulted in the mortality of 67.11 percent followed by *B. bassiana* ($1\times10^8$ cfu/g) and *M. anisopliae* ($1\times10^8$ cfu/g) with 62.85 and 56.52 percent mortality, respectively. Among the three tested fungal pathogens *L. lecanii* @ $1\times10^8$ spores $ml^{-1}$ was proved to be efficient entomopathogen with increased efficiency as the period prolongs.



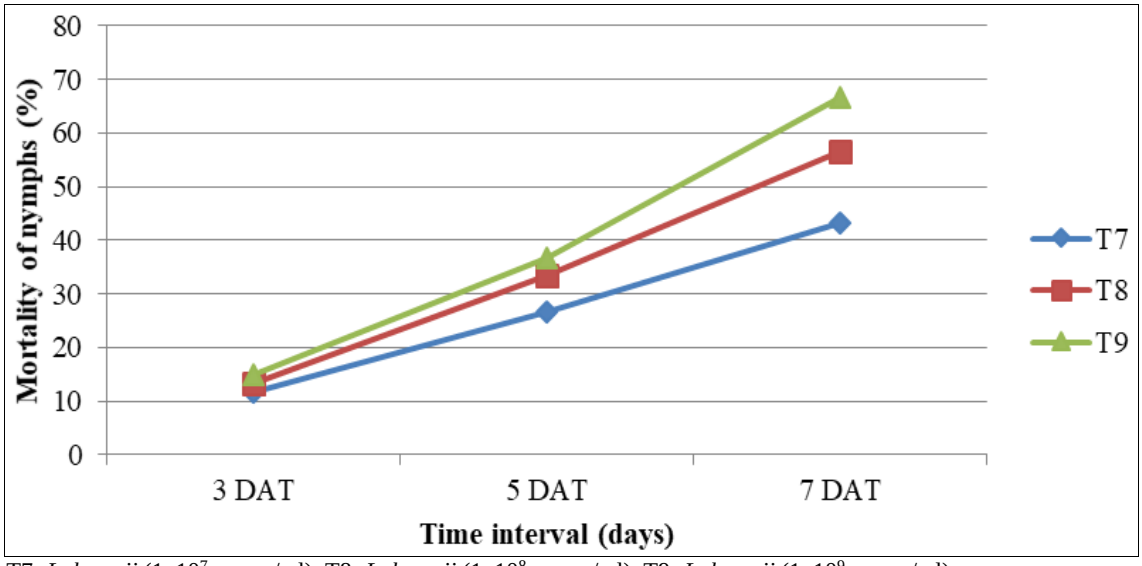

T1- *M. anisopliae* ($1x10^7$ spores/ml), T2- *M. anisopliae* ($1x10^8$ spores/ml), T3- *M. anisopliae* ($1x10^9$ spores/ml)

**Fig 1:** Effect of concentrations of *Metarhizium anisopliae* on *Dysmicoccus brevipes* at different under laboratory conditions




T4- *B. bassiana* ($1x10^7$ spores/ml), T5- *B. bassiana* ($1x10^8$ spores/ml), T6- *B. bassiana* ($1x10^9$ spores/ml)

**Fig 2:** Effect of different concentrations of *Beauveria bassiana* on *Dysmicoccus brevipes* under laboratory conditions




T7- *L. lecanii* (1x10$^{7}$ spores/ml), T8- *L. lecanii* (1x10$^{8}$ spores/ml), T9- *L. lecanii* (1x10$^{9}$ spores/ml)

**Fig 3:** Effect of different concentrations of *Lecanicillium lecanii* on *Dysmicoccus brevipes* under laboratory conditions

**4.2 Evaluation of entomopathogenic fungi under pot culture experiment**

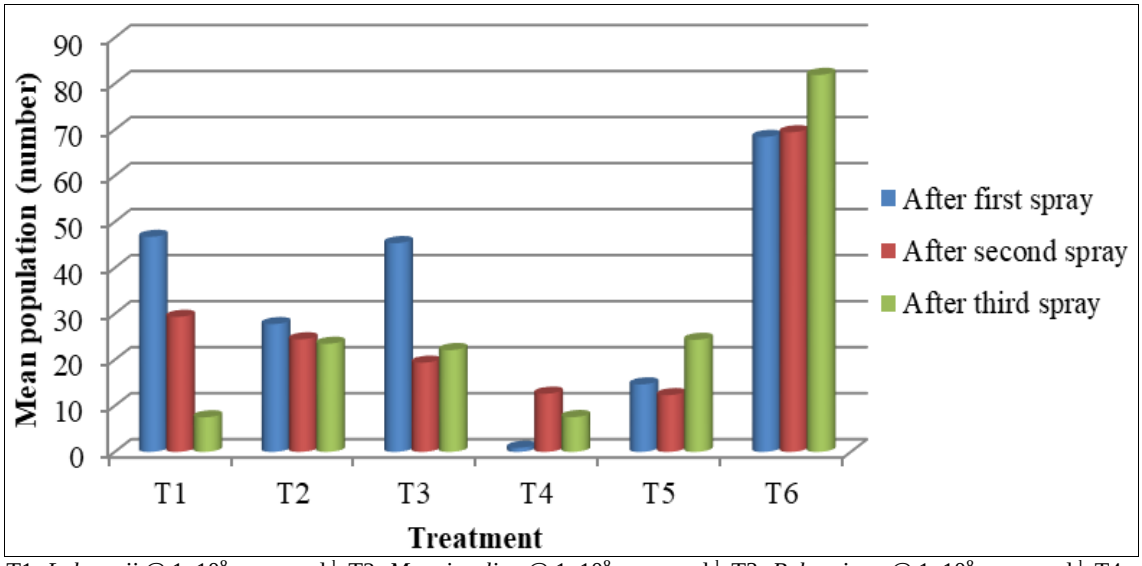
Efficacy of the *Lecanicillium lecanii*, *Beauveria bassiana* and *Metarhizium anisopliae* @ 1x10$^{8}$ spores ml$^{-1}$ (three sprays) in reducing the population of mealybugs was evaluated with the three sprays of azadirachtin (1% @ 0.005%) and a standard check quinalphos (25 EC @ 0.05%) with a single spray. The observation on the percent reduction of the mealybugs was taken at 10 days after each treatment.

Single spray of quinalphos was the most effective in reducing the number of mealybugs upto 40$^{th}$ day after the first spraying (Fig. 4 and 5). After the first spray, the highest reduction of 96.73 and 87.75 percent mortality of mealybug was recorded with quinalphos and azadirachtin, respectively. It might be due to the fact that chemical insecticides and botanicals were the most effective in controlling the mealybugs, because of their immediate action on interrupting the physiology of the insects. Efficiency of the insecticide was proved earlier by Kumar *et al*. [13] where the maximum mortality of 58.66 and 61.33 percent when acephate (75% SP @ 5 ml/l) and chlorpyrifos (20% EC @ 8 ml/l) were sprayed, and Halder *et al*. [12] who obtained a mortality of 70.29 percent of the *Phenococcus solenopsis* on application of neem oil (5%) while entomopathogenic fungi recorded the least mortality (62.85, 56.52 and 67.11 percent with *B. bassiana*, *M. anisopliae* and *V. lecanii*, respectively) over the control which was in conformity with the present finding.

Among the microbial pesticides, after the first application *M. anisopliae* recorded the maximum reduction of the mealybugs with 59.29 percent mortality followed by *B. bassiana* (30.13%) which was on par with each other. *L. lecanii* recorded a mortality of 17.26 percent. The present finding was in conformity with the report of Pandher *et al.* [14], where seven days after the application of treatment *B. bassiana* @ 10 g/l recorded the maximum mortality (34%) of *P. solenopsis* followed by *M. anisolpliae* (28.88%) and *V. lecanii* (25.38%). The results of the present study could also be supported by finding of Amutha and Banu [15] where they observed variation in the time and duration of various phases of mycosis of the *M. anisopliae*, *B. bassiana* and *V. lecanii* against the adults of the *P. marginatus*. *M. anisopliae* and *B. bassiana* were reported to possess rapid adhesion and penetration of the conidial spores on integument could be within 24 h and 48 to 96 h, respectively after the spraying of the fungus, whereas, in *V. lecanii*, hyphal penetration requires 48 h after the spraying lasting for 120 h. As the time required for the infection of *V. lecanii* was more, time required for causing the mortality of mealy bugs increased.

