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BIOLOGICAL CONTROL OF YELLOW SUGARCANE APHIDS, *SIPHA FLAVA* (HOMOPTERA: APHIDIDAE) USING A COMMERCIAL STRAIN OF *BEAUVERIA BASSIANA* (HYPOCREALES: CORDYCIPITACEAE)

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ABSTRACT

The yellow sugarcane aphid, *Sipha flava* (Homoptera: Aphididae) is a major insect pest of sugarcane in Eswatini. Insecticides are used to prevent honeydew build up and yield loss before harvest but they are not preferred due to resistance problems, disruptive effects on natural control of other sugarcane pests, pesticide residue and high production costs. The use of the entomopathogenic fungi as a biocontrol agent of sugarcane aphid is an environmentally friendly management tactic. The current study evaluated the mortality effects of four concentrations [Control, 1.25 g/L (2.5×10^6 spores/ml), 2.5 g/L (5×10^6 spores/ml) and 5g/L (1×10^7 spores/ml)] of commercially available *Beauveria bassiana* (Eco-Bb®) strain R444 on adult yellow sugarcane aphids in laboratory and leaf bioassays. The application of 5g/L (1×10^7 spores/ml) of *B. bassiana* resulted in mortality that was not significantly different from 2.5g/L (5×10^6 spores/ml) but was significantly ($P<0.05$) different to 1.25g/L (2.5×10^6 spores/ml) and the control in the laboratory bioassay. The application of 5g/L (1×10^7 spores/ml) of *B. bassiana* resulted in mortality that was significantly ($P<0.05$) higher from all the other concentrations in the leaf bioassay. The higher concentration of the fungus can be recommended to control *S. flava*. However, large-scale field trials and combining *B. bassiana* with other biocontrol agents is recommended to demonstrate the potential use of the biocontrol agent within an integrated pest management program.

Keywords: *Beauveria bassiana*, biocontrol agent, entomopathogenic fungi, integrated pest management program, yellow sugarcane aphid.

1. INTRODUCTION

The first presence of the sugarcane aphid, *Sipha flava* (Homoptera: Aphididae) in Africa was recorded in 2006 in Morocco (Abdelmajid, 2008). It was later recorded in the South African Sugarcane Industry for the first time during the 2013 growing season (Munywa, 2007) and was subsequently detected across the Kingdom of Eswatini and Zimbabwe (SASA, 2012). Sugarcane aphids have been known to feed on a variety of grasses including rice, sugarcane, sorghum, millet, Bermuda grass, Johnson grass and others (DeSouza, 2018; Nuessly, 2019). Yellow Sugarcane aphids infest the lower leaves of a sugarcane plant and as colonies grow, the aphids make their way up the plant (Knutson et al., 2016). Feeding of *S. flava* initially causes yellowing and reddening of sugarcane leaves, and prolonged feeding leads to early senescence of leaves and stalk death of the cane (Nuessly, 2019). Drastic yield reductions normally occur due to feeding damage to early plant growth stages, including reduced tillering (Hall, 2001). Yields are

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reduced by up to 6% following aphid-induced death within the first three months of growth (Nuessly and Hentz, 2002).

Feeding on sugarcane leaves by *S. flava* causes minimal symptoms until heavy infestations produce honeydew which leads to the development of black sooty mould on leaves (Wilson, 2019). Akbar et al. (2010) indicated that aphid-feeding reduces chlorophyll content in leaves and removes amino acids while also causing yellow to red or brown leaf discolorations on both sides. Wilson (2019) further states that the formation of sooty mould results from growth of a complex of fungi on honeydew and is thought to reduce the surface area available for photosynthesis. As a result, the development of sooty mould reduces photosynthesis and negatively impact plant growth. The vector potential of the sugarcane aphid is of great importance in the sugarcane industry as it can vector the Sugarcane Yellow Leaf Virus (ScYLV), red-millet virus and the sugarcane mosaic virus (Wilson, 2019).

