Field Bio-Efficacy of “Brigade-BL” (Beauveria bassiana) an Entomopathogenic Fungi for the Management of Mealy Bugs on Thompson Seedless Grapes

Sandeepa Kanitkar¹, V. M. Raut², S. D. Sawant³, D. S. Yadav⁴, Medha Kulkarni⁵ and Meghraj Kadam⁶

¹Kan Biosys Pvt. Ltd., Kanitkar Path, F. C. Road, Pune – 411004, INDIA
²Kan Biosys Pvt. Ltd., Kanitkar Path, F. C. Road, Pune – 411004, INDIA
³National Research Station for Grapes, Manjri Farm Post, Solapur Road, Pune – 412 307, INDIA
⁴National Research Station for Grapes, Manjri Farm Post, Solapur Road, Pune – 412 307, INDIA
⁵Kan Biosys Pvt. Ltd., Kanitkar Path, F. C. Road, Pune – 411004, INDIA
⁶Kan Biosys Pvt. Ltd., Kanitkar Path, F. C. Road, Pune – 411004, INDIA

Corresponding Author: meghraj.kadam@kanbiosys.com

ABSTRACT
A field bio-efficacy of Brigade-BL (Beauveria bassiana) an entomopathogenic fungi was evaluated against mealy bugs (Meconellicoccus hirsutus) on Thompson Seedless grapes at National Research Centre on Grapes, Manjri, Pune during 2014-15. The experiment was conducted in RBD with five replications on foundation pruning and fruit pruning. Results obtained revealed that application of Brigade-BL @ 5.0 ml/L with two sprays resulted in reduction of mealy bug colonies upto 67.82% in foundation pruning as compared to control. Likewise, at fruiting pruning, after fifth spray of Brigade-BL @ 5.0 ml/L recorded 75.68% reduction in mealy bug population and increase in fruit yield of 92.44% over control. Similarly, there was no any abnormality and phytotoxic effect observed by spraying Brigade-BL on grapevines. Likewise, Brigade-BL was found compatible with Buprofezin 25 SC chemical insecticide.

Keywords: Brigade-BL, Beauveria bassiana, Thompson Seedless, Mealy Bugs, Phytotoxicity, Compatibility.

I. INTRODUCTION

Grapes (Vitis vinifera L.) are the world’s most economically important fruit crop with good nutritional value and is consumed fresh or processed into raisins and wine. Viticulture is one of the most significant activities worldwide in terms of, not only the area but also the amount of money involved in this market in wine producing countries. However, this huge production presents pest problems. Around 150 arthropod species worldwide have been considered as vineyard pests, being phytophagous mites, leafhoppers, grape moths and mealy bugs among the main pests that attack vineyards. Mealybugs are phytophagous insects that constitute a family with about 2000 species, many of which are major pests of other agricultural plants (Gullan and Kosztarab, 1997) and Mansour (2017). Vine mealybugs could cause direct damage through sap suction and injection of phytotoxic saliva, affecting the normal growth and development of the plants (Singh, 2007). In addition, this insect could cause indirect damages due to the transference of virus between plants, which produces a decreased sugar content and diminished pigmentation, reducing the productivity and altering the quality of the grapes (Bertin 2016, Vega et al. 2011).

