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Effectiveness of *Beauveria* and *Metarhizium* in the biological control of the fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

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ABSTRACT: Entomopathogenic fungi are promising alternatives to chemical insecticides for biocontrol. In this study, we evaluated the pathogenicity of three strains of *Beauveria bassiana* (BCC4742, BCC2779, and BCC1495) and two strains of *Metarhizium anisopliae* (BCC 30455 and BCC 16762) against the fruit fly *Bactrocera dorsalis* (Hendel), a major agricultural pest. Fruit flies that were directly exposed to the conidial suspension had mortality rates of 64–93% using *B. bassiana* and 16–64% using *M. anisopliae*. The mortality rates were higher for the adult feeding bioassay than for direct exposure; approximately 90% of *B. bassiana* and 80% of *M. anisopliae*. Moreover, horizontal transmission of fungi was investigated, in which treated flies were maintained with untreated flies of the opposite sex, they were able to transmit the infection. The highest mortality rates were observed from treatment of *B. bassiana* (BCC4742 and BCC2779), which average >80% mortality for both sexes. These results suggested that all *B. bassiana* strains (BCC4742 and BCC2779) are potential candidates for *B. dorsalis* control programs using various application strategies.

Keywords: *Beauveria bassiana*; *Metarhizium anisopliae*; *Bactrocera dorsalis* (Hendel); biological control; entomopathogenic fungi

Introduction

The oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is one of the most destructive agricultural pests, particularly in Asiatic countries. This fruit fly species is capable of causing serious damage to more than 250 species of commercially valuable tropical and subtropical crops (Clarke et al., 2005; Stephens et al., 2007; Liu et al., 2017). Enormous economic losses result from direct infestation as well as the loss of potential market owing to quarantine regulations to prevent the entry of exotic pests from importing countries (Dimbi et al., 2003).

Currently, a multiple-technique approach or integrated pest management program is recommended for the control of fruit flies (Vargas et al., 2010; Boulahia-Kheder et al., 2012). However, chemical insecticides are frequently used. Synthetic insecticides are associated with various ecological problems and negative effects on human health (Desneux et al., 2007). Moreover, Tephritidae fruit flies have developed insecticide resistance (Nadeem et al., 2014). Various alternatives to insecticidal control are being developed, such as sterile insect techniques, mass trapping, and biological control methods. Biological control using specific parasitoids and entomopathogens could provide a promising, environmentally safe alternative to synthetic insecticides (Zenil et al., 2004; Daniel and Wyss, 2009; Martinez-Ferrer et al., 2012).

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Received: date; December 24, 2020 Accepted: date; July 27, 2021 Published: date; December 29, 2021

Entomopathogenic fungi (EPFs), including more than 700 species, are common natural enemies of arthropods worldwide and are potential biological control agents. Among these, fungal entomopathogens such as *Beauveria bassiana*, *B. brongniartii*, *Isaria farinosa*, *I. fumosorosea*, *Lecanicillium* spp. And *Metarhizium* spp. play an important role in the regulation of insect populations (Shah and Pell, 2003; Khan et al., 2012; Lacey et al., 2015). In particular, *B. bassiana* and *M. anisopliae* are being developed for the control of agricultural pests and some products are now available commercially. They are a successful alternative to chemical insecticides in organic agriculture or in cases where pesticide resistance and environmental concerns limit the use of synthetic products (Khan et al., 2012; Lacey et al., 2015). Unlike typical entomopathogenic microbial organisms, fungal entomopathogens can infect hosts via contact, invade via the epicuticle of the integument, and do not need to be ingested by the insect to cause infection (Goettel et al.,2005; Ali et al., 2010; Lacey et al., 2015). Therefore, entomopathogenic fungi is one of suitable options as alternatives to chemical control and as a component of integrated management control strategies for fruit fly pest.