Temperature is an important environmental factor for the development, survival and reproduction of aphids (DeSouza, 2018); a milder winter allows the sugarcane aphid to infest earlier than a colder winter when high mortality occurs (DeSouza, 2018). The nymphs and adults of *S. flava* feed on the phloem by sucking the sap from the under-surface of the leaves (DeSouza, 2018). The feeding on plant sap cause reddening of leaves which results to leaf tissue necrosis (Knutson et al., 2016). The infested leaves dry up and turn yellow or brown. Under heavy infestation of *S. flava*, the plants may be severely stunted (Singh et al., 2004).

The use of natural enemies in controlling insect pests is an important component of any Integrated Pest Management (IPM) control strategy (Padmanabhan et al., 1989). A range of natural enemies have been shown to help in reducing populations of sugarcane aphids including several lady beetle species (Coleoptera: Coccinellidae); green lacewings, *Chrysoperla rufilabris* (Neuroptera : Chrysopidae); hoverflies, *Eupeodes* spp. (Diptera: Syrphidae) and *Aphelinus certus* (Yasnosh) (Hymenoptera: Aphelinidae) (Reay-Jones and Greene, 2017). In cases where the population of sugarcane aphids increase rapidly, the use of insecticides may be needed to prevent honeydew build up and yield loss before harvest (Reay-Jones and Greene, 2017). Chemical control of insect pests of sugarcane has given non-significant results and has added to the higher costs of production (Padmanabhan et al., 1989). Often, insecticides only provide fair control and pre-harvest intervals are lengthy even at the lowest rates (Reay-Jones and Greene, 2017).

Since Yellow sugarcane aphids are still considered as a new pest particularly in the southern African region, it is too early, after the invasion, to develop a defined and/or specific management strategy for this pest in southern Africa. The use of insecticides to control the sugarcane aphids is coupled with several disadvantages including the emergence of secondary pests, insecticide resistance, market restrictions, and their inability to kill the yellow sugarcane aphids on the under-surface of the leaves (Dlamini et al., 2020). Yellow sugarcane aphids can overwinter on volunteer sugarcane plants and weeds setting up the following year's sugarcane crop for an early infestation (Way et al., 2015). This means that it is important for the sugarcane producers have to constantly scout their fields and rogue out any volunteer sugarcane plants (Way et al., 2015).

There is need, therefore, for environmentally-friendly sugarcane aphid control strategies. The use of entomopathogenic fungi (EPF) has been successfully used to control a number of insect pests and is an eco-friendly alternative method that can be used to control sugarcane aphids. EPF are microorganisms found in almost all ecosystems infecting several groups of insect pests (Dent,

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1999; Quesada-Béjar et al., 2019; Dlamini et al., 2020). There are a number of EPF causing epizootics but the most commonly used commercial EPF include *Beauveria bassiana* (Bals.-Criv.) Vuill, 1912 (Hypocreales: Clavicipitaceae); *Metarhizium anisopliae* (Metchnikoff) Sorokin, 1883 (Hypocreales: Clavicipitaceae); and *Metarhizium acridum* (Driver and Milner) (Hypocreales: Clavicipitaceae) (Quesada-Béjar et al., 2019; Hatting et al., 2019). For bio-insecticides to perform effectively in IPM program, it is normally recommendable to determine the most lethal concentration of the bio-insecticides in order to achieve maximum control of the insect pests in the field. In the current study, the main objective was to investigate the effect of different concentrations of an entomopathogenic fungus (Eco-Bb®) on the mortality of yellow sugarcane aphids in laboratory bioassays.

2.MATERIALS AND METHODS

2.1 Source of insects

Yellow sugarcane aphids were reared in a greenhouse (natural sunlight, no supplemental lighting) at the National Crop Production Centre, Faculty of Agriculture, University of Eswatini, Kingdom of Eswatini. Adult yellow sugarcane aphids used in the experiment were collected from sugarcane plants at Siteki by taking of the infested sugarcane leaves. The aphids were kept in Perspex boxes measuring 15 cm × 20 cm and were allowed to feed on the sugarcane branches. To mass rear the sugarcane aphids, sorghum were planted in organic soil using DY- hydroponic seed foam trays (66 x 34 cm plastic tray, 200 cells per tray, individual rectangular cells 4.8 cm deep). The seedling trays with sorghum were placed in trays containing water and the water was replaced daily throughout the duration of the experiment. Aphids collected from sugarcane plants in the field were introduced onto each tray of sorghum plants a couple of days after the sorghum plants emerged. The aphids were allowed to feed on the plants to increase their densities. A brushing procedure was frequently used for collecting aphids from leaves of infested sorghum, although the brushing procedure killed some aphids.