Grape is attacked by a number of insect pests. Among them, mealybugs are very important and cause considerable damage to the grapes. Pink mealy bug (Macconellicoccus hirsutus) is considered to be the most serious pests of grape in India. Both adult and nymph cause damage by sucking the sap from the leaves, stem, cordon as well as from bunches with their styles. The infested surface is marked by the accumulation of honey dew droplets which further coalesce and spreads on other plant parts thereby resulted in the development of black sooty mold, which can be detected from a distance. Leaves covered with sooty mold lose their phytosynthetic ability and also interfere with development of bunches. The heavy infestation leads to production of malformed shoot tips with wrinkling and stunting appearances. Initial colonies were found inside the loose bark of stem but it migrated towards new flush and later on bunches of grape. Heavy infested bunches are unit for human consumption. The grape mealybug alone is able to cause 50-100 per cent yield losses in the field. Mealybugs are seen throughout the year in the vineyards. In peninsular Indian conditions mealybugs found to be active from June to August and again from November to March. Hot and dry weather seems to be highly favorable for mealybugs. Numerous setbacks, including pollution, toxicity to animals and plants and insecticide resistance, are associated with the use of synthetic insecticides (Aktar et al. 2009). Knowledge gained over the years on the influence of endophytic microbial symbionts on plant defense mechanisms vis-à-vis insect herbivory have opened up opportunities for management of insect pests using fungal endophytes. Endophytic fungi occur in plants and colonies them without adverse effects.
meanwhile, plants serve as a host and provide nutrient to these fungi. Through this mutual relationship with their plant hosts, endophytes enhance plants tolerance to biotic and abiotic stresses (Lugtenberg et al. 2016). Endophytic fungi have also been reported to induce increased growth in plants, such as grasses. (Clay, 1988). The growth enhancing effect can be attributed to the ability of fungi to mobilize valuable nutrients for plant growth, for example *Metarhizium robertsi* promotes root growth and nitrogen absorption in switchgrass and haricot bean (Sasan and Bidochka, 2012). A study by Dara et. al. (2016) demonstrated improved plant health coupled with an increase in shoot-root ratio for plants treated with the fungus *Beauveria bassiana*. *Metarhizium robertsi* and *B. bassiana*, two well known entomopathogenic fungal species, have strains that can cause natural epizootic death in many insects (Sandhu et. al., 2012). Plant growth and productivity, as well as defense, could be enhanced by exploring the plant-fungus interaction.

Therefore, it is most appropriate to investigate on the viable, effective and safer effect of endophytic *Beauveria bassiana* formulation for grape vines pest management. Keeping this in view, the present study was undertaken to explore the Brigade-B (*Beauveria bassiana*) against mealybugs of grapevines.

### II. MATERIAL AND METHODS

A field experiment was conducted during foundation pruning 2014 and fruiting season 2014-15 on Thompson Seedless grape variety trained to ‘Y’ system at National Research Centre for Grapes, Pune, Manjri Farm, Pune-412 307. The experiment was laid out in a Randomised Block Design (RBD) with five replications. The vines were spaced at 2.4 m between rows and 1.2 m within rows. A basal fertilizer dose of NPK was applied as per the recommendations to all the plots. The treatment details are as below:-

| Treatment No. | Treatment details | Dose ml/L |
|---------------|-------------------|-----------|
| T-1           | Brigade-B (L) foliar spray | 2.50      |
| T-2           | Brigade-B (L) foliar spray | 5.00      |
| T-3           | Brigade-B (L) foliar spray | 7.50      |
| T-4           | T-1 + T-7          | 2.5 + 1.0 |
| T-5           | T-2 + T-7          | 5.00 + 1.0|
| T-6           | T-3 + T-7          | 7.5 + 1.0 |
| T-7           | Buprofezin 25 E.C. | 1.0       |
| T-8           | Control (Water spray) | -         |

Two treatment applications were made during foundation pruning and five treatment applications were made during fruit pruning 2014-15 from berry development stage to post-version stage for evaluating bio-efficacy against mealybugs. Spray volume of one litre per vine was used with knap sack sprayer. Pre-counts of number of mealybug colonies per vine were taken before treatment and at 3, 5 and 10 days after each treatment applications.

Brigade-BL formulation contains spores of *Beauveria bassiana* in a liquid formulation. It is bio-insecticide for management of soil and foliar insect pest infestation of crops. The active principles in Brigade-BL (Spores of *Beauveria bassiana*) germinate or stick to the insect. These spores germinate on the insect body, penetrate and destroy the tissues. The fungi proliferate in the body of the insect causing mycosis so that the insect stop feeding, resulting in insect’s death. Under moist conditions fluffs of fungus forms sores on the dead insect causing spread of fungus and affect other insects. Likewise, it is residue free, non-toxic, no pre-harvest interval, organic certified and environmentally free and safe for foliar application.

