

Efficacy of Banana spray oil, Mineral oil and water formulations of *Beauveria bassiana* Balsamo for the control of *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) in *Musa* spp.

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Abstract: Banana spray oil, Mineral oil and water were evaluated as carriers for *Beauveria bassiana* spores in an effort to increase its virulence against the banana borer weevil, *Cosmopolites sordidus*. Suspensions of Banana spray oil and *B. bassiana*, Mineral oil and *B. bassiana* and water and *B. bassiana* were made at concentrations of 10^8 , 10^7 , 10^6 , 10^5 , and 10^4 spores/ml. Mineral oil at a concentration of 10^4 spores/ml was found to cause highest mortality to *C. sordidus* in the shortest time. An LD_{50} of 6.55×10^4 spores/ml of *B. bassiana* in Mineral oil was found to be most virulent. The LT_{50} for *C. sordidus* at this concentration was 13.16 days post inoculation.

Keywords: Banana spray oil, mineral oil, *Beauveria bassiana*, *Cosmopolites sordidus*, *Musa*.

INTRODUCTION

The banana (*Musa sapientum* L.) is thought to have been first cultivated in Southeast Asia (Simmonds and Shepherd, 1955) but reference to this plant can be found dating back to the sixth century BC in India (Heslop-Harrison and Schwarzacher, 2007). Today, the banana plant can be found cultivated throughout tropical and subtropical climates of the world (Simmonds and Shepherd, 1955).

In the Caribbean, the economies of the Windward Islands, Belize, Jamaica and Suriname are all strongly dependent on the sale of bananas to the developed countries of North America and Europe. Banana export accounts for more than 50% of the foreign exchange revenue for some of these states (Arias *et al.*, 2003) consequently, the importance of banana production to the Caribbean region as a whole cannot be disregarded.

Cosmopolites sordidus (Germar) (Coleoptera: Curculionidae) the banana borer weevil is

distributed in all areas where banana is cultivated and is considered to be one of the most destructive insect pests of banana and plantain (Bujulu *et al.* 1983; CABI, 2014). Infested plants show decreased vigour and general decline with concomitant reduced bunch sizes.

Damage is caused by both larval and adult stages of the pest, both of which tunnel through the rhizome and destroy conductive tissue, generally weakening the pseudostem. In younger plants, this leads to stunted growth and yellow, dry leaves. The weakening of the pseudostem also leads to the entire banana plants being susceptible to toppling, which sometimes occur under windy conditions and with the increased weight of mature bunches of bananas (Bujulu *et al.* 1983; Gold *et al.*, 2005).

Various methods including chemical, cultural and biological control are currently used for the management of *C. sordidus*. However, *C. sordidus* has long since developed resistance to most insecticides

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(Collins *et al.*, 1991). Phytosanitary methods largely involve the removal of cut pseudostems and other litter, thus reducing the presence of refugia in the field. Some researchers have reported the use of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) for the control of the pest *C. sordidus* (Gold *et al.*, 2001; Khan and Gangapersad, 2001). The present study was undertaken to evaluate the efficacy of mineral oil, banana spray oil and water formulations of *B. bassiana* when used against *C. sordidus*.

MATERIALS AND METHODS

Field Collection

Field collection of *C. sordidus* was done at the University of the West Indies Field Station banana (*Musa spp.*) germplasm collection located at Valsayn, Trinidad at 10.38°N latitude, 61.23°W longitude and with an elevation of 15.3 m above sea level. Adult *C. sordidus* collected using the disc-on-stump method of Vilardebo (1973) were maintained in 10 L plastic containers (25 cm × 25 cm × 40 cm) containing freshly cut pseudostem as a food source. They were kept in this manner until needed for laboratory bioassays. Care was taken to remove excess water produced by the decaying pseudostem in the containers.

Beauveria bassiana

Cultures of *B. bassiana* (IMI-351833) were produced on Potato Dextrose Agar (PDA) using inoculum from the pure culture maintained at the University of the West Indies, Faculty of Science and Technology Mycology Laboratory. *B. bassiana* was cultured for 14 days on PDA in 10 petri dishes (9cm) to increase the quantity of inoculum available. Plates were examined to ensure that they were not contaminated and then the spores harvested by adding a mixture of sterile water and Tween 80® to petri dishes containing pure cultures. The sterile water and Tween 80® reduced the surface tension of the spores and facilitated separation from the agar. A sterile wire loop was used to gently agitate the spores and assist in their suspension. Suspended spores from 5 petri dishes were poured into a 100 ml beaker on a magnetic agitator and left for 30 minutes. A small quantity of sterile water was used to wash the sides

of the beaker to ensure that all the spores went into suspension. After 30 minutes a 1 ml sample of spore suspension was placed on an improved Neubauer haemocytometer and the concentration of spores per millimeter determined. Serial dilutions (10^4 , 10^5 , 10^6 , 10^7 , 10^8 spores/ml) were made in mineral oil, banana spray oil and water using the stock suspension.