Entomopathogenic fungi including *B. bassiana* and *M. anisopliae* are widely explored for management of a wide range of fruit fly pests (Ekesi et al., 2002; Daniel and Wyss, 2009; Beris et al., 2013; Sookar et al., 2014). Although, the pathogenicity of locally isolated entomopathogenic fungi to fruit fly has not yet been studied. Especially, there have been a few studies for for the feeding bioassays and horizontal transmission. Thus, the objective of this laboratory experiment was to determine the effects of five local strains of *M. anisopliae* and *B . bassiana* on fruit fly *B. dorsalis*. To develop an effective biocontrol agent from native strains, we determined the effectiveness of these fungal taxa by spore suspension contact, feeding bioassays, and horizontal transmission.

Materials and Methods

Fungal isolates and preparation of Conidial Suspensions

The fungi included two strains of *M. anisopliae* (BCC 30455and BCC 16762) and three strains of *B. bassiana* (BCC4742, BCC2779, and BCC1495), which isolated from insects (Order Hemiptera). All strains were obtained from the National Center for Genetic Engineering and Biotechnology, Thailand. Fungal strains were cultured on potato dextrose agar (PDA) in Petri dishes and maintained in incubator which temperature-controlled at 25°C for 2–3 weeks in complete darkness. After that, fungi conidia were harvested from surface cultures by scraping and were suspended in 10 ml of sterile distilled water containing 0.1% Tween-80 in glass vials. The conidial suspensions were sieved with a layer of sieve cloth, then vortexed to produce a homogeneous suspension. Conidia were counted using a haemocytometer following serial dilution in sterile distilled water containing 0.1% Tween-80. The viability of conidia was determined by the plate count method on PDA following the methods of Sookar et al. (2008) with slight modifications.

Insect mass rearing

Colonies of *B. dorsalis* were obtained from the Plant Protection Promotion and Soil-Fertilizer Management Division, Department of Agricultural Extension, Bangkok Thailand. Adults were placed in metal cages (45 × 50 × 60 cm) containing an artificial diet of yeast hydrolysate and sugar at a 1:3 ratio and a sponge soaked in water as the water source. After mating, females were allowed to oviposit on ripe bananas and hatched larvae were fed until they developed to the full-grown larval stage. They were then moved to dry areas for pupation. Mature pupae were collected daily and placed in the cage allow adult fly emergence

Bioassay

Conidia were quantified using a haemocytometer following serial dilution 1×10^{9}, 1×10^{8}, 1×10^{7}, and 1×10^{6} spores/ml for each isolate. Adults (5-7 days old) were anaesthetised by freezing at -20°C for 90 s, after which 1 ml of suspension was applied to adults using a hand-sprayer. Sterile distilled water containing 0.1% Tween-80 was

applied as the control treatment. A completely randomized design (CRD) was used, with five replicates of 20 adults (ten each for males and females) per strain and concentration. Duration of spraying the suspension was approximately 1-2 minutes for each replicate. Treated adults were released to plastic cages (9 cm in diameter × 15 cm in length), which open at top ends and covered with a layer of sieve cloth. The cages were containing an artificial diet of yeast hydrolysate and sugar at a 1:3 ratio and a sponge soaked in water as the water source.

Adult mortality was observed and recorded every 2 days for 14 days. To assess fungal infection (mycosis), dead insects were removed and immediately washed by rinse three time with sterile deionized water. Cadavers were then incubated in sterile Petri dishes on wet filter paper, sealed with Parafilm, and maintained at 25°C for 5 days to allow the growth of surface mycelia.

Adult feeding bioassay

For efficacy testing of fungi isolates by feeding bioassay, the equivalent concentration of conidial suspension for each fungal strain was assigned at (1×10^8 spores/ml). Then, solution was mixed with artificial diet (yeast hydrolysate and sugar at a 1:3 ratio) for fruit fly adults in a 1:1 ratio. The mixture (1 ml) was poured into plates 3 cm in diameter and provided to fruit fly adults in plastic cages (9 cm × 15 cm). The plates with the food mixture were kept in the cage for 24 h and then replaced with plates with standard food only. A CRD was used with five replicates of 20 adults (ten each for males and females) for each fungal strain. The mortality was recorded every 2 days for 14 days. To assess fungal infection (mycosis), dead insects were removed and analysed according to the methods used in the bioassay. Standard adult food mixed with sterile distilled water was applied to the controls at the same amount using for the fungal treatments.