2.2 Fungal cultures

Fungi used in this study included the commercially available formulation of Eco-Bb® with the active ingredient *Beauveria bassiana* strain R444 (Hypocreales: Cordycipitaceae) (Hatting et al., 2019). Conidia for the assays were obtained by culturing the EPF on Sabouraud Dextrose Agar (SDA) medium (supplemented with 1ml dodine, 50 mg/l chloramphenicol, and 50 mg/l ampicillin or 50 mg/l rifampicin) for 14 days at 25 ± 1 °C (Goettel & Inglis, 1997). The EPF cultures were grown at 25 °C for 3 to 4 weeks.

2.3 Conidial concentrations and viability

EPF cultures (3–4 weeks old) were used to harvest conidia by scraping the surface of Petri dish cultures with a glass rod as describe by Dlamini et al. (2020). The conidia were suspended in 20 ml sterilised distilled water augmented with 0.01% Tween® 20 (Merck, Kommanditgesellschaft auf Aktien (KGaA), Darmstadt, Germany) in sterile McCartney bottles. The 0.01% Tween 80 was added in order to uniformly disperse the conidia in the conidial suspension. The bottles were sealed and vortexed for 2 min to obtain a homogeneous suspension. The concentration of conidial suspensions was determined using a haemocytometer. Before the chamber was used, it was rinsed with 70 % ethanol. To determine the germination response of the four Eco-Bb®

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concentrations, 100 µl of each Eco-Bb® spore suspension (1×10^6 conidia/ml) was spread onto a SDA plate in a Petri dish (60×15 mm). A cover slip was placed on each plate and the Petri dishes were incubated at 25 °C. The percentage of germination was determined after 24 h by examining 100 spores from each isolate under a microscope at ×40 magnification and counting the number of spores that had germinated (Ekesi et al., 2002).

2.4 Laboratory bioassay

The 24-well bioassay trays were used in the experiment as described by Dlamini et al. (2020). Each alternate well was lined with a piece of 13 mm diameter filter paper to remove excess spore suspension from the aphid. Adults of yellow sugarcane aphids (1 day old) were individually immersed in a 100 ml conidial suspension with 1.25g/L (2.5×10^6 spores/ml), 2.5g/L (5×10^6 spores/ml) and 5g/L (1×10^7 spores/ml) for 60 sec which is half the recommended, recommended and double the recommended rate, respectively. The controls were immersed in a 100 ml solution of distilled water and 0.01 % Tween® 20. Twelve treated yellow sugarcane aphids were placed singly in alternate wells in 24-well bioassay trays, kept at 25 °C in a growth chamber for a period of 21 days. For each concentration, five replicate plates with 12 treated yellow sugarcane aphids were prepared per treatment. The entire bioassay was repeated for each Eco-Bb® concentration on a different test date with newly prepared conidial suspension, resulting in a total of 120 yellow sugarcane aphids for each treatment. The 24-well bioassay trays were inspected every seven days for 21 days and the number of dead sugarcane aphids was recorded. The yellow sugarcane aphids were considered infected if signs of overt fungal mycosis and sporulation were observed. Yellow sugarcane aphids without signs of overt fungal mycosis and sporulation excluded.

2.5 Leaf bioassay

Yellow sugarcane aphids were mass reared on sorghum leaves and a brush was used to remove the sugarcane aphids from the sorghum leaves. Twelve aphids were placed on the lower side of freshly cut sugarcane leaves. Each sugarcane leaf containing *S. flava* was placed in a petri dish and the different concentrations of Eco-Bb® {1.25g/L (2.5×10^6 spores/ml), 2.5g/L (5×10^6 spores/ml) and 5g/L (1×10^7 spores/ml)} were prepared and sprayed using the Potter spray tower (Rothamsted Experimental Station, Harpenden, Herts, England) on both sides of the fresh sugarcane leaves. Distilled water and 0.01 % Tween® 20 was sprayed using the Potter spray tower on both sides of the fresh sugarcane leaves on the control. The petri dishes were then closed and kept in an incubator at 25°C. Leaf bioassays were replicated five times for each treatment, also making a total of 60 aphids per treatment. Petri dishes, with the sprayed sugarcane leaves, were then placed in one plastic container per treatment, lined with wet paper towels to ensure high humidity, and kept in an incubator at 25°C. Data was collected in 7-day intervals for 21 days after the application of the different concentrations of Eco-Bb®. The development of mycosis on the sugarcane aphid's body was used to identify aphid death due to the EPFs. Yellow sugarcane aphids without signs of overt fungal mycosis and sporulation were excluded.