Treatments

Fresh banana pseudostems were cut in the field on the morning of the bioassay. Discs measuring 2.5 cm in thickness and 6 cm in diameter were cut from the fresh banana pseudostem and placed in 9cm petri dishes and 2 ml of the different concentrations of *B. bassiana* added to the pseudostem. Five concentrations (10^4 - 10^8 spores/ml) of *B. bassiana* were used for each treatment and each treatment was replicated five times. An additional 5 petri dishes was needed for the control for each treatment.

Each of the treatments had 5 adult *C. sordidus*. Thus 150 *C. sordidus* beetles were needed for each carrier and a total of 450 beetles were required for the experiment. The banana pseudostem in the petri dishes simulated field conditions under which the beetles picked up spores of *B. bassiana* from the pseudostem. This arrangement also reduced the stress on *C. sordidus* from dipping the beetles into the various treatments as was done in previous studies. The petri dishes for one carrier (banana spray oil, mineral oil or water) were stacked in groups of 5 and placed into a 10 L covered plastic container, the base of which was covered with gravel to a depth of 5 cm and a 4 cm deep layer of water was added. This increased the relative humidity in the container, prevented desiccation of the beetles, aided in the germination of the fungal spores and simulated the high relative humidity found naturally in banana fields. *C. sordidus* mortality was recorded daily.

RESULTS AND DISCUSSION

While a wide variety of factors including temperature, low humidity and UV radiation affect the performance of entomopathogenic fungi (Hicks, 2016); formulations also play a pivotal role in their efficacy. The present study investigated the effect of formulations of *B. bassiana* (IMI 351833) in banana

spray oil, mineral oil and water against adult *C. sordidus*. The LC_{50} values for banana spray oil (1.51×10^5 spores/ml) and mineral oil (6.55×10^4 spores/ml) formulations of *B. bassiana* were not significantly different ($P > 0.05$) from each other, however, the LC_{50} for *B. bassiana* spores suspended in water (2.00×10^6 spores/ml) was significantly higher ($P < 0.05$) than both oils (Table 1). This is in accordance with Bandani and Esmailpour (2006) who note that oil-based formulations of entomopathogenic fungi have increased virulence to insects due in large measure to the improved adhesion to the insect's waxy epicuticle compared to water-based formulations.

The 50% Lethal Times (LT_{50}) for the three carriers were significantly different from each other ($P < 0.05$) (Table 2). *B. bassiana* suspended in banana spray oil took the longest time to achieve 50% mortality with an LT_{50} of 16.75 days, while *B. bassiana* spores in mineral oil had the lowest LT_{50} (13.16 days). Water suspended spores had an intermediate LT_{50} of 14.47 days (Table 2). The LT_{50} for mineral oil was achieved 3.6 days earlier than that for banana spray oil; while 50% mortality for mineral oil suspended spores was achieved 1.3 days earlier than that for water. These results are similar to that obtained by Batista Filho and Leite (1995) who found that there was a synergistic and cumulative effect of mineral oil on the pathogenicity of *B. bassiana* spores to *C. sordidus* adults with 96% mortality

observed 12 days post treatment with a concentration of 10^6 spores/ml.

Variation of corrected mortality with time for *C. sordidus* adults treated with *B. bassiana* spores in banana spray oil, mineral oil and water at different concentrations is presented in Figures 1-3. In the present study, higher concentration of spores generally resulted in higher and faster overall mortality (Figures 1-3). Khan and Gangapersad (2001) observed that the use of an aqueous suspension of *B. bassiana* at a concentration of 10^8 spores/ml resulted in total mortality of the test population in 16 days.

