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Mortality in Tephritid Fruit Fry Puparia and Adults Caused by Metarhizium Anisopliae, Paecilomyces Fumosoroseus and Beauveria Bassiana

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Abstract

Tephritid fruit flies are recognized as the most economically important group of phytophagous Diptera, and cause large losses to fruits and vegetables throughout the world. In the small developing island state, Mauritius, the key major pest of fruits and vegetables are *Bactrocera zonata* (Saunders) and *B. cucurbitae* (Coquillett), respectively. At present, growers have recourse to chemical pesticides which are hazardous to both the environment and human health. The objective of the study was to evaluate entomopathogenic fungi isolated from the soils of Mauritius as biocontrol agents of fruit flies. The pathogenicity of six isolates of *M. anisopliae*, three isolates of *B. bassiana* and one isolate of *P. fumosoroseus* were determined for late third-instar larvae, puparia and emerging adults of *B. zonata* and *B. cucurbitae.* A standard concentration of 1 x 10^8 conidia/ml (5 ml) was used

to inoculate 50 g lots of sand. Twenty mature third-instar larvae of either *B. zonata* or *B. cucurbitae* that were ready to pupate within the next 24 h were then introduced into each Petri dish for pupation. The overall result showed a significant reduction in adult emergence for both fruit fly species. For *B. zonata*, the percentage adult emergence varied from 60 to 93% in fungal-treated sand; while, 1 to 30% of the puparia showed visible signs of mycosis. As regards *B. cucurbitae*, adult emergence ranged from 52 to 92% in fungal-treated sand and the highest percentage of mycosed puparia recorded with an isolate of *M. anisopliae* was 48%. Given the fact that there was significant reduction in adult emergence and a corresponding large mortality on puparia, the most virulent isolates could be potential candidates for soil application against fruit flies.

Keywords: *Bactrocera cucurbitae, Bactrocera zonata*, fruit flies, pupae, biocontrol

1.0 Introduction

During development, third-instar larvae of most fruit fly species drop from fruits to the ground, burrow into the soil and form a puparium (Christenson & Foote, 1960; White & Elson-Harris, 1992). An important part of a fruit fly suppression and eradication programme therefore includes soil treatment with insecticides beneath host trees to kill fruit fly larvae and puparia (Roessler, 1989; Permalloo *et al.* 1998; Seewooruthun *et al.* 1998). The insecticide imidacloprid was used against fruit fly larvae/puparia in the eradication campaign of the notorious Oriental fruit fly, *Bactrocera dorsalis,* in Mauritius (Seewooruthun *et al.* 1998). The use of synthetic insecticides for pest control is, however, associated with various ecological problems such as environmental contamination, adverse effects on non-target organisms and the development of resistance.

As an alternative to chemical control or as part of integrated management programmes, there is a resurgence of interest in the use of biological control of insect pests. Fungal agents are among the most promising group of biological control agents. The Deuteromycete fungi are known to cause epizootics that significantly reduce host populations under laboratory and field conditions (Lacey *et al.* 1994; Fargues *et al.* 1997; Crawford *et al.* 1998; Negasi *et al.* 1998; Lecuona *et al.* 2001). *M. anisopliae, B. bassiana* and *Paecilomyces fumosoroseus* (Wize) Brown & Smith have been recognized as some of the most important entomopathogens of dipteran insects (Sweeney, 1983; Ferron *et al.* 1991; De La Rosa *et al.* 2000).

Several studies have shown the success of soil treatment with fungal pathogens for the control of different agricultural pests (Krueger *et al.* 1991; Villani *et al.* 1994; Booth & Shanks, 1998). Published research on fungal pathogens of tephritids is limited mainly to the pathogenicity of *M.*

anisopliae, B. bassiana or *P. fumosoroseus* against *Anastrepha ludens* (Lezama-Cutiérrez *et al.* 2000; De La Rosa *et al.* 2002; Toledo *et al.* 2007), *Ceratitis capitata* (Castillo *et al.* 2000), *C. rosa* and *C. cosyra* (Ekesi *et al.* 2002). Fungal attack resulted in mortality or reduced fecundity and fertility. Since most entomopathogenic fungi are soil-borne microorganisms, their incorporation into the soil to target pupariating larvae and puparia can form an important component of an integrated pest management strategy.