Horizontal transmission of fungus

After adults emerged, they were separated by sex and placed in separate cages containing an artificial diet of yeast hydrolysate and sugar at a 1:3 ratio and a sponge soaked in water as the water source until they reached sexual maturity (10–12 days old). Conidia of each isolate were then quantified using a haemocytometer at two concentrations of 1×10^8 and 1×10^6 spores/ml.

Mature virgin ten males were anaesthetised by freezing at -20°C for 90 s, after which 1 ml of suspension was applied using a hand sprayer. After exposure to fungal spores, treated males were released into the plastic cages (9 cm in diameter × 15 cm in length) containing an artificial diet (yeast hydrolysate and sugar at a 1:3 ratio) and a sponge soaked in water as the water source. An equal number of untreated females was released into the cages. The insects were maintained together for 24 h to allow mating and were then separated. Mortality rates for both sexes were recorded for 14 days. The experiment included five replicates per strain and concentration. In another experiment, female flies were treated and held with untreated males following the experimental procedure described above.

Statistical analysis

Mortality was adjusted for control-mortality using Abbott's formula (Abbott, 1925).

The normality of the data obtained was first checked using the Kolmogorov-Smirnov test.

The differences among treatments were determined by one-way analysis of variance (ANOVA) followed by Duncan's new multiple-range test. Mean lethal concentrations were estimated using a probit analysis for correlation data. All analyses were conducted using SPSS 14.0 (2004; SPSS, Chicago, IL, USA).

Results and Discussion

Virulence of fungal isolates

Conidial viability of all fungal isolates was assessed before bioassays; greater than 90% of the conidia germinated at 24 h. With respect to virulence, all tested fungal isolates were pathogenic to adult fruit flies. However,

the pathogenic activity varied among isolates. The mortality levels of treated flies ranged from 16% to 93. At the lowest concentration, *B. bassiana* BCC2779 and BCC4742 showed the highest efficacy, with mortality rates of 89% and 87%, respectively. At higher concentrations, these two strains and *B. bassiana* BCC1495 cause 93% mortality, which was significantly different from the rates for the two strains of *M. anisopliae* ($P < 0.05$) (**Table 1**).

In these studies, we confirmed the susceptibility of *B. dorsalis* adults to all native *B. bassiana* and *M. anisopliae* strains. The susceptibility of Tephritidae fruit flies to *Beaveria* and *Metarhizium* has been reported in previous studies (Ekesi et al., 2002; Dimbi et al., 2003; Daniel and Wyss, 2009; Bedini et al., 2018). However, the mortality caused by suspension contact varied among strains. *B. bassiana* showed a higher level of virulence (64-93% mortality) than that of *M. anisopliae* (16–64% mortality). The efficacy of EPFs may vary among species within a genus and even among isolates of the same species. Differences in mortality rates may be related to differences in conidial attachment to the insect cuticle, the germination rate, and the suppression of the host immune system. In addition, enzymes produced by EPFs may contribute to variation in mortality (Khan et al., 2012).

Table 1 Mortality (%) ± SE of adult of fruit fly *B. dorsalis* after 14 days of exposure to five fungal strains at different four concentrations

Fungal species	Strain	Conidial concentration (spores/ml) [1/]			
		1×10^6	1×10^7	1×10^8	1×10^9
M. anisopliae	BCC16762	16 ± 1.34^c	22 ± 0.84^d	32 ± 2.39^c	43 ± 1.58^c
	BCC30455	18 ± 2.45^c	40 ± 0.51^c	52 ± 3.42^b	64 ± 2.49^b
B. bassiana	BCC1495	64 ± 1.79^b	83 ± 1.58^b	93 ± 0.55^a	93 ± 1.25^a
	BCC2779	89 ± 0.84^a	93 ± 0.45^a	93 ± 1.52^a	93 ± 0.85^a
	BCC4742	87 ± 0.45^a	92 ± 0.45^a	93 ± 0.55^a	93 ± 0.51^a
F-test [2/]		7.21	9.97	7.71	4.62
C.V. (%)		13.54	10.28	12.72	8.79