These results are similar to those observed in the present study with total *C. sordidus* adult mortality occurring in 14 days at a concentration of 10^8 spores/ml in water. Mortality was also faster at higher spore concentrations with first adult *C. sordidus* death occurring 2 days post treatment in both banana spray oil and mineral oil (Figures 1 and 2) compared with water where first adult mortality occurred 7 days post treatment at the highest spore concentration (Figure 3). Research done on the use of different formulations of the fungus suggests that the carrier used may have an effect on the pathogenicity of the fungus. Carballo and Arias de Lopez (1994) reported that when two formulations (rice substrate and powder) of the fungus were used in Costa Rica, 30.7% mortality was observed on the rice substrate in 10 days, while 63% mortality was

Table 1
 LC_{50} values for *Cosmopolites sordidus* adults treated with *Beauveria bassiana* (IMI 351833) in three carriers

Carrier	Probit equation	LC_{50} (spores/ml)*	S.E. of LC_{50}	χ^2	95% C.I
Banana spray oil	$Y = 0.75x + 1.13$	1.51×10^5 a	1.54	1.30	$6.48 \times 10^4, 3.54 \times 10^5$
Mineral oil	$Y = 0.89x + 0.73$	6.55×10^4 a	1.49	1.06	$3.00 \times 10^4, 1.43 \times 10^5$
Water	$Y = 0.72x + 0.48$	2.00×10^6 b	1.54	0.82	$8.53 \times 10^5, 4.67 \times 10^6$

* Values followed by the same letter are not significantly different from each other based on Tukey's test ($P > 0.05$)

Table 2
 LT_{50} values for *Cosmopolites sordidus* adults treated with *Beauveria bassiana* (IMI 351833) in three carriers

Carrier	Probit equation	LT_{50} (days)*	S.E. of LT_{50}	χ^2	95% C.I
Banana spray oil	$Y = 12.25x - 9.99$	16.75 a	1.02	2.62	16.24, 17.27
Mineral oil	$Y = 12.09x - 8.53$	13.16 b	1.02	1.93	12.71, 13.62
Water	$Y = 10.44x - 7.11$	14.47 c	1.10	6.07	13.93, 15.03

* Values followed by the same letter are not significantly different from each other based on Tukey's test ($P > 0.05$)