The objective of this study was to evaluate the effect of six isolates of *M. anisopliae*, three isolates of *B. bassiana* and one isolate of *P. fumosoroseus* that were collected from soils in Mauritius (Sookar *et al.* 2008) on late third-instar larvae, puparia and emerging adults of *B. zonata* and *B. cucurbitae.*

2.0 Materials and methods

2.1 Insects

Larvae of *B. cucurbitae* and *B. zonata* were from the mass rearing colony maintained at the Entomology Division, Ministry of Agro Industry Food Production & Security. The larvae were reared on an artificial diet composed of sugar cane bagasse (6%), ground maize (6%), sugar (11%), waste brewer's yeast (6%), wheat bran (6%), sodium benzoate (0.1%), nipagen (0.1%) and water (64.8%). Adult flies were fed *ad libitum* with a mixture of enzymatic yeast hydrolysate (ICN Biochemical) and sugar (1:3). Water was provided in plastic containers with sponge.

2.2 Fungi

The 10 fungal isolates used in the experiments were obtained from the soils of Mauritius (Sookar *et al.* 2008). The fungi were grown on Sabouraud dextrose agar (SDA) in Petri dishes and maintained at ambient temperature (22-28^{0}C) in complete darkness.

2.3 Preparation of Conidial Suspension

Conidia were harvested by scraping the surfaces of 3-week old cultures Spores were suspended in 20-ml sterile distilled water containing 0.05% Triton X-100 in glass bottles containing 3 mm glass beads. Bottles were stoppered and vortexed for 5 min to produce a homogeneous conidial suspension. Conidia were then quantified with a haemocytometer following serial dilution in sterile distilled water. The viability of conidia was determined by spread-plating 0.1 ml of conidial suspension (titrated to $3x10^6$ conidia ml^{-1}) on four SDA plates. Sterile microscope cover slips were placed on each plate. The plates were incubated at 24-29^{0}C and examined after 20 h. Percentage germination was determined by counting approximately 100 spores for each plate at 200 x magnification. Each plate served as a replicate with four replications per isolate.

Inoculation of Insects: A standard concentration of 1 x 10^8 conidia/ml (5 ml) was used to inoculate 50 g lots of sand which were then vigorously mixed with a spatula. Control lots were treated with sterile distilled water containing 0.05% Tween-80.Twenty mature third-instar larvae of *B. zonata* and *B. cucurbitae* that were ready to pupate within the next 24 h were then introduced into each Petri dish for pupation and the dishes were placed inside a humid transparent container maintained at room temperature. Seven days after the introduction of the larvae, puparia were removed from the treated sand and transferred into another Petri dish containing untreated sand and placed in cages. Water and a 3:1 mixture of sugar and yeast hydrolysate were provided in the cage as a food source for adults. The number of adult flies that emerged from treated and control sand was recorded daily until 14 days after the first emergence. Records were also kept for the number of puparia that failed to emerge. Adult flies that died during this period and puparia that failed to emerge were surfaced sterilized

in 2% sodium hypochlorite followed by two rinses with sterile distilled water and transferred to Petri dishes lined with moistened filter paper. The criteria for scoring mycoses were (1) failure of puparia to develop, accompanied by fungal sporulation and colonized puparia and (2) death of adults accompanied with fungal sporulation on the cadavers. Each treatment was replicated four times with 20 insects per replicate.

3.0 Statistical analysis

Percentage mortality data were corrected for control mortality (Abbott, 1925) and the data were normalized through angular transformation and then subjected to analysis of variance (ANOVA) followed by mean separation by the Student-Newmann-Keuls' test (P = 0.05) using the ANOVA procedure. All analyses were performed using the SAS (1996) package.