**Phytotoxicity**

Brigade-BL @ 7.50 ml/L and Brigade-BL + Buprofezin @ 7.50 ml/L + 1.00 ml/L were used for phytotoxicity observations and vines were critically observed for presence of phytotoxic effects such as chlorosis, tip burning, necrosis on leaves and berries, epinasty and russetting on berries. Observations were recorded in the form of visual ratings in 0-10 scale as given in Table-3 on 10th day after last spray.

**Compatibility studies**

Compatibility of *Beauveria bassiana* from Brigade-BL was evaluated with Buprofezin 25SC @ 1.00 ml/L in vitro and data was analyzed using TwoSample T-test.

### III. RESULTS AND DISCUSSION

**Bio-efficacy of Brigade-BL against mealybugs**

**A) Foundation pruning**

Pink mealybug, *Maconellicoccus hirsutus* (Green) species of mealybug was found in the experimental plot. Results presented in Table-1 and Fig 1a and 1b indicated that at one day after first spray, T-2
(Brigade-BL @ 5.0 ml/L) recorded lowest population of mealybugs (16.58/vine) followed by T-1 (Brigade-BL @ 2.50 ml/L) with 17.69 and T-5 (Brigade-BL @ 5.00 ml/L + Buprofezin @ 1.0 ml/L) with 20.77. At three days after first spraying, T-7 (Buprofezin 25 SC @ 1.0 ml/L) recorded lowest population of mealybugs (9.17) and was at par with T-2 (Brigade-BL @ 5.0 ml/L) with 13.64 and all other doses of Brigade BL + Buprofezin 25 SC. At five days after first spray also, T-7 recorded lowest population of mealybugs (2.68) and at par with all the doses of Brigade-BL + Buprofezin 25 SC and was superior over all other treatments. Likewise T-2 (Brigade-BL @ 5.0 ml/L) recorded minimum mealybugs (7.04) among other Brigade-BL doses.

Reduction in the mealybug colonies per vine was recorded at one day after second spray; T-3 (Brigade-BL @ 7.5 ml/L) recorded lowest population of mealybugs (6.22) and was at par with all other treatments. At three days after second spray also, T-3 (Brigade-BL @ 7.5 ml/L) was at par with all other treatments. At five days after second spray T-6 (Brigade-BL @ 7.5 ml/L + Buprofezin @ 1.0 ml/L) recorded minimum population of mealybugs (2.38) and was at par with its other doses of 2.5 ml/L, 1.0 ml/L and 5.0 ml/L + 1.00 ml/L and standard check Buprofezin.
### Table 1: Bio-efficacy of Brigade-BL against mealybug in grapes during foundation pruning

| No. | Treatment name | Dose (ml or g/L) | Covariance adjusted mean number of mealybug colonies per vine | % reduction in mealybug over control after 1st spray |
|-----|----------------|-----------------|----------------------------------------------------------------|-------------------------------------------------|
|     |                |                 | First spray | 1 DAS | 3 DAS | 5 DAS | 1 DAS | 3 DAS | 5 DAS |
| T-1 | Brigade B      | 2.50            | 17.69 bc | 5.96 b | 70.50 |
| T-2 | Brigade B      | 5.00            | 16.58 c  | 6.09 b | 67.82 |
| T-3 | Brigade B      | 7.50            | 25.26 a  | 5.27 b | 77.48 |
| T-4 | Brigade B + Buprofezin 25 SC | 2.50+1.00 | 22.68 ab | 6.67 b | 82.87 |
| T-5 | Brigade B + Buprofezin 25 SC | 5.00+1.00 | 20.77 abc | 6.65 b | 85.05 |
| T-6 | Brigade B + Buprofezin 25 SC | 7.50+1.00 | 22.46 ab | 6.33 b | 88.22 |
| T-7 | Buprofezin 25 SC | 1.00           | 22.15 abc | 7.69 b | 84.46 |
| T-8 | control        | -               | 23.00 ab  | 21.12 a | -    |

|     | F value | DF  | P>F     |
|-----|---------|-----|---------|
|     |         | 12.27 | <.0001  |

F value: 10.45, 4.5, 15.37, 7.22, 10.17, 14.02

DAS – Days after spraying
The data were analyzed by ANCOVA using actual values which are presented above.