2.6 Field trial

The experimental design consisted of eight young yellow sugarcane plants (0.5m) for each

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treatment (with a total of 32 plants), with two buffer sugarcane plants between each treatment plant, in four alternate rows, in a completely randomised design (Malan et al., 2016). Twelve aphids were placed on the lower side of sugarcane leaves and the plant was covered with a pre-prepared cage made out of a white sheet to prevent the aphids from escaping. Each sugarcane plant containing the 12 *S. flava* was sprayed with the different concentrations of Eco-Bb® {1.25g/L (2.5×10^6 spores/ml), 2.5g/L (5×10^6 spores/ml) and 5g/L (1×10^7 spores/ml)} prepared in 200 mL water. Data was collected in 7-day intervals for 21 days after the application of the different concentrations of Eco-Bb® in the field. The development of mycosis on the yellow sugarcane aphid's body was used to identify aphid death due to the EPFs. Yellow sugarcane aphids without signs of overt fungal mycosis and sporulation were excluded.

2.7 Data analysis

Virulence assays were analysed using analysis of variance (ANOVA); if the F-value was significant ($P < 0.05$), the means were differentiated using the LSD (SAS Institute, 1985). The data obtained from the efficacy of the different concentrations of Eco-Bb® on *S. flava* was corrected for the corresponding control mortality using the Abbot equation: CM (%)={(T-C)/(100-C)}×100, where CM is the corrected mortality, T is the percentage mortality in treated insects, and C is the percentage mortality in untreated insects (Abbot, 1925). If no significant interactions occurred between the main effect of the test dates and the treatments, the data obtained from the two test dates were pooled and analysed using a one-way ANOVA.

3. RESULTS

3.1 Laboratory bioassay

Eco-Bb® was able to infect and kill *S. flava* adults (Table 1; Fig 1). The results showed that there was a significant difference on the mortality of *S. flava* between the different concentrations of Eco-Bb® after 7 and 14 days (Table. 1). At 7 days after the application of the different concentrations of Eco-Bb®, the mean mortality of *S. flava* ranged from 2.0% to 51.7%. Analysis using a one-way ANOVA showed that the different concentrations of *B. bassiana* had a significant effect on the percentage mortality of *S. flava*. The application of 5g/L (1×10^7 spores/ml) (51.7%) of *B. bassiana* resulted in mortality that was not significantly different to 2.5g/L (5×10^6 spores/ml) (50%) but was significantly ($P<0.01$) different to 1.25g/L (2.5×10^6 spores/ml) (38.3%) and the control (Table. 1). The application of 1.25g/L (2.5×10^6 spores/ml) (38.3%) of *B. bassiana* resulted in mortality that was significantly ($P<0.01$) different from all the concentrations of Eco-Bb®.

At 14 days after the application of the different concentrations of Eco-Bb®, the mean mortality of *S. flava* ranged from 2.3% to 81.7%. Analysis using a one-way ANOVA showed that the different concentrations of *B. bassiana* had a significant effect on the percentage mortality of *S. flava*. The application of 0g/L, 1.25g/L, 2.5g/L and 5g/L (2.3, 50, 68.3 and 81.7%, respectively) of *B. bassiana* resulted in mortality that was all significantly ($P<0.01$) different from each other (Table. 1).