4.0 Results

In viability tests, germination for all the isolates ranged from 94 to 100%. All pupariating larvae treated with fungi pupated normally, but infections became established in puparia (Figure I) and emerging adults for both fruit fly species. In *B. zonata*, percentage adult emergence was 98% in the control treatment and varied from 60 to 93% in fungal-treated sand (F = 34.4; df = 10, 79; $P < 0.0001$) (Figure II). Percentage of puparia with visible signs of mycosis ranged from 1 to 30% (F = 17.6; df = 9, 39; $P < 0.0001$) (Figure III). Isolates M394 and M65 were the most pathogenic to *B. zonata* followed by M196, M421, M603, M499, M265, M103, M235 and M397 (Figure III). Deferred mortality in *B. zonata* adult flies ranged from 9 to 68% (Figure IV) and natural mortality in the control did not exceed 3%.

Figure I. Pupae of *Bactrocera zonata* mycosed by *Metarhizium anisopliae* (5 to 6 mm in length)

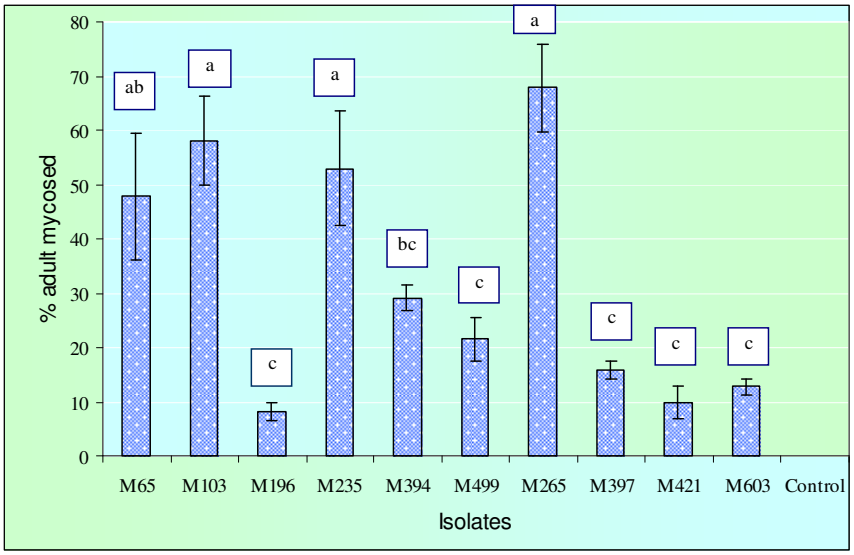

Figure II. Pathogenicity of different isolates of *Metarhizium anisopliae* (M65, M 103,M196, M235, M394 and M499), *Paecilomyces fumosoroseus* (M603) and *Beauveria bassiana* (M265, M397 and M421) to mean (% ± SE) adult *Bactrocera zonata* mycosed.

Bars with the same letter do not differ significantly by SNK (P=0.05)

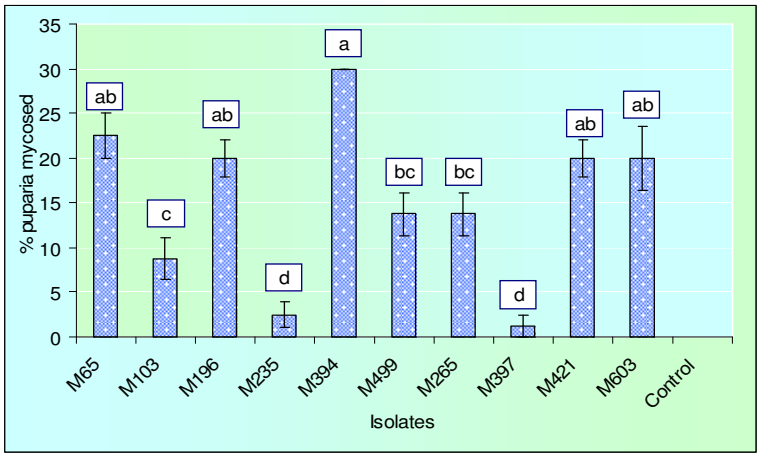

Figure III. Pathogenicity of different isolates of *Metarhizium anisopliae* (M65, M 103, M235, M394 and M499), *Paecilomyces fumosoroseus* (M603) and *Beauveria bassiana* (M265, M397 and M421) to mean (% ± SE) *Bactrocera zonata* puparia mycosed.

