PATHOGENICITY OF ISOLATES OF *BEAUVERIA BASSIANA* TO THE BANANA WEEVIL *COSMOPOLITES SORDIDUS*

C. A. Omukoko[1], K. N. Maniania[2], J. M Wesonga[3], E. M. Kahangi[4] and L. S. Wamocho[5]
[1,3,4,5]Department of Horticulture Jomo Kenyatta University of Agriculture and Technology, Kenya
[1,2] International Centre of Insect Physiology and Ecology (ICIPE), Kenya
E-mail: canaye3@yahoo.com

Abstract

Bananas constitute a major staple food crop for millions of people in developing countries, providing energy as well as important vitamins and minerals. Its production in Kenya has been on the decline due to, among other factors, pests and disease of which the banana weevil, *Cosmopolites sordidus,* is the most important. The aim of this study was to screen ten isolates of the entomopathogenic fungus *Beauveria bassiana* for pathogencity against *C.sordidus.* Twenty weevils were inoculated with the fungus by dipping them into a fungal suspension containing 1×10^8 conidia ml^{-1} for 11 seconds. The suspension was then drained out and pieces of banana corm introduced as food for the weevils. All the ten isolates of *B. bassiana* tested were found to be pathogenic to adult banana weevil causing mortalities of between 20-50% by 40 days post exposure. ICIPE 273 was the most pathogenic isolate killing 50% of adults, followed by M313 at 36% and M207 at 30%. Isolates KE300, M221, ICIPE 50, M573, M618, M470 and ICIPE 279 killed less than 30% of the weevils, ICIPE 279 was the least pathogenic isolate to the adult *C. sordidus* killing 6% of the inoculated weevils. The dead weevils were incubated in sterile moist filter papers in Petri dishes for two weeks and fungal mycelia was observed on the surface of the weevils starting from intersegmental junctions. Mortality caused by fungus was confirmed by microscopic examination. Since isolate ICIPE 273 has reasonable pathogenicity to *C.sordidus* and field/screenhouse studies are recommended to validate the findings.

Key words: biocontrol, entomopathogens, pest management

1.0 Introduction

Banana (*Musa sapientum, M. acuminata*) constitutes a major staple food crop for millions of people in developing countries providing energy as well as important vitamins and minerals. Majority of producers are small-scale farmers growing the crop either for home consumption or for local markets as a source of income for rural people (Karamura, 1998). This crop is also considered a key component of sustainable agricultural systems in densely populated rainfall zones. On steep slopes, bananas reduce soil erosion and are a principal source of mulch for maintaining and improving soil fertility (Akello, 2008).

Banana production in Kenya has been declining due to environmental stresses, declining soil fertility, poor crop management as well as lack of clean planting material (Seshu *et al.,* 1998).Pest and disease pressures have also increased, reducing the life span of banana orchards (ISAAA, 1996). Important banana diseases include black Sigatoka caused by the fungus *Mycosphaerella fijiensis* and fusarium wilt caused by *Fusarium oxysporum* f.sp. *cubense* (Seshu *et al.*, 1998).

Out of about 200 insect pests reported to attack banana, the most important is the banana weevil, *Cosmopolites sordidus* (Germar) (Godonou *et al.*, 2000). The other pests of economic importance are the parasitic nematodes (*Radopholus similis, Pratylenchus spp. and Helicotylenchus multicinctus*). Both the weevil and nematode infestation interfere with nutrient uptake and transport resulting in slow growth, reduced fruit filling and susceptibility to wind lodging hence the need to device control measures for sustainable banana production (Gold *et al.*, 2003).

The yield losses associated with the banana weevil range from 40% to 100% in severe infestations (Seshu *et al.*, 1998). Banana weevil attack in newly-planted banana stands can lead to poor crop establishment. In established fields, weevil damage can result in plant loss due to snapping and toppling, lower bunch weight, mat die-out and shortened plantation life (Gold *et al.*, 2003).