[1/] Values in a column followed by different letters are significantly at $p<0.05$ (Duncan's Multiple Range Test)

[2/] Mean (±SE) differ significantly at $p<0.05$ (*) (ANOVA)

The entomopathogenic activity of tested isolates was confirmed by the presence of fungal hyphae and spores on the bodies of dead flies. Mycosis was confirmed for more than 80% of dead treated flies. Many factors might affect the ability of the mycelium to develop in cadavers (Shimazu and Takatsuka, 2006; Beris et al., 2013). No mycosis was observed on dead untreated flies in any experiments.

The mortality rate was correlated with the conidial lethal concentrations (LC_{50}) that is required to kill 50% of tested flies. The log of the LC_{50} ranged from 5.6 to 9.5 (conidia/ml) with variation among isolates. The lowest required concentration (5.6) was recorded for *B. bassiana* BCC2779. The highest required concentration (9.5) was recorded for *M. anisopliae* BCC16762. According to the 50% lethal concentration, *B. bassiana* BCC2779 was highly pathogenic to fruit fly adults, followed by *B. bassiana* BCC4742 and *B. bassiana* BCC1495. The 90% lethal concentration was 6.1–15.5 (log concentrations, conidia/ml) and the values for different strains were correlated with LC_{50} values (**Table 2**).

Table 2 Lethal concentrations of each strain on adult of fruit fly *B. dorsalis* over 14 days of treatment

Fungal species	Strain	log of conidia/ml [1/]			
		LC_{50} (±SE)	Slope (±SE)	LC_{90} (±SE)	Slope (±SE)
M. anisopliae	BCC16762	9.5 ± 0.45^{c}	0.56 ± 0.03	15.5 ± 0.27^{c}	1.59 ± 0.15
	BCC30455	7.5 ± 0.87^{ab}	0.33 ± 0.05	11.1 ± 0.56^{b}	0.98 ± 0.12
B. bassiana	BCC1495	6.6 ± 0.63^{a}	0.23 ± 0.03	7.4 ± 0.27^{a}	0.33 ± 0.05
	BCC2779	5.6 ± 0.30^{a}	0.65 ± 0.07	6.1 ± 0.61^{a}	1.16 ± 0.28
	BCC4742	6.2 ± 0.12^{a}	0.67 ± 0.06	7.1 ± 0.45^{a}	0.48 ± 0.07
	F-test [2/]	3.41		2.31	
	C.V. (%)	3.86		5.94	

[1/] Values in a column followed by different letters are significantly at $p<0.05$ (Duncan's Multiple Range Test).

[2/] Mean (±SE) differ significantly at $p<0.05$ (*) (ANOVA)

Feeding bioassay

In addition to spraying the conidia suspension, the fungal isolates can be administered by feeding bioassay. Average mortality rates of *B. dorsalis* adults after feeding 24 h on a mixture of the conidial suspension and artificial diet were 81–93% depending on the fungal strain. The virulence levels determined by the feeding method were correlated with levels obtained by contact with the spore suspension. The highest adult mortality was 93% using *B. bassiana* BCC2779 and BCC4742. The lowest values were obtained for adults fed on the conidia of *M. anisopliae* BCC16762 (**Table 3**). Mortality rates were higher in the feeding bioassay than after exposure to the conidial suspension. This may be explained by the ease of evasion via the mouthpart opening than via the cuticle of fruit flies. Accordingly, a bait station is also suggested as a potential strategy for the successful use of these fungal strains against adult *B. dorsalis*.