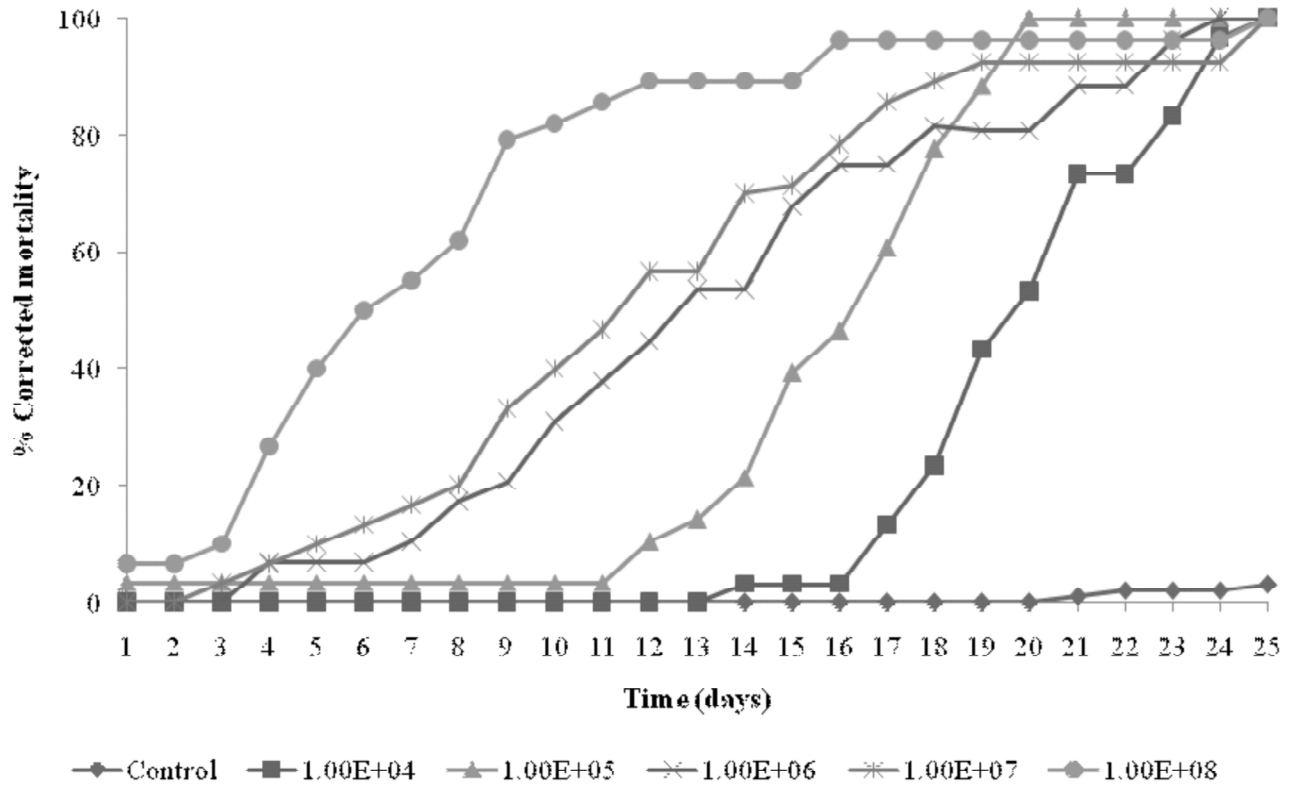


Figure 1: Percent corrected mortality of *Cosmopolites sordidus* adults over time with different concentrations of *Beauveria bassiana* (IMI 351833) in banana spray oil

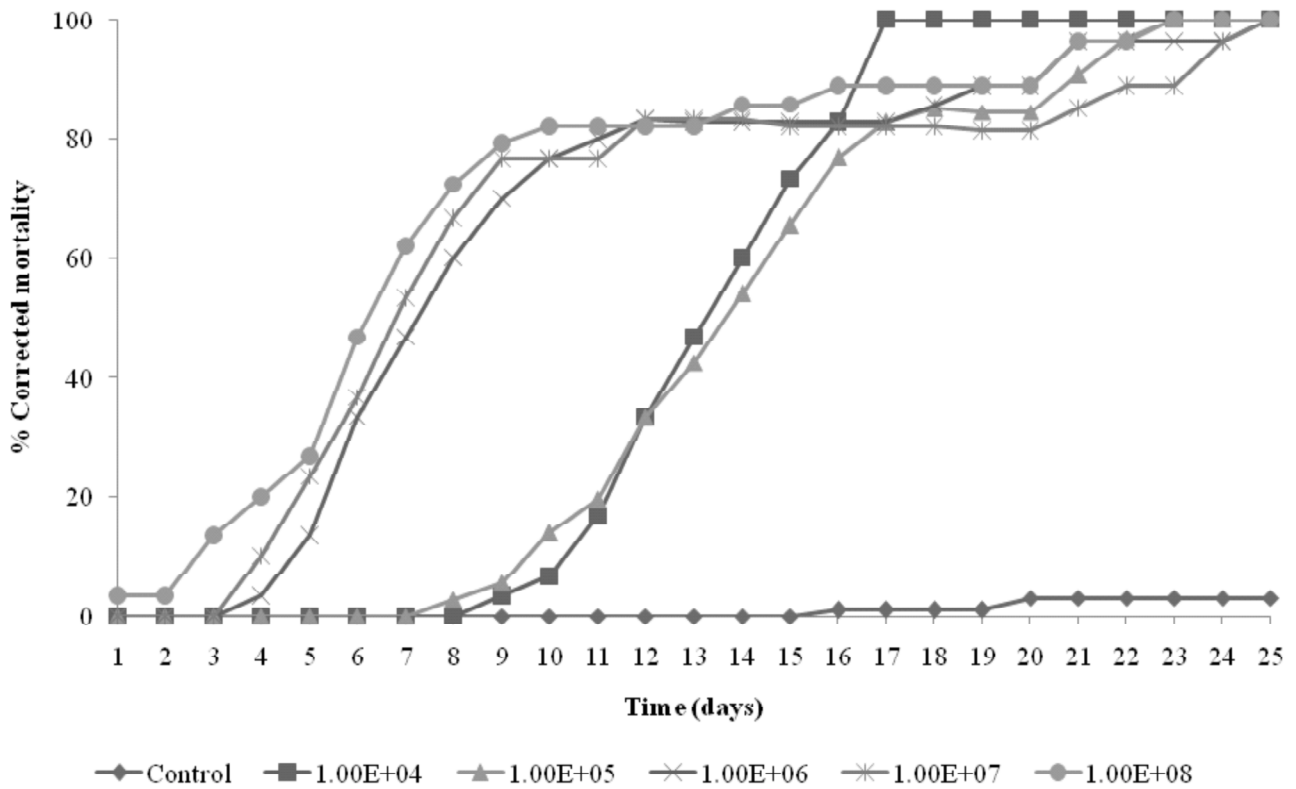


Figure 2: Percent corrected mortality of *Cosmopolites sordidus* adults over time with different concentrations of *Beauveria bassiana* (IMI 351833) in mineral oil