Figures with same letter (a) do not differ significantly from each other.

B) Fruit Pruning
Pink mealybug, *Maconillicoccus hirsutus* (Green) species of mealybug was found in the experiment. There was no any significant difference was found for mealybug population up to second spray (Table 2). However, there was significant reduction in the population of mealybugs three days after third spray (Table 2 and Fig 2a, 2b and 2c). Brigade-BL @ 5.0 ml/L (T-2) recorded lowest population of mealybugs (8.0) and was at par with its other doses of 2.50 ml/L (T-1) and 7.50 ml/L (T-3).
At five and ten days after third spraying, T-7 recorded minimum population of mealybugs with 2.2 and 1.4 respectively. Likewise, at 3, 5 and 10 days after fourth and fifth spraying, all the treatments recorded significantly less mealybugs population over untreated control. Similarly, T-3 (Brigade-BL @ 7.50 ml/L), T-5, T-6 and T-7 recorded 91.89% reduction in mealybug population as compared to control treatment. However, T-2 (Brigade-BL @ 5.00 ml/L) recorded 75.68% reduction of mealybug population over control treatment.

When compared for economic yield, all the treatments were at par with each other and standard check (T-7) but superior over control. T-2 (Brigade-BL @ 5.00 ml/L) recorded highest yield of 10.18 T/ha with 92.44% higher yield than control treatment (5.29 T/ha).
Table 2: Bio-efficacy of Brigade-BL against mealybug in grapes during fruit pruning

| Tr. No | Pre count | Mean number of mealybug colonies as vine | % reduction in mealybugs over control | Yield (t/ha) | % increase in yield over control |
|--------|-----------|------------------------------------------|--------------------------------------|-------------|---------------------------------|
|        | Days after 1st spray | Days after 2nd spray | Days after 3rd spray | Days after 4th spray | Days after 5th spray |
| T1     | 34.8a     | 18.4a | 18.0a | 11.6a | 16.8a | 12.0a | 12.2a | 8.6ab | 8.0ab | 6.8b | 5.6b | 4.4b | 5.0ab | 4.2ab | 3.4ab | 3.8ab | 48.65 | 10.04a | 89.79 |
| T2     | 26.2a     | 20.2a | 17.4a | 13.2a | 13.6a | 12.8a | 10.2a | 8.0ab | 6.8bc | 5.8bc | 5.0bc | 4.2bc | 3.6b | 3.0b | 2.2b | 2.1b | 1.8b | 75.68 |
| T3     | 26.6a     | 20.2a | 17.6a | 14.2a | 12.2a | 11.2a | 10.8a | 8.8ab | 6.4bc | 4.4bc | 4.0bc | 3.4bc | 2.0b | 1.0b | 0.6b | 0.9a | 91.89 |
| T4     | 27.4a     | 19.8a | 16.3a | 12.0a | 13.94a | 8.6a | 6.8b | 3.4bc | 3.4bc | 3.4bc | 3.0bc | 2.6bc | 2.6b | 2.0b | 1.6b | 1.8b | 75.68 |
| T5     | 25.6a     | 16.4a | 13.9a | 12.4a | 12.6a | 9.0a | 6.4a | 5.2bc | 3.6bc | 2.4bc | 1.8c | 1.6b | 2.0b | 1.0b | 0.6b | 0.6b | 91.89 |
| T6     | 27.2a     | 20.8a | 21.4a | 12.4a | 13.2a | 11.4a | 8.0a | 5.4bc | 3.8bc | 1.4bc | 2.0b | 1.8b | 2.0b | 1.4b | 0.8b | 0.6b | 91.89 |
| T7     | 31.6a     | 20.8a | 21.4a | 12.4a | 13.2a | 11.4a | 8.0a | 5.4b | 2.2c | 1.4c | 1.4c | 1.4bc | 1.6b | 1.2b | 0.8b | 0.6b | 91.89 |
| T8     | 19.8a     | 21.6a | 15.0a | 16.8 | 15.2a | 14.6a | 12.8a | 14.8a | 13.2a | 12.4a | 11.2a | 10.0a | 7.8a | 10.6a | 7.6a | 7.4a | 5.29b |
| F valve | 1.32      | 0.33 | 0.94 | 1.07 | 0.72 | 0.71 | 1.29 | 3.19 | 6.03 | 4.63 | 10.84 | 5.15 | 3.14 | 4.44 | 4.34 | 8.59 |
| Pr>F    | 0.2639    | 0.9713 | 0.54 | 0.40 | 0.72 | 0.60 | 0.72 | 0.72 | 0.60 | 0.60 | 0.02 | 0.00 | 0.00 | 0.00 | <.0001 | 0.0008 | <.0001 |
| DF (Mod. Err.) | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 | 11.28 |
| LSD at 5% | 15.55 | 13.85 | 10.62 | 8.26 | 9.13 | 8.43 | 7.70 | 6.82 | 5.37 | 5.21 | 3.63 | 4.94 | 5.77 | 3.61 | 4.16 | 4.21 | 2.15 |

Figures with same letter/s do not differ significantly from each other.
Phytotoxicity

Results presented below indicated that among the tested treatments of Brigade-BL, no treatment have shown any type of phytotoxic symptoms either on leaves or berries during the period of observation (T-3).

Table 3: Phytotoxicity of Brigade-BL on leaves and berries.

| No. | Treatment          | Dose ml/L | Leaves/Berries                  |
|-----|--------------------|-----------|---------------------------------|
|     |                    |           | Leaf Chlorosis | Tip burning | Necrosis | Epinasty | Russetting |
| 1   | Brigade-BL         | 7.5       | 0.00           | 0.33        | 0.07     | 0.00     | 0.00       |
| 2   | Brigade-BL + Buprofezin 25 SC | 7.5 + 1.0 | 0.00           | 0.40        | 0.00     | 0.20     | 0.00       |
| 3   | Control            | -         | 0.00           | 0.47        | 0.00     | 0.13     | 0.00       |

Significance: NS - Not significant at 5% level of significance.

Compatibility studies

The results presented below revealed that there was not much difference in the diameter of mycelia growth of Beauveria bassiana on PDA amended plates. No significant difference found in test as compared to control statistically.

Table 4: Percent growth in hibition of Beauveria bassiana with Buprofezin 25 SC after 15 days of inoculation.

| Replication | % inhibition in growth as compared to control | Control fungus diameter (mm) X | Test fungal diameter (mm) Y |
|-------------|---------------------------------------------|-------------------------------|---------------------------|
| 1           | 8.57                                        | 35                            | 32                        |
| 2           | 8.57                                        | 35                            | 32                        |
| 3           | 0.00                                        | 31                            | 31                        |

Samples are not significantly different.

In the present study, Brigade-BL formulation containing Beauveria bassiana resulted in reduction of mealybug colonies during foundation and fruit pruning. Application of Brigade-BL @ 5.00 ml/L with two sprays resulted in reduction of mealybug colonies up to 67.82% as compared to control in foundation pruning. Likewise, at fruit pruning, after fifth spray of Brigade-BL @ 5.00 ml/L recorded 75.68% reduction in mealybug population and at 7.50 ml/L. recorded 91.89% reduction in mealybug population as compare to control with increase in fruit yield of 92.44% and 86.39% respectively over control treatment.

Economic losses resulting from vineyard mealybug infestations have increased dramatically during the past decade. In response, there has been a cosmopolitan effect to improve control strategies with better understand mealybug biology and ecology as well as their role as vectors of plant pathogens. Mealybugs are named for the powdery secretions covering their bodies. The most important vineyard mealybugs belong to the subfamily Pseudococcinae (Hardy et. al. 2008). Severe mealybug outbreaks have been reported in India’s vineyards, adversely affecting grape production by as much as 90% in extreme cases in the state of Andhra Pradesh. Among the eight mealybug species that have been reported on vines in India, Maconellicoccus hirsutus, Planococcus citri, Nipaecoccus viridis and the root mealybug, X. annandaleti are the primary mealybug pests. Cultivars harvested in late fall suffer heavily from mealybug damage. The increasing mealybug problem in recent years may be due to frequent and indiscriminate use of insecticides to control other pests, which may disrupt natural enemies responsible for the suppression of mealybugs. The root mealybugs damage by this pest reduces vine vigour and yield, and shortsens fruit-bearing canes (Rajgopal et. al. 1997).