Bars with the same letter do not differ significantly by SNK (P=0.05)

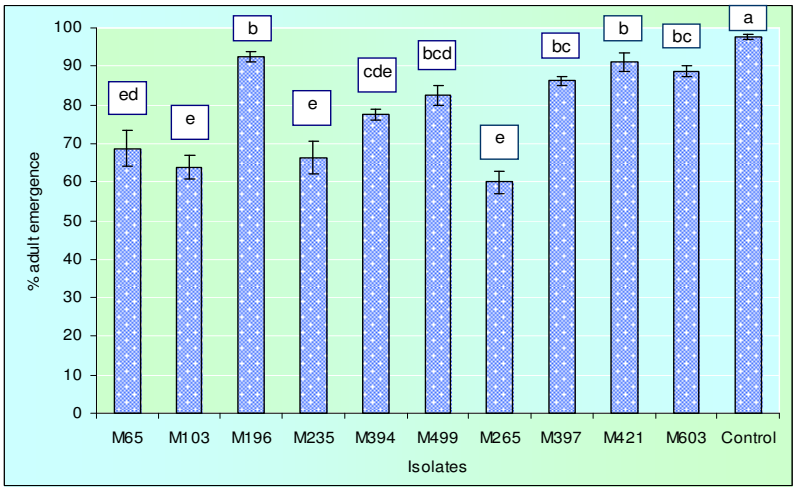

Figure IV. Pathogenicity of different isolates of *Metarhizium anisopliae* (M65, M 103, M235, M394 and M499), *Paecilomyces fumosoroseus* (M603) and *Beauveria bassiana* (M265, M397 and M421) to mean (% ± SE) emergence of *Bactrocera zonata*.

Bars with the same letter do not differ significantly by SNK (P=0.05)

In *B. cucurbitae*, adult emergence was 99% in the control and ranged from 52 to 92% in fungal treated sand (F = 65.94; df = 10, 79; $P < 0.0001$) (Figure V). The percentage of mycosed puparia was highest in M103 (48%) followed by M265, M235, M196, M499, M65, M394, M421, M603 and M397 (F = 12.42; df = 9, 39; $P < 0.0001$) (Figure VI). Deferred morality in adults due to mycosis ranged from 9% to 49% in all the isolates (Figure VII) (F = 6.14; df = 9, 39; $P < 0.0001$) and natural mortality in the control was 3%.

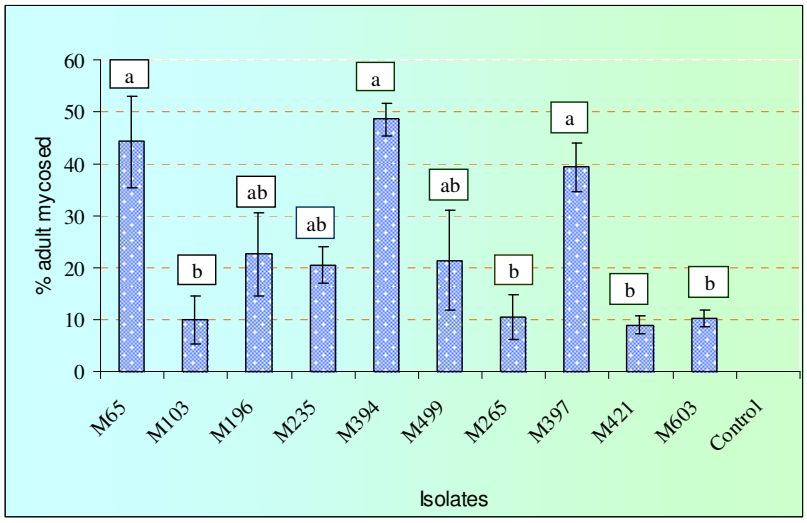

Figure V. Pathogenicity of different isolates of *Metarhizium anisopliae* (M65, M 103, M235, M394 and M499), *Paecilomyces fumosoroseus* (M603) and *Beauveria bassiana* (M265, M397 and M421) to mean (% ± SE) adult *Bactrocera cucurbitae* mycosed.
Bars with the same letter do not differ significantly by SNK (P=0.05)