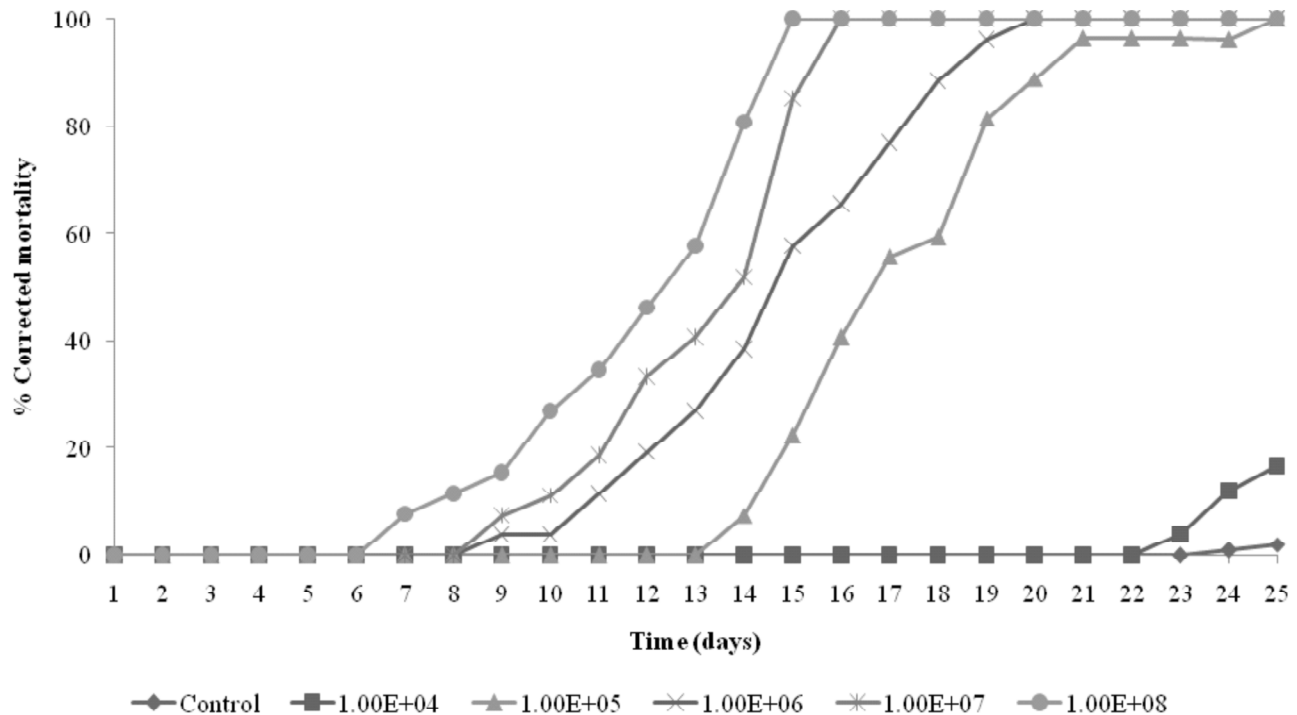


Figure 3: Percent corrected mortality of *Cosmopolites sordidus* adults over time with different concentrations of *Beauveria bassiana* (IMI 351833) in water

recorded after 13 days using the powder formulation.

The combination of lower spore concentration (6.55×10^4 spores/ml) and significantly faster ($P < 0.05$) mortality as evidenced by the lower LT_{50} of 13.16 days of the mineral oil formulation of *B. bassiana* as compared with that of the banana spray oil formulation ($LC_{50} = 1.31 \times 10^5$ spores/ml and $LT_{50} = 16.57$ days) suggest that use of the mineral oil formulation in the field may be desirable. The water-based *B. bassiana* formulation had a significantly higher ($P < 0.05$) LC_{50} (2.00×10^6 spores/ml) and LT_{50} (14.47 days) compared with the mineral oil formulation and coupled with its decreased adhesion to the insect's cuticle (Bandani and Esmailpour, 2006; Ummidi and Vadlamani, 2014) its use under field conditions may not be advocated. Use of mineral oil formulation of *B. bassiana* should result in increased mortality of the population and thus decreased damage to plants in the field. This hypothesis needs to be confirmed using field trials to determine the effectiveness of the oils under these conditions. Additional research needs to be conducted under field conditions to ascertain the increased effectiveness and duration

of the residual control of *C. sordidus* using these oils. Higher concentration of spores may also result in increased mortality of *C. sordidus* adults as was achieved by Khan and Gangapersad (2001), with total mortality of *C. sordidus* test population in 7 days using a *B. bassiana* concentration of 10^{10} spores/ml in water.

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