Table 3 Average mortality of adult of fruit fly *B. dorsalis* after feeding on a conidial suspension in an artificial diet

Fungal species	Strain	% Mortality ± SE[1/]
M. anisopliae	BCC16762	81 ± 1.64^{c}
	BCC30455	83 ± 1.30^{c}
B. bassiana	BCC1495	90 ± 0.89^{ab}
	BCC2779	93 ± 1.20^{a}
	BCC4742	93 ± 0.52^{a}
F-test[2/]		5.05
C.V. (%)		15.93

[1/] Values in a column followed by different letters are significantly at $p<0.05$ (Duncan's Multiple Range Test)

[2/] Mean (±SE) differ significantly at $p<0.05$ (*) (ANOVA)

Horizontal transmission of fungi between *B. dorsalis* adults

Treated male and female flies that were directly exposed to the conidial suspension became infected and died at different rates. When treated flies were maintained together with untreated flies of the opposite sex, they were able to transmit the infection. At a low concentration of 1x 10^{6} spores/ml, the mortality rates of treated males differed significantly among fungal strains ($P < 0.05$) (**Table 4**). The three strains of *Beauveria* caused 56–88% mortality, and these rates were higher than those for *Metarhizium* (18–24% mortality). Untreated females maintained together also received the infection, resulting in mortality rates of 48–84% and 20–26% for *Beauveria* and

Metarhizium, respectively. The transmission was confirmed using a conidia suspension with a higher concentration of 1 x10^8 spores/ml and the mortality results for treated males reared with untreated females were consistence with those obtained using a lower concentration. The mortality rates differed significantly between the three strains of *Beauveria* and the two strains of *Metarhizium* ($P < 0.05$).

Similarly, treated female flies became infected and were able to transmit the infection to male recipients (**Table 5**). The mortality rates of treated females and untreated males differed significantly depending on the fungal strain ($P < 0.05$). At 1x10^6 spores/ml, the mortality rates of treated females ranged from 62% to 88% for *Beauveria*, which were higher than those for *Metarhizium* (i.e. 14 and 20%). The mortality rates of untreated males mixed together ranged from 48% to 84% for *Beauveria* and 16%-24% for *Metarhizium*. Transmission was also confirmed using a concentration of 1x10^8 spores/ml, and mortality for treated females and untreated males caused by the three *Beauveria* strains was significantly different from mortality caused by two *Metarhizium* strains ($P < 0.05$).

Horizontal transmission in fungi is useful for the biocontrol of insect pests belonging to several orders, including Blattodea (Quesada-Moraga et al., 2004), Lepidoptera (Furlong and Pell, 2001), Coleoptera (Shimazu and Takatsuka, 2006), and Diptera (Toledo et al., 2007; Quesada-Moraga et al., 2008; Dimbi et al., 2013; Sookar et al., 2014). The fungus-infected flies could be transmitted to uncontaminated flies of the opposite sex by physical contact during mating or other behavioural interactions among individuals of the same or opposite sex (Quesada-Moraga et al., 2008; Dimbi et al., 2013; Sookar et al., 2014). Thus, sterile flies infected with these fungi, in particularly, *B. bassiana* BCC2779 and BCC4742 can be used as vectors to transmit the infection to the opposite sex. However, mating duration may affect on the horizontal transmission, in which longer period of courtship allows more occasion of fungi transmitted to the opposite sex.

We identified two possible limitations of this study. First, the lethal time (LT) or time measurement which indicate the virulence after infection of fungi that can kill the fruit flies was not determined in this experiment. To improve the accuracy data on the virulent fungi, LT50-LT90 will be further studies. Second, the limitation for horizontal transmission experiment is that small size of the cages which using for maintain the treated and untreated-fruit fly. Resulting in more opportunity for mating or contacting due to restricted space. More accurate data can be acquired by using larger size of rearing cage as well as evaluate the efficacy of horizontal transmission in greenhouse to confirm the laboratory results.