The results showed that there was a significant difference on the mortality of *S. flava* between the different concentrations of Eco-Bb® after 21 days (Fig. 1). The mean mortality of *S. flava* ranged from 3% to 86.7% after 21 days. Analysis using a one-way ANOVA showed that the different concentrations of *B. bassiana* had a significant effect on the percentage mortality of *S. flava*. The

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application of 5g/L (1×10^7 spores/ml) (86.7%) of *B. bassiana* resulted in mortality that was not significantly different to 2.5g/L (5×10^6 spores/ml) (75%) but was significantly ($P<0.01$) different to 1.25g/L (2.5×10^6 spores/ml) (58.3%) (Fig. 1). The control (distilled water and 0.01 % Tween® 20) (3%) gave mortality that was significantly ($P<0.01$) different to all three Eco-Bb® concentrations (Fig. 1). Natural mortality in the control was 3% after a period of 21 days.

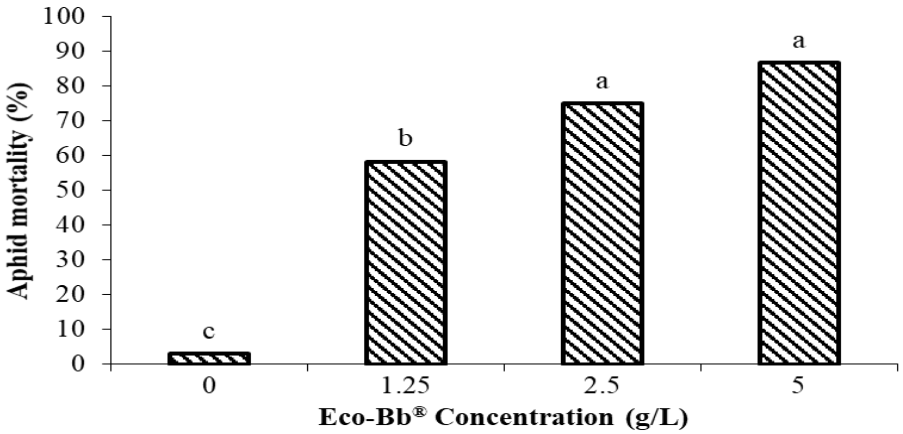

Figure 1. Mean percentage mortality of *S. flava* (99 % confidence level) after 21 days of the application of different concentrations of Eco-Bb® in a laboratory bioassay. Different letters indicate significant differences ($P < 0.01$) between concentrations of Eco-Bb® and mortality of *S. flava*.

Table 1: Mean mortality of yellow sugarcane aphids at different day intervals in aphid bioassays.

Concentration (g/L)	Mean mortality (%) at different day intervals		
	Day 7	Day 14	Day 21
0 (control)	2.04^c	2.32^d	3.01^d
1.25	38.33^b	50.00^c	58.33^c
2.5	50.00^a	68.33^b	75^b
5	51.67^a	81.67^a	86.67^a
cv (%)	**10.0**	**5.7**	**3.6**
LSD	**4.775**	**3.850**	**2.696**
F. pr	**<.001**	**<.001**	**<.001**

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3.2 Leaf bioassay

Eco-Bb® was able to infect and kill *S. flava* adults (Table 2; Fig 2). The results showed that there was a significant difference on the mortality of *S. flava* between the different concentrations of Eco-Bb® after 7 and 14 days (Table. 2). At 7 days after the application of the different concentrations of Eco-Bb®, the mean mortality of *S. flava* ranged from 2.0% to 41.7%. Analysis using a one-way ANOVA showed that the different concentrations of *B. bassiana* had a significant effect on the percentage mortality of *S. flava*. The application of 5g/L (1×10^7 spores/ml) (41.7%) of *B. bassiana* resulted in mortality that was not significantly different from 2.5g/L (5×10^6 spores/ml) (35%) but significantly ($P<0.01$) different from the control (2%) and 1.25g/L (2.5×10^6 spores/ml) (33.3%) (Table. 2). The application of 2.5g/L (5×10^6 spores/ml) of *B. bassiana* resulted in mortality that was not significantly different to 1.25g/L (2.5×10^6 spores/ml) but was significantly ($P<0.01$) different to the control (Table. 2).

At 14 days after the application of the different concentrations of Eco-Bb®, the mean mortality of *S. flava* ranged from 2.4% to 70%. Analysis using a one-way ANOVA showed that the different concentrations of *B. bassiana* had a significant effect on the percentage mortality of *S. flava*. The application of 5g/L (1×10^7 spores/ml) (70%) of *B. bassiana* resulted in mortality that was significantly ($P<0.01$) different to all the other concentrations of Eco-Bb® (Table. 2). The application of 2.5g/L (5×10^6 spores/ml) (46.5%) of *B. bassiana* resulted in mortality that was not significantly different to 1.25g/L (2.5×10^6 spores/ml) (45%) but was significantly ($P<0.01$) different to the control (Table. 2).

The mean mortality of *S. flava* ranged from 3.3% to 91.67% after 21 days (Fig. 2). Analysis using a one-way ANOVA showed that the different concentrations of *B. bassiana* had a significant effect on the percentage mortality of *S. flava*. The application of 5g/L (1×10^7 spores/ml) (91.7%) of *B. bassiana* resulted in mortality that was significantly ($P<0.01$) higher from all the other concentrations (Fig. 2). The application of 1.25g/L (2.5×10^6 spores/ml) (48.3%) and 2.5g/L (5×10^6 spores/ml) (58.3%) were not significantly different to each other but were both significantly ($P<0.01$) different to the 5g/L (1×10^7 spores/ml) and the control (Fig. 2). The control (distilled water and 0.01 % Tween® 20) (3.3%) gave mortality that was significantly ($P<0.01$) different to all three Eco-Bb® concentrations (Fig. 2). Natural mortality in the control was 3% after a period of 21 days.

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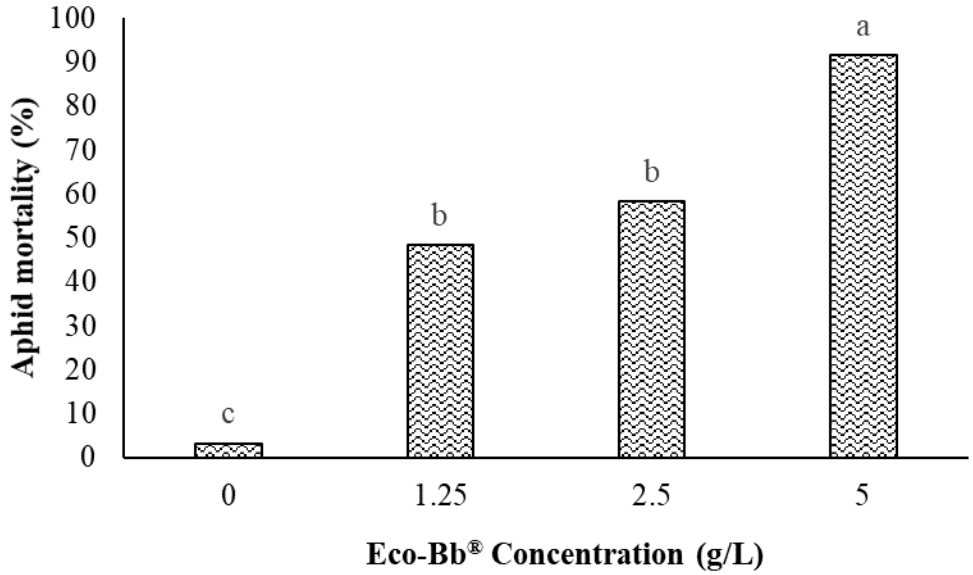

Figure 2: Mean mortality of *S. flava* (99 % confidence level) after 21 days of the application of different concentrations of Eco-Bb® in the leaf bioassay experiment. Different letters indicate significant differences ($P < 0.01$) between concentrations of Eco-Bb® and mortality of *S. flava*.