Chemical pesticides, have been used extensively for the control of *C. sordidus* but environmental pollution and widespread resistance to these pesticides have developed in most parts of the world (Allard *et al.*,1991).The most commonly used cultural control methods include the use of clean planting material, crop hygiene, agronomic practices to improve plant vigor and tolerance to weevil attack. Application of these methods have labour requirements and adoption by resource poor subsistence farmers is often limited (Nankinga *et al.*, 1998).

There is need therefore to develop safer and cheaper control alternatives that can be used to complement existing control methods (Nankinga *et al.*, 1998). Entomopathogenic fungi have been used successfully to control various agricultural pests (Kaaya and Hassan 2000), including banana weevils (Gold *et al.*,

2003). *Beauveria bassiana* has for instance been researched extensively as an alternative means for controlling the banana weevil (Nankinga *et al*., 1994). Isolates often perform well in short -term laboratory bioassays causing high mortality of >90.0% within 2 weeks but field applications do not perform well (Kaaya *et al*., 1993; Nankinga *et al*., 1994, 1999;Godonou *et al*., 2000).

Laboratory pathogenicity tests using different strains of *Beauveria bassiana* in Uganda, (Nankinga *et al.,* 1994) and in West Africa (Godonou *et al.,* 2000) produced 50%-100% mortality in banana weevil adults in 14 days (Magara *et al.,* 2004). The aim of this study was therefore to assess the pathogenicity of ten *Beauveria bassiana* isolates to the banana weevil, *C. sordidus.*

2.0 Materials and Methods

2.1 Trapping of Banana Weevils

Adult banana weevils were obtained from naturally infested banana plants in Kenya Agriculture Research Institute banana farm in Mwea, Central Kenya. Split pseudostem (approximately 45cm) traps were used to attract and capture adults of banana weevil in the field that was infested with weevils. The two half cylinders were placed flat (cut) surface down, on either side of the plant. Pseudostem used for the traps were obtained from freshly harvested plants. Traps were left in place for three days before examination of weevils. Weevils were collected in 10litre plastic buckets. The weevils were then transferred in plastic buckets at room temperature (27 ± 2^oC) in the laboratory for one week before being used in the experiments. The covers of the containers were perforated for ventilation. Banana suckers of susceptible variety were cut into small pieces and corm tissue placed in the containers and used as food for the weevils.

2.2 Fungal Isolates Used in the Study

The following ten isolates of *Beauveria bassiana* were used in the bioassays: ICIPE 273, ICIPE 279, ICIPE 50, M470, KE300, M313, M207, M573, M618 and M221. They were obtained from the International Centre for Insect Physiology and Ecology, (*icipe*'s)Arthropod Germplasm Centre, Duduville, Nairobi, Kenya. The original cultures were stored at -85^oC in 10% sterile glycerol. Details of the date of isolation, host and site where the isolates were sampled are presented in Table 1.

Table 1: Date of isolation, substrate and sampling site for Beauveria bassiana isolates used in the study

Isolates	Year of Isolation	Host/Substrate	Locality /Country
ICIPE 273	2006	Soil	Mbita/Kenya
ICIPE 279	2005	Coleopteran larvae	Kericho/Kenya
M573	2005	Soil	Mauritius
KE 300	2007	Hymenoptera	Taita hills/Kenya
M221	2005	Soil	Mauritius
M618	2005	Soil	Mauritius
M313	2005	Soil	Mauritius
M207	2005	Soil	Mauritius
ICIPE 50	1996	*Rhipicephalus appendiculatus*	Rusinga island/Kenya
M470	2005	Soil	Mauritius

2.3 Culturing the *Beauveria bassiana*

The fungal isolates had been stored at –85°C in 10% sterile glycerol in the germplasm at *icipe*.They were then transferred to a deep freezer at -4 °C for 12 hours before being placed in a refrigerator at 5- 10 °C for 4 hours. Sabouraud Dextrose Agar (SDA) was prepared using the standard procedure, antibiotic chloramphenical added and dispensed into 90 mm Petri dishes and left to solidify. The fungal isolates were then cultured on Sabouraud Dextrose Agar (SDA) for three weeks for sporulation in an incubator at 26°C and 70 % relative humidity for 21 days to allow for sporulation to take place (Inglis *et al.*, 1996).