Table 4 Mortality (%) of adult of fruit fly *B. dorsalis* induced by the horizontal transmission of each fungal strain from treated male flies to untreated females using conidial suspensions at two concentrations

Fungal species	Strain	%Mortality ± SE[1/]			
		1×10^6 conidia ml^{-1}		1×10^8 conidia ml^{-1}	
		male	female	male	female
M. anisopliae	BCC16762	24 ± 2.97^{c}	16 ± 2.51^{dc}	28 ± 2.49^{c}	20 ± 3.46^{dc}
	BCC30455	18 ± 1.48^{dc}	10 ± 0.71^{dc}	30 ± 0.71^{c}	22 ± 1.1^{dc}
B. bassiana	BCC1495	56 ± 1.14^{b}	48 ± 0.84^{b}	88 ± 0.45^{a}	60 ± 0.71^{b}
	BCC2779	84 ± 0.9^{a}	76 ± 1.95^{a}	90 ± 0.57^{a}	90 ± 0.55^{a}
	BCC4742	88 ± 0.45^{a}	84 ± 0.55^{a}	90 ± 1.20^{a}	84 ± 0.89^{a}
	F-test [2/]	4.91	3.43	3.04	4.32
	C.V. (%)	17.88	22.42	32.50	33.75

[1/] Values in a column followed by different letters are significantly at $p<0.05$ (Duncan's Multiple Range Test)

[2/] Mean (±SE) differ significantly at $p<0.05$ (*) (ANOVA)

Table 5 Mortality (%) of adult of fruit fly *B. dorsalis* induced by the horizontal transmission of each fungal strain from treated female flies to untreated males using conidia suspensions at two concentrations.

Fungal species	Strain	%Mortality ± SE[1/]			
		1×10^6 conidia ml^{-1}		1×10^8 conidia ml^{-1}	
		male	female	male	female
M. anisopliae	BCC16762	6 ± 1.14^{e}	14 ± 0.55^{dc}	8 ± 0.45^{d}	16 ± 1.14^{dc}
	BCC30455	14 ± 1.52^{d}	20 ± 0.71^{dc}	24 ± 0.55^{c}	26 ± 1.14^{c}
B. bassiana	BCC1495	48 ±1.1^{c}	62 ± 0.84^{b}	60 ± 1.87^{b}	90 ±1.75^{a}
	BCC2779	84 ± 0.89^{a}	88 ± 0.89^{a}	86 ± 0.55^{a}	90 ±0.75^{a}
	BCC4742	80 ± 1.22^{a}	86 ± 0.55^{a}	80 ± 1.22^{a}	88 ± 0.45^{a}
	F-test	8.55	5.01	5.50	8.32
	C.V. (%)	18.21	15.58	12.90	20.75

[1/] Values in a column followed by different letters are significantly at $p<0.05$ (Duncan's Multiple Range Test).

[2/] Mean (±SE) differ significantly at $p<0.05$ (*) (ANOVA).

Conclusion

These results indicate that *B. bassiana* (BCC4742 and BCC2779) are promising biological control agents against adults of the fruit fly *B. dorsalis* under different modes of application, such as cover spray, bait, and fly-to-fly conidial transmission. However, further investigations are necessary to evaluate the efficacy of these strain in greenhouse to confirm the laboratory results.

Acknowledgements

The authors gratefully acknowledge the financial support provided by Thammasat University Research Fund under TU Research Scholar, Contract No. 16/2562.

References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 18: 265-267.

Ali, S., Z. Huang, and S. Ren. 2010. Production of cuticle degrading enzymes by *Isaria fumosorosea* and their evaluation as a biological agent against diamondback moth. Journal of Pest Science. 83: 361-370.

Bedini, S., S. Sarrocco, R. Baroncelli, G. Vannacci, and B. Conti. 2018. Pathogenic potential of *Beauveria pseudobassiana* as bioinsecticide in protein baits for the control of the medfly *Ceratitis capitata*. Bulletin of Insectology. 71: 31-38.

Beris, E. I., D. P. Papachristos, A. Fytrou, S. A. Antonatos, and D. C. Kontodimas. 2013. Pathogenicity of three entomopathogenic fungi on pupae and adults of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). Journal of Pest Science. 86: 275.

Boulahia-Kheder, S., F. Loussaïef, A. Benhmidène, I. Trabelsi, F. Jrad, Y. Akkri, and M. Fezzani. 2012. Evaluation of two IPM programs based on mass-trapping against the Mediterranean fruit fly *Ceratitis capitata* on citrus orchards. Tunisian Journal Plant Protection. 7: 55-68.

Clarke, A. R., K. F. Armstrong, A. E. Carmichael, J. R. Milne, S. Raghu, and G. K. Roderick. 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. Annual Review of Entomology. 50: 293-319.

Daniel, C., and E. Wyss. 2009. Susceptibility of different life stages of the European cherry fruit fly, *Rhagoletis cerasi*, to entomopathogenic fungi. Journal of Applied Entomology. 133: 473-483.

Desneux, N., A. Decourtye, and J. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annual Review of Entomology. 52: 81-106.

Dimbi, S., N. K. Maniania, and S. Ekesi. 2013. Horizontal transmission of *Metarhizium anisopliae* in fruit flies and effect of fungal infection on egg laying and fertility. Insects. 4(2): 206-216.

Dimbi, S., N. K. Maniania, S. A. Lux, and J. M. Mueke. 2003. Host species, age and sex as factors affecting the susceptibility of the African tephritid fruit fly species, *Ceratitis capitata*, *C. cosyra* and *C. fasciventris* to infection by *Metarhizium anisopliae*. Journal of Pest Science. 76: 113-117.

Dimbi, S., N. K. Maniania, S. A. Lux, S. Ekesi, and J. M. Mueke. 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) *Vuillemin*, to three adult fruit fly species: *Ceratitis capitata* (Wiedemann), *C. rosa* var. fasciventris Karsch and *C. cosyra* (Walker) (Diptera: Tephritidae). Mycopathologia. 156: 375-382.

Ekesi, S., N. K. Maniania, and S. A. Lux. 2002. Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Science and Technology. 12(1): 7-17.

Furlong, M. J., and J. K. Pell. 2001. Horizontal transmission of entomopathogenic fungi by the diamondback moth. Biological Control. 22(3): 288-299.

Goettel, M. S., J. Eilenberg, and T. R. Glare. 2005. Entomopathogenic fungi and their role in regulation of insect populations. Comprehensive Molecular Insect Science. 6: 361-406.

Khan, S., L. Guo, Y. Maimaiti, M. Mijit, and D. Qiu. 2012. Entomopathogenic fungi as microbial biocontrol agents. Molecular Plant Breeding. 3: 63-79.

Lacey, L., D. Grzywacz, D. I. Shapiro-Ilan, R. Frutos, M. Brownbridge, and M. S. Goettel. 2015. Insect pathogens as biological control agents: Back to the future. Journal of Invertebrate Pathology. 132: 1-41.

Liu, H., X. F. Zhao, L. Fu, Y. Y. Han, J. Chen, and Y. Y. Lu. 2017. BdorOBP2 plays an in dispensable role in the perception of methyl eugenol by mature males of *Bactrocera dorsalis* (Hendel). Scientific Reports. 7: 15894.

Martinez-Ferrer, M. T., J. M. Campos, and J. M. Fibla. 2012. Field efficacy of *Ceratitis capitata* (Diptera: Tephritidae) mass trapping technique on clementine groves in Spain. Journal of Applied Entomology. 136(3): 181-190.

Nadeem, M. K., S. Ahmed, S. Nadeem, M. Ishfaq, and M. Fiaz. 2014. Assessment of insecticides resistance in field population of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). Journal of Animal and Plant Science. 24(1): 172-178.

Quesada-Moraga, E., R. Santos-Quiros, P. Valverde-Garcia, and C. Santiago-Alvarez. 2004. Virulence, horizontