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VIRULENCE OF THREE STRAINS OF *Beauveria bassiana* AGAINST THE BANANA WEEVIL

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ABSTRACT

The banana weevil, *Cosmopolites sordidus* Germar, is the most important insect pest of banana and plantain. *Beauveria bassiana* is the most researched and commercialized fungal biopesticide effective against a variety of insects. Laboratory studies have revealed a great potential of this entomopathogen for use against the banana weevil, *Cosmopolites sordidus* in banana. Use of traps for collecting adults or infecting them with biopesticides will most likely capture only those weevils in the immediate vicinity of the traps. The indiscriminate use of chemicals has resulted in the development of resistance in insect pests, adverse ecological events, affecting beneficial fauna, and accumulation of residues in the environment. There is considerable need therefore to develop safe and cheaper biocontrol alternatives that can be used to complement existing control methods. The potential of utilizing the entomopathogen *B. bassiana* for control of banana weevil was evaluated by testing the virulence of three isolates of *Beauveria bassiana* (ICIPE 273, M353 and M207) at three concentration (10^8, $3x10^8$ and 10^9). From previous pathogenicity tests these three isolates were the most pathogenic. At higher fungal concentrations of $3x10^8$ and 10^9 adult mortality for all the three isolates was between 35%-70%. The highest mortality was achieved using an elevated concentration of 10^9, causing mortalities varying from 50-70% 40 days after exposure depending on the isolate. ICIPE 273 was the most virulent, killing 70% of adults followed by M353 (65% mortality) and M207 (51% mortality). This was far much greater than when a standard concentration of 10^8 was used (mortalities ranged between 28%-50%). Differences in virulence among the tested isolates were due to their geographical origins.

Keywords: *Beauveria bassiana*, banana, biological control, bioassays, entomopathogens.

INTRODUCTION

The banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is a major pest of bananas. Weevil damage is primarily the result of destruction of the corm tissue sometimes accentuated by secondary attacks by other insects and micro organisms leading to an increased risk of toppling (Godonou *et al*., 2000). Control of the banana weevil relies on costly chemicals which are beyond reach for most small scale farmers (Gold *et al.*, 2000).

The indiscriminate use of chemicals has also resulted in the development of resistance in insect pests, adverse ecological events, affecting beneficial fauna and accumulation of residues in the environment (Gold and Messiaen, 2000). 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 and pasture pests. *Beauveria bassiana* and *Metarhizium anisoplie* have gained considerable attention as biological control agents for weevils and other agricultural pests (Kaaya and Hassan, 2000).

In pathogenicity tests conducted previously *B. bassiana* isolates ICIPE 273, M353 and M207 were identified as possible biocontrol agents based on their virulence against the banana weevil. This paper describes the experiment that was undertaken to determine the virulence of these three isolates against the banana weevil under laboratory conditions in Kenya.

The objective of our research was to evaluate further the virulence of three isolates of *B. bassiana* to the banana weevil. The $LC_{50's}$ and $LT_{50's}$ of the three isolates were determined and compared.

MATERIALS AND METHODS

Insects

Adults of banana weevils were obtained from naturally infested banana plants at Kenya Agricultural Research Institute Mwea. The weevils were maintained in the laboratory in plastic containers at room temperature for one week before being used in the experiments. The covers of the containers were perforated for ventilation. Banana suckers of susceptible variety were pared and corm tissue placed in the containers.

Fungal isolates and cultures

Isolates of *Beauveria bassiana* ICIPE 273, M313 and M207 were obtained from the ICIPE's Arthropod Germplasm Centre, Duduville, Nairobi, Kenya. The isolates were cultured on Sabouraud Dextrose Agar (SDA) medium for three weeks for complete sporulation in an incubator (27°C). Antibiotic chloramphenical was added to the medium to keep off any bacterial contamination. 65 g SDA was weighed and dissolved in 1000 ml boiled distilled water in a conical flask a magnetic stirrer was

added and the flask placed on hot plate for the SDA to dissolve completely. The media was autoclaved at 121°C for 20 min and left to cool to 50-60°C before adding antibiotics and pouring. Media was poured into petri dishes on a clean bench and left overnight to cool and solidify. Streaking was done by putting the inoculating loop on a hot flame till it turned red hot. A plate with *Beauveria bassiana* grown for three weeks was used for sub culturing. The petri dish was wiped with cotton wool soaked in 70% alcohol and opened inside the lamina flow cabinet and the inoculation loop used to pick *Beauveria bassiana* and streak on the solidified plates. The petri dishes were incubated for three weeks at 26°C and 70% relative humidity for 21 days for sporulation to take place (Inglis *et al*., 1996). Long term storage of the original isolates of all fungi was stored at -85^{o}C in 10% sterile glycerol. Subcultures were made from original cultures for each new experiment.

Inoculation of the weevils

Three *Beauveria bassiana* isolates (ICIPE 273, M353, and M207) were selected from the pathogenicity screen and evaluated in dose-response bioassay against *Cosmopolites sordidus* adults. The isolates were evaluated at three concentrations $1x10^8$, $3x10^8$, $1x10^9$ of conidia each with four replicates. For each strain conidia were gently scrapped from fungal cultures and suspended in 10 ml of 0.01% Tween-20 surfactant until all the spores had been harvested. The conidial suspension was transferred with a sterile pasture pipette in to 20 ml sterile universal bottles with glass beads to prepare the stock solution. From the stock solution different dilutions (10^8, 109, $3x10^8$ conidia/ml) were prepared. A control treatment, sterile distilled water with 0.01% Tween 20, was also prepared. The weevils were sorted in four batches of 80 adults. The four batches were arranged randomly among different treatments. For inoculation each batch of banana weevils was placed in a petri dish and 10 ml of the appropriate conidial suspension was gently poured in immersing the banana weevils. To obtain rapid immersion the Petri dish was shaken gently for 11 s after which the suspensions were poured out and the infected weevils introduced to plastic containers with a piece of banana corm as a source of food. The treated banana weevils were observed daily for 40days to record mortality

Fungus-induced mortality assessment

Dead insects were monitored for fungal growth for two weeks and observations recorded. To assess mycosis dead insects were surface sterilized in 2% sodium hypochlorite, 70% alcohol and two rinses of sterilized water for 15 s before placing them in Petri dishes with moist sterile filter papers. Only dead insects with fungal growth were considered to have been killed by the fungus.

Mean lethal concentration

In studies of dose-response relationships, the terms LC_{50} and LD_{50} are the most common expressions of virulence. LC_{50} is the concentration of a given insect pathogen required to kill 50% of the test insect population within a given period of time. With respect to hyphomycete fungi, LC_{50} is the appropriate term since the methodology only admits an estimate of the concentration used and not of the dose actually received by the test insects (Goettel and Inglis, 1997). In studies of time-response relationships the terms average survival time (AST) and median lethal time (LT_{50}) are the most common expressions of the time required to kill a given insect. LT_{50} is defined as the time period required to kill 50 % of the test insect population when subjected to a given concentration or dose of an insect pathogen.

Statistical analysis

Data obtained from the bioassay to determine LC_{50} and LC_{50} were corrected using Abbott's formula (1925) and then analyzed with the statistical package pc Probit which compared the Fiducial limits at 95%. Using the same program, logarithmic relationship (linear regression) between doses and mortality was determined and the most toxic strains identified. The percentage of inoculated weevils was calculated.

RESULTS

Mean lethal time (LT_{50})

Time taken for each of the three strains to kill 50% of the banana weevils are shown in Table-1. The average lethal time across strains was as follows:

Table-1. Mean lethal time LT_{50} for banana weevils inoculated with three strains of *B. bassiana.*

Strains	LT_{50}	FL (95%) days
ICIPE 273	31.25 a	30.67-31.87
M353	34.74 b	33.84 -35.65
M207	51.07 c	49.31- 53.03

Values followed by the same letter are not significantly different as determined by examination of 95% FL of calculated LC_{50} values.

The results indicate that isolate ICIPE 273 had the lowest LT_{50} of 31 days, M353 34 days and M207 had the highest of 51days.All these three isolates were significantly different since their was no overlap of Fiducial limits.

Mean lethal concentration (LC_{50})

The LC_{50} values for the three strains of *B. bassiana* that proved to be most virulent are presented in Table-2. The LC_{50} values were $5.34x10^6$, $4.22x10^8$ and $8.89x10^8$ conidia/ml for ICIPE273, M353 and M207 strains respectively. As mentioned by Ferron (1978), virulence measured in terms of lethal concentration will depend on the strain, the species of insect and mode of application. Other factors may include successful conidial attachment to the cuticle, the utilization of cuticular

surface fatty acids, prepenetration growth and other extracellular toxins.

Table-2. Median lethal concentrations of three *B.bassiana* strains applied against the adult banana weevil.

Strains	LC_{50} (Conidia/ml)	95% FL limits
ICIPE 273	5.34×10^6 a	4.54×10^6 - 6.24×10^6
M353	4.22×10^8b	3.45×10^8 - 4.95 $\times 10^8$
M207	8.89×10^8c	8.05×10^8 - 9.96×10^8

Values followed by the same letter are not significantly different as determined by examination of 95%FL of calculated LC_{50} values.

All these three isolates were significantly different since there was no overlap of Fiducial limits. ICIPE 273 was considered the best of the three strains because of its very high level of virulence towards, the banana weevil as shown by the LC_{50} values. The difference in virulence observed between the strains tested in these experiments may be due to differences in production of enzymes which degrade the cuticle of the potential host, such as chitinases chyemolestase, chymotrpsin and esterase which are considered an essential prerequisite for successful fungi infection (Ferron 1978,St Leger *et al.,* 1994)

Sporulation of *B. bassiana* on adult banana weevils

Figure-1. Adult *C. sordidus* infected with *B. bassiana*. Notice fungal growth at intersegmental junctions (arrows).

DISCUSSIONS

Virulence is the ability of an entomopathogenic fungus to cause death and is commonly measured as the rate at which death occurs. Fungal pathogenesis is the biochemical, physiological and genetic process during infection and disease formation. A virulence factor is defined as the process that leads to insects' death. All the *B. bassiana* isolates were pathogenic to the banana weevils; however the virulence differed significantly with different conidia concentrations. Within the limitations of laboratory bioassays our results suggest that *B. bassiana* isolate ICIPE 273 is the best biocontrol agent for the management of *C. sordidus* based on it's virulence against the insect pest. There is however need for screenhouse evaluation to determine the impact of the fungus on banana weevil under semi field conditions. Biological control with pathogenic fungi may provide long lasting insect control without damaging the environment.

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