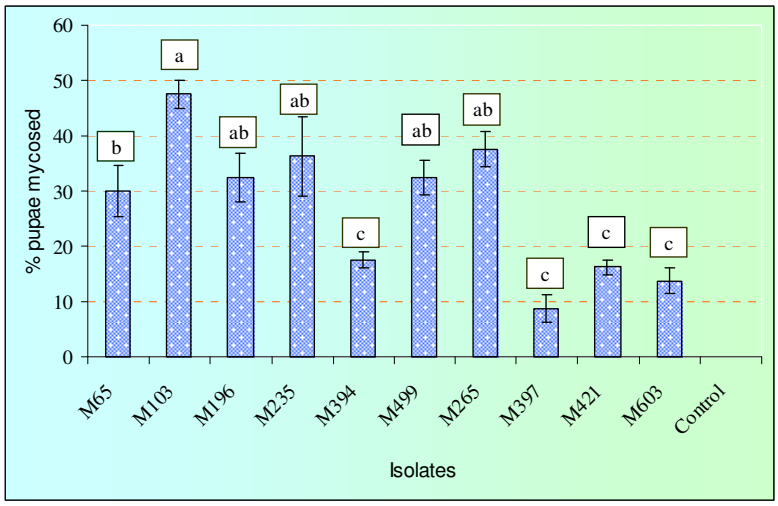

Figure VI. Pathogenicity of different isolates of *Metarhizium anisopliae* (M65, M 103, M235, M394 and M499), *Paecilomyces fumosoroseus* (M603) and *Beauveria bassiana* (M265, M397 and M421) to mean (% ± SE) *Bactrocera cucurbitae* puparia mycosed.

Bars with the same letter do not differ significantly by SNK (P=0.05)

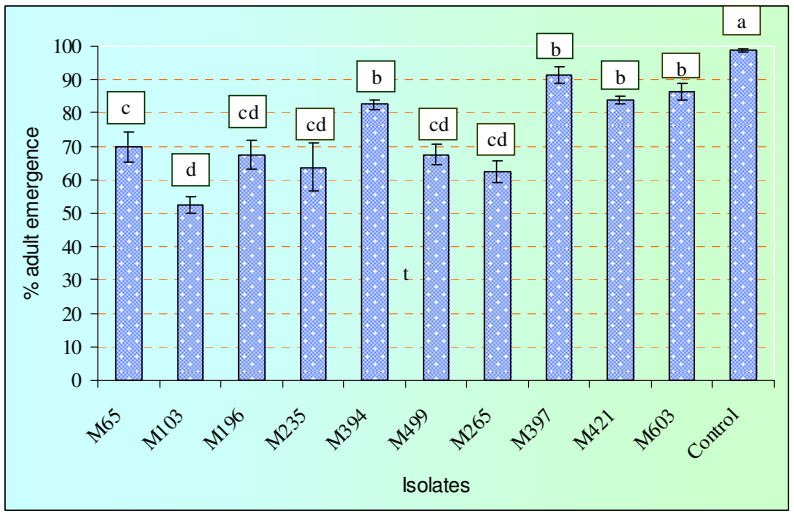

Figure VII. Pathogenicity of different isolates of *Metarhizium anisopliae* (M65, M 103, M235, M394 and M499), *Paecilomyces fumosoroseus* (M603) and *Beauveria bassiana* (M265, M397 and M421) to mean (% ± SE) emergence of *Bactrocera cucurbitae*.

Bars with the same letter do not differ significantly by SNK (P=0.05)

5.0 Discussion and conclusion

The local entomopathogenic fungi that were used induced mortality in puparia of *B. zonata* and *B. cucurbitae* when pupariating third-instar larvae were exposed to sand treated with conidial suspension. The overall effect of this treatment led to a significant reduction in adult emergence in both fruit fly species tested. Out of the 10 isolates evaluated against *B. zonata,* seven isolates (*M. anisopliae* M65, M196, M394 and M499, *B. bassiana* M265 and M421 and *P. fumosoroseus* M603) caused significantly higher pupal mortality. Six isolates (*M. anisopliae* M65, M103, M196, M235 and M499, and *B. bassiana* M265) were found to be significantly more pathogenic to puparia of *B. cucurbitae* as compared to the other tested isolates.

Ferron (1985) reported that, generally, fruit fly larvae treated with entomopathogenic fungi pupated normally. However, two days after pupation, spots of melanisation were observed on the integument of the puparia. In this study, mycelial growth followed by profused sporulation was observed on colonized puparia at five days after pupation. A high level of sporulation is an important criterion for selected isolates. Each dying puparium constitutes an infection focus, which will serve as a source of inoculum for infecting other healthy pupariating larvae. A high level of sporulating puparia occurring in the soil will ensure that threshold and contact chances between conidia and healthy pupariating larvae and puparia is increased thus ensuring that disease is transmitted through generations of the pests. Gottwald and Tedders (1984) reported that *B. bassiana* hyphae can spread rapidly from infected weevil cadavers through the soil.

Ekesi *et al.* (2002) found that, in laboratory tests, isolates of *M. anisopliae* and *B. bassiana* caused a significant reduction in adult emergence and a corresponding large mortality of puparia of *C. capitata* and *C.* var. *rosa*

fasciventris (Bezzi) when exposed as late third-instar larvae in sand. In this study, adult emergence for *B. zonata* and *B. cucurbitae* varied from 60 to 93% and 52 to 92%, respectively, in fungal-treated sand. However, Ekesi *et al.* (2002) reported less than 68% adult emergence of *C. capitata* and *C.* var. *rosa fasciventris* in fungal treated sand. Furthermore, the percentage of mycosed puparia found was up to 94% and a deferred adult mortality reached 100%. In this study, the percentage of puparia with visible signs of mycosis did not exceed 48%, while deferred adult mortality was less than 68%. The difference in pathogenicity between the two studies could be explained by differences in genus of fruit flies tested. The genus *Ceratitis* could be more susceptible than the genus *Bactrocera.* It could also be due to the fact that isolates used by Ekesi *et al.* (2002) are more virulent than those used in this study. It might be interesting to consider if fruit flies or more, or less, susceptible to local vs "exotic" fungal strains. There is a sporadic debate over whether or not co-evolved pathogens are more virulent. Admittedly, there hasn't been much time for an evolutionary history to emerge on Mauritius…but perhaps Indo-Australian fungi would give a different result.

There is increasing interest in the exploitation of entomopathogenic fungi for the control of different agricultural pests and some products are available commercially (Lacey & Goettel, 1995; Butt *et al.* 2001). Their safety and selectivity to non-target beneficial organisms make them ideal candidates for integration into various pest management programmes (Goettel & Johnson, 1992; Moore & Prior, 1993; Lacey *et al.* 2001). Furthermore, their production is easy and cheap and does not require high input technology (Prior, 1988). This study suggests that soil drenches with entomopathogenic fungi may be an effective integrated pest management component for the control of *B. zonata* and *B. cucurbitae* in fruit tree orchards and cucurbit plantations, respectively. Soil application of

entomopathogenic fungi has been undertaken in various parts of the world as a cost-effective management technique for many insect pests. In Australia, Rath and Bullard (1997) reported that a granular formulation of *M. anisopliae*, BioGreen^{TM}, can persist in pasture ecosystem for at least 7.5 years at the equivalent of the applied dose thus controlling the population of the redheaded pasture cockchafer. Watt and Le Brun (1984) in USA demonstrated that soil of *B. bassiana* successfully controlled first and second generation Colorado potato beetle with 74 and 77% reductions in populations, respectively. Hence, entomopathogenic fungi can suppress soil-inhabiting insect pests and provide long-term control.

Given the fact that there was significant reduction in adult emergence and a corresponding large mortality on puparia of *B. zonata* and *B. cucurbitae* when exposed as late third instar larvae in sand in this study, the most virulent isolates could be potential candidates for soil application against fruit flies.

6.0 Acknowledgement

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