2.4 Inoculum Preparation

Conidia were gently scrapped from Petri dishes and suspended in sterile 10 ml of 0.01% Tween-20. The suspension was then transferred with a sterile pasteur pipette into 20 ml sterile universal bottles with glass beads. For counting the spores a dilution of x100 was prepared by removing 0.1ml using a pasteur pipette from 10 ml sterile distilled water with Tween-20.0.1 ml was picked from the stock solution and added into the diluent. Vortexing was done for three minutes. Conidial density for each strain was then estimated by placing 0.1 ml on the improved Neubauer haemocytometer and counting the spores. For a concentration of 1 x 10^8 the concentration formula was used. The spores counted from x100 dilution were multiplied by a constant 2.5 x 10^5 and x100. This gives the spores in the standard 1 x 10^8. From the standard concentration C_1V_1 and C_2V_2 the number of spores needed for each isolate were then calculated.

2.5 Spore Germination Test

Sabouraud Dextrose Agar was prepared as described above and dispensed into 90 mm diameter petri dishes. A conidial suspension with a concentration of $3x10^6$ conidia ml^{-1} was prepared. The viability of conidia was determined by spread plating 0.1 ml of conidial suspension *B. bassiana* on SDA plates using a sterile glass rod. For each of the ten isolates, three 90 mm Petri dishes were prepared and incubated at 27 ± 2 °C for 16 hours. The culture was then fixed using a few drops of lactophenol cotton blue, cover slips were placed over four sections of the Petri dish with germinated spores. Conidial germination was assessed by counting a total of 100 spores, i.e the number of conidia that germinated plus those that had not germinated in four different fields under a dissecting microscope. Conidial germination was characterised by germ tube development and these were categorized as viable conidia while the non-germinated conidia that lacked the germ tube were categorized as non-viable. The germination test is important since it indicates which isolates are viable and therefore can be used in the experiment. Percentage conidial germination was determined by counting 100 spores for each plate. Four replicates were used for each plate, the percentage germination was then calculated as: (viable conidia/total conidia) x100

2.6 Inoculation of Weevils with *Beauveria bassiana*

Weevils were inoculated by dipping them into 20 ml fungal suspension with a conidia concentration of 10^8 conidia ml^{-1} for 11 seconds. The suspension was then drained off and weevils were transferred to plastic containers. Banana corms were then placed in the plastic containers as food for the weevils and placed in the laboratory in a dark room. Control weevils used as a check were dipped in sterile distilled water containing 0.01% Tween 20. Mortality of the weevils was recorded every 3days for 40 days. The experiment consisted of five replicates for ICIPE 279, M573, ICIPE 273 and KE300, and four replicates for ICIPE 50, M470, M207, M618, M221 and M313.

Figure 1: Banana weevils infected with Beauveria bassiana and introduced into plastic containers with banana corm.

To assess fungus induced mortality, dead weevils were surface-sterilized in 2% sodium hypochlorite, 70% alcohol and rinsed twice with sterilized water for 15 seconds before placing them in clean petri dishes with moist sterile filter papers. Dead insects were monitored for fungal growth for two weeks and observations recorded. Only dead insects with fungal growth were considered to have been killed by the fungus.

2.7 Statistical Analysis

All data for mortality was analysed with analysis of variance using the PROC GLM of SAS statistical software and the treatment means were separated using Student-Newman-Keuls (SNK) (SAS version 9.1).