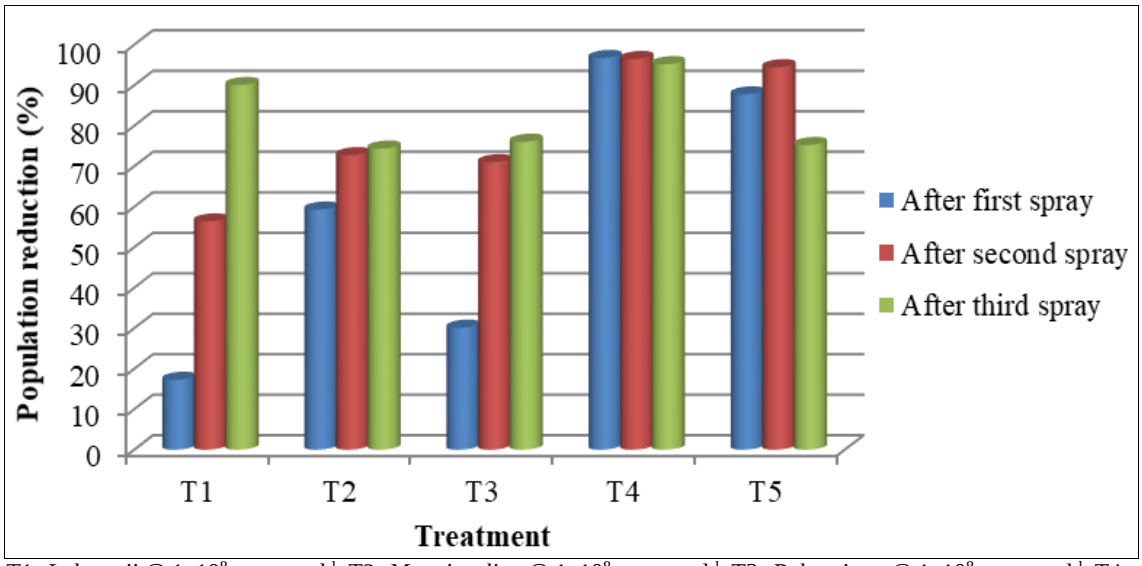
After the second spray, azadiractin gave the maximum mortality of 94.44 percent of the mealy bugs. Among three EPF sprayed *M. anisopliae* recorded the maximum mortality of 72.72 percent followed by *B. bassiana* (70.98%) which was on par with each other. The present result was in agreement with the findings Surulivelu *et al*. [16] who observed the maximum reduction of the *P. solenopsis* infesting on Bt cotton with 93.8 and 87.1 percent mortality with synthetic insecticide acephate and chlorpyrifos, while moderate level with *B. bassiana*, *V. lecanii* and *M. anisopliae* resulting in 39.1, 30.9 and 28.2 percent mortality, respectively.

After the third spray, *L. lecanii* had the highest percent mortality (90.04%) of mealybugs which was on par with the effect of quinalphos (95.2%). Effect of *L. lecanii* was followed by *B. bassiana* and *M. anisopliae.* Ghelani *et al*. [17] also reported that *V. lecanii* @ 2.5 kg/ha gave the highest mortality of 48.2 percent mortality of the *Aphis gossypii* infested on Bt cotton, while 44.6 and 37.7 percent of mortality was obtained with the spraying of *B. bassiana* @ 2.5 kg/ha and neem oil (1%), respectively which was in line with the present findings. As observed in the laboratory experiments, *L. lecanii* was found to be the most effective as that of synthetic insecticides quinalphos after three sprays.




T1- *L. lecanii* @ 1x10$^{8}$ spores ml$^{-1}$, T2- *M. anisopliae* @ 1x10$^{8}$ spores ml$^{-1}$, T3- *B. bassiana* @ 1x10$^{8}$ spores ml$^{-1}$, T4- Quinalphos 25EC @0.05%, T5- Azadirachtin 1% @ 0.005%, T6-Control

**Fig 4:** Effectiveness of the entopathogenic fungi, azadirachtin and quinalphos on *Dysmicoccus brevipes* in pot culture experiment




T1- *L. lecanii* @ 1x10$^{8}$ spores ml$^{-1}$, T2- *M. anisopliae* @ 1x10$^{8}$ spores ml$^{-1}$, T3- *B. bassiana* @ 1x10$^{8}$ spores ml$^{-1}$, T4- Quinalphos 25EC @0.05%, T5- Azadirachtin 1% @ 0.005%

**Fig 5:** Percent reduction in population of *Dysmicoccus brevipes* over control by entomopathogenic fungi in pot culture experiment

## 5. Conclusion

In the present study three entomopathogenic fungi *viz.*, *M. anisopliae*, *B. bassiana* and *L. lecanii* evaluated at concentrations 1×10$^{9}$ spores ml$^{-1}$ along with a botanical insecticide and a standard check (quinalphos 25EC @ 0.05%) under pot culture experiment. Among three EPF *L. lecanii* at 1×10$^{9}$ spores ml$^{-1}$ was found effective compared to *M. anisopliae* and *B. bassiana* at 1×10$^{9}$ spores ml$^{-1}$. Hence, it is concluded *L. lecanii* was effective and resulted in highest reduction (90.04%) of mealybugs which was on par with the reduction obtained by quinalphos (95.20%) application.

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