Maconellicoccus hirsutus is the most important of the vineyard mealybugs in Peninsula India, with severe infestations leading to berry and shoot damage. The mealybugs occurs on the vine throughout the year (Mani and Thontadarya 1987). Mealybugs stick up sap from inflorescence, tender leaves, shoots and fruit panicles. As a result, the affected inflorescences are shrivelled and get dried severe attack affect the fruit set and causes fruit drop. They also secrete honey dew on leaves, fruits and shoots consequently sooty mould develops on these plant parts (Karar et. al. 2010). Some plant extracts are used as biopesticides that control insect population by non-toxic mechanisms (Schmiutterer, 1990). Bio-pesticides are the substances or mixture of substances used to prevent, destroy, repel, attract, sterilize or mitigate the pests (Khathe, 2011).
Azadirachtin is a non-toxic plant material to birds and mammals and is non-carcinogenic that is derived from the neem tree (*Azadirachtaindica*), which affects reproductive and digestive process of a number of important pests (Kalra and Khanuja, 2007). Neem products have a repellent effect on some mealybugs (Saxena, 2002). *Eucalyptus camaldulensis* and some other botanicals are also used as weed control in farmlands to maximize yields (Yadav et. al., 2008). Atar (2014) evaluated the bio-efficacy of ten botanicals and two bio-agents in vitro against *Xanthomonas axonopodispv.punicae*. Among them, neem oil was best effective for control of disease at all concentrations recording 14.47% inhibition followed by garlic extract. This leaf extract, ginger extract, guava leaf extract and aloe vera extract with 12.86%, 12.28%, 11.70%, 9.36% and 7.34% average inhibition respectively. On the other hand, *Azadirachta indica* was the most used plant species in the different studies on mealybugs followed by *Eucalyptus globules*. Also, Azadirachtin is the most important of the neem limonoids, (*A.indica*) which has a repellent and insecticidal action against many insect species (Badchah, 2015, Nisbet, 2000). The effectiveness of a product can be increased by adding a low concentration soap solution to the botanical products (Sola, 2014, Prishanthini and Vinobaba, 2014).

Grapevine plants exposed to *B.bassiana* inoculums had higher tissue nutrient contents of Ca and Mg compared to the control plants have been reported by Moloinyane and Nchu (2019). They also suggested that fungus inoculation influences nutrient accumulation in the tissue of grapevine plants. Endophytic fungi have also been reported to induce increased growth of Plants (Dara, 2016, Clay 1998). A strain of the endophytic fungus *B.bassiana* reduced infestation rates on potted grapevine plants (Rondot and Reineke, 2018). Some endophytic fungi have been reported to produce metabolites that can reduce insect infestations on their host plants (Jaber, 2018). It is believed that increased in quantity and diversity of secondary metabolites in endophyte-containing plants is somewhat responsible for the reduction of insect herbivory on plants (Hartley and Gange, 2009). Fungal endophytes can increase the production of antioxidant in plants as well as produce antioxidant compounds such as phenolics. They also produce plant growth hormones and enhance plant nutrient absorption, favoring increased germination success and growth rate (Jallow et. al. 2008).

The grapevine mealybug is a sap sucking insect that is difficult to control (Goodfrey et. al. 2008.), individuals at this species often hide in plant crevices and have a protective waxy layer. It is among the most serious pests of vineyards causing substantial losses globally (Berlinger, 1977). Based on the results of present investigation, it was concluded that the product Brigade-BL (*Beauveria bassiana*) tested may provide viable alternative to synthetic insecticides used in the control of mealybug.

In the present study ‘Brigade-BL’ @ 5.00 ml/L resulted in reduction of mealybug colonies during foundation and fruit pruning stage with increase in yield upto 92.44% over control treatment. Similarly, there was no any abnormality and phytotoxic effect by spraying Brigade-BL on grapevines. Similarly, Brigade-BL was found compatible with Buprofezin 25 SC @ 1.00 ml/L water.

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