Table 2: Mean mortality of yellow sugarcane aphids at different day intervals in leaf bioassays

Concentration (g/L)	Mean mortality (%) at different day intervals		
	Day 7	Day 14	Day 21
0 (control)	1.96^{c}	2.48^{c}	3.26^{d}
1.25	33.33^{b}	45.00^{b}	48.33^{c}
2.5	35.00^{ab}	46.47^{b}	58.33^{b}
5	41.67^{a}	70^{a}	91.67^{a}
cv	**21.6**	**10.4**	**5.3**
LSD	**8.10**	**5.696**	**3.594**
F. pr	**<.001**	**<.001**	**<.001**

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3.4 Field trial

The mean mortality of *S. flava* ranged from 0% to 83% after 21 days (Fig. 3). Analysis using a one-way ANOVA showed that the different concentrations of *B. bassiana* had a significant effect on the percentage mortality of *S. flava*. The application of 5g/L (1×10^7 spores/ml) (83%) of *B. bassiana* resulted in mortality that was significantly ($P<0.01$) higher from all the other concentrations (Fig. 3). The application of 1.25g/L (2.5×10^6 spores/ml) (46%) and 2.5g/L (5×10^6 spores/ml) (55%) were not significantly different to each other but were both significantly ($P<0.01$) different to the 5g/L (1×10^7 spores/ml) and the control (Fig. 3). The control (distilled water and 0.01 % Tween® 20) (0%) gave mortality that was significantly ($P<0.01$) different to all three Eco-Bb® concentrations (Fig. 3).

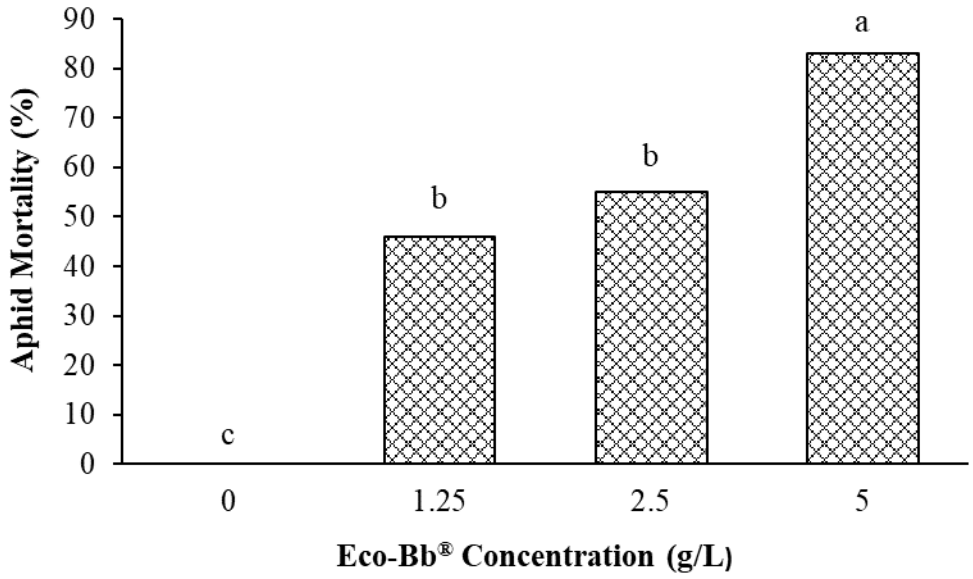

Figure 3: Mean mortality of *S. flava* (99 % confidence level) after 21 days of the application of different concentrations of Eco-Bb® in the field experiment. Different letters indicate significant differences ($P < 0.01$) between concentrations of Eco-Bb® and mortality of *S. flava*.

4. DISCUSSIONS

In the current study, the pathogenicity of the commercially available *B. bassiana* (Eco-Bb®) strain R444 was tested against adult yellow sugarcane aphids. The results from the study indicated that all the concentrations of *B. bassiana* (Eco-Bb®) was able to kill *S. flava* adults. The results are in line with the findings of Sayed et al. (2019), who found that the mortality of *Macrosiphum rosae* L. (Aphididae: Hemiptera) was influenced by the concentration of *B. bassiana*. In the same study the mean mortality of *M. rosae* increased with increasing concentrations of *B. bassiana*. The author further reported that a mortality percentage of >90% was recorded on rose leaves at a concentration of 4.6×10^6 spores/ml, which is in consistent with the mortality percentage of 91.67% on the bioassay tray experiment at an almost similar

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concentration (5×10^6 spores/ml). From the results obtained, it is evident that the mortality percentage of the aphids decreased with decreasing concentrations of *B. bassiana*. As the concentration of *B. bassiana* increased from 1.25g/L to 5g/L, the mortality also increased from 58.33% to 86.67% on the leaf bioassay experiment and from 48.33% to 91.67% on the laboratory bioassay. These findings correspond with the findings of Selvaraj et al. (2012), who reported an increase in mortality of coriander aphid, *Hydaphis coriandri* (Das) (Aphididae: Homoptera), with an increase in concentrations of *B. bassiana*. Selvaraj et al. (2012) also reported the highest percent mortality of *Hydaphis coriandri* at the highest concentration of *Beauveria bassiana* (1×10^8).

The mortality of sugarcane aphids showed an increasing trend to both the concentration and exposure time. As the concentration, together with the number, of days increased, the mortality of *S. flava* increased. These observations were similar to those reported by Stokwe and Malan (2017), who reported an increase in mortality of the Woolly Apple Aphid (WAA) to an increase in concentration and exposure time of the WAA in response to the commercially available *B. bassiana* (Eco-Bb®, strain R444). Stokwe and Malan (2017) also observed the highest mortality on the highest concentration of *B. bassiana* and longest exposure time. Sayed et al (2019) used different isolates of *B. bassiana* to control rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae) infestation on rose plants in the field, and the efficacy of the local isolates was compared with a commercial strain (Naturalis®) with two concentrations for each isolate (2.3×10^6 and 4.6×10^6 conidia/ml). The author reported that the highest concentration of 4.6×10^6 conidia/ml for both the indigenous and commercial strains gave the highest reductions of rose aphid infestation in lab bioassay and field application. Such results concurs with results from the current study where double the recommended rate (5g/L (1×10^7 spores/ml) gave the highest mortality of *S. flava.*

The immersion of insect pests in the conidial suspension, as conducted in the aphid bioassay in the current study, to infect sugarcane aphids gives out different results from spraying on leaf surfaces, as is the case in field conditions. Therefore, the efficacy of the EPF in controlling sugarcane aphids is, to a particular extent, open to doubt, regardless of the susceptibility of *S. flava* to the EPF at adult stage. Dlamini et al. (2020) postulated that various factors affect the field efficacy of EPF, emphasising on the behaviour of the insect host in its natural habitat as an important factor. Dlamini et al. (2020) also stated that since the natural habitat of EPFs is the soil, perhaps their efficacy would be more suitably justified when applied to the soil itself. The author further supported this theory by implementing fungal control of Banded Fruit Weevil (BFW) larvae (since they are soil-borne) in the soil, reporting a mortality of up to 85%. Godonoua et al. (2009) stated that EPF is beneficial in managing insect pests is that they possess the ability to kill their insect host and spread the disease quickly. The results from the current study showed that the sugarcane adult aphid was infected by the EPF. Results further showed that Eco-Bb®, at a concentration of 5g/L (1×10^7 spores/ml), gave the best control against *S. flava*.

5. CONCLUSION

The study revealed that the commercially available *B. bassiana* (Eco-Bb®) has the potential to cause mortality on the adult sugarcane aphid in laboratory conditions. The overall efficacy of the different concentrations of Eco-Bb® was better on the aphid bioassays instead of the leaf bioassays. An increase in concentration of Eco-Bb® resulted in an increase in mortality of *S.*

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flava and double the recommended concentration, 5g/L (1×10^7 spores/ml) of Eco-Bb®, was found to be the most efficient in controlling yellow sugarcane aphids after 21 days of dipping in conidial suspension. Based on the results of the experiment, Eco-Bb® can be used as a biopesticide for the control of sugarcane aphids. This study determined the mortality effect of Eco-Bb® alone. Further research can be done using Eco-Bb® with other EPFs and/or Entomopathogenic nematodes (EPNs) as a means of biological control of *S. flava* in the sugarcane industry.

6. ABBREVIATION

ANOVA:	Analysis of Varience
BFW :	Banded Fruit Weevil
EPF:	Entomopathogenic fungi
EPNs:	Entomopathogenic nematodes
IPM:	Integrated Pest Management
KGaA:	Kommanditgesellschaft auf Aktien
SDA:	Sabouraud Dextrose Agar
ScYLV:	Sugarcane Yellow Leaf Virus
WAA:	Woolly Apple Aphid

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