3.0 Results

3.1 Conidial Germination Experiment

In germination tests 81.2-92.7% of spores germinated, for all *B. bassiana* isolates 16 h after exposure on SDA media. There was no significant difference (P=0.37) in percentage conidial germination among the isolates. However there were slight differences among the isolates in germination M 313 and ICIPE 273 gave the best germination of spores (92 %), followed by M470, M207, ICIPE 50 (87 % - 89 %).The rest ICIPE 279, M573, KE 300, M 221 and M618 had germination percent that ranged between (81.3 %-86.6 %).These results are shown in Table 2.

Table 2: Beauveria bassiana isolates used in the study and their % germination SDA

Isolates	% Germination ± SE
ICIPE 273	92.2 ± 3.2
ICIPE 279	86.8 ± 4.5
M573	85.4 ± 3.7
KE 300	86.6 ± 3.5
M221	82.3 ± 3.7
M618	81.3 ± 3.5
M313	92.7 ± 2.1
M207	88.0 ± 2.6
ICIPE 50	87.3 ± 2.6
M470	89.0 ± 2.4

3.2 Pathogenicity Test

All the ten isolates of *Beauveria bassiana* were pathogenic to the adult *Cosmopolites sordidus*, causing mortalities varying from 20-50% by 40 days post-exposure . ICIPE 273 killed 50% of adults followed by M313 36% and M207 30% (Figure 2). The rest KE 300, M 221, ICIPE 50, M573, M 618, M 470 and ICIPE 279 killed < 30% of the inoculated weevils. Isolate ICIPE 279 was the least pathogenic to *C. sordidus* causing only 6% mortality.

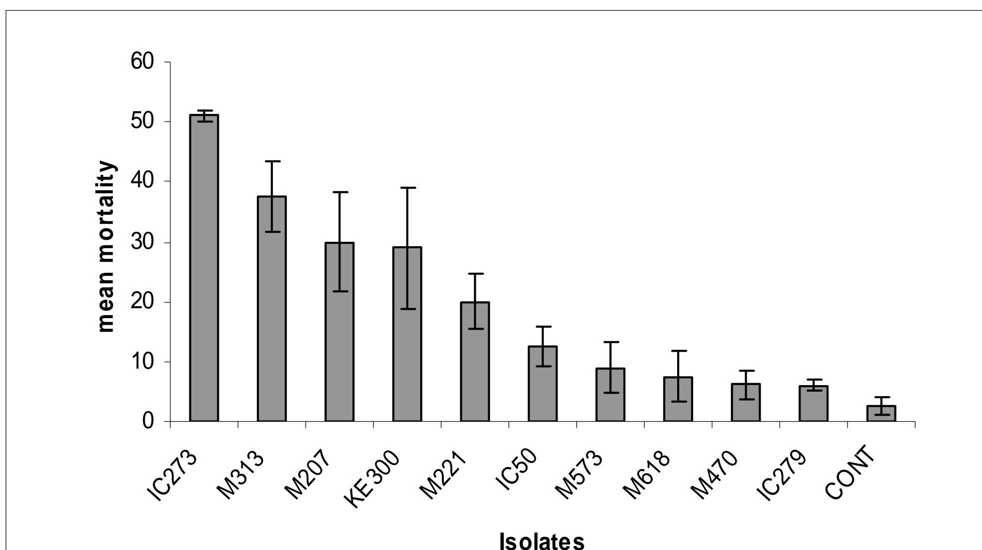

Figure 2: Mortalities in adult weevils inoculated with 10 isolates of Beauveria bassiana 40 days post exposure.

Incubation of the dead weevils in humidified chambers resulted in development of mycelia on the surface of the cadaver, starting from the intersegmental junctions of the body and legs (Figure 2). No fungi grew on dead weevils in the controls since they died due to other causes apart from the fungus.

Plate 1: Adult C. sordidus infected with B. bassiana. Notice fungal growth at intersegmental junctions (arrows)

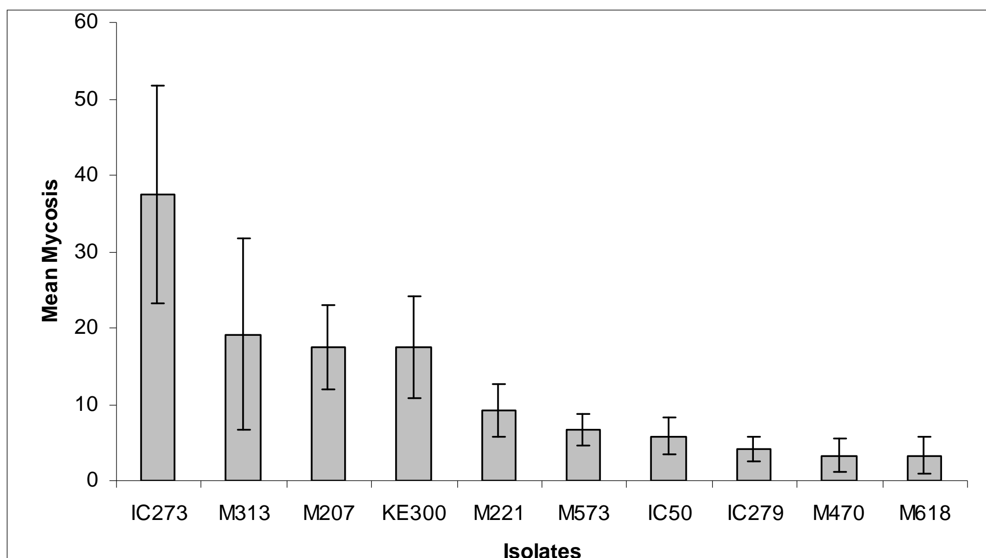

Figure 3: Percent Mean mycosis of adult banana weevils infected with different isolates of B. bassiana

ICIPE 273 had over 38% mycosis on the dead insects, while M313, M207, KE 300, had between 18% - 19% mycosis. All the other isolates had mycosis that ranged between 5 % -10% mycosis. No fungi grew on dead weevils in the controls.

4.0 Discussion

Laboratory pathogenicity studies indicate that *B. bassiana* isolate ICIPE 273 isolated from soil in Mbita, Kenya gave better results among the tested isolates since it gave 50% mortality when a standard concentration of 1 x 10^8 for 11 seconds was used. This is acceptable for entomopathogens when used as biological control agents since reduction of the pest population by 50% and above is usually targetted. The level of effectiveness obtained in our study compares favourably with those of Nankinga *et al.* (1994), Pena *et al.* (1991) and Godonou *et al.* (2000). Isolates from Mauritius M313 had mortality of 36% while M207 had 30% which was comparatively low. For all the tested isolates, dead insects kept in humidified chambers developed surface mycelia. Mortality caused by *B. bassiana* was confirmed by microscopic examination of the dead banana weevils.

5.0 Conclusions

All isolates of *Beauveria bassiana* tested were pathogenic to banana weevils causing mortalities of 20% to 50% with the best isolates being ICIPE 273, M313 and M207.The rest KE 300, M221, ICIPE 50, M470, M618, M573 and ICIPE 279 killed less than 20% of the banana weevils with ICIPE 279 being the least pathogenic to the adult banana weevil *Cosmopolites sordidus.*

6.0 Recommendations

ICIPE 273 has reasonable pathogenicity to *C.sordidus* and field/screenhouse studies are recommended to validate the findings.

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Appendix

Pathogenicity mortality

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Treatment	10	11242.91	1124.29	9.46	<.0001
Error	37	4398.75	118.88		
Corrected Total	47	15641.66			

Pathogenicity mycoses

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Treatment	9	6278.75	697.63	2.47	0.020
Error	50	14145.83	282.91		
Corrected Total	59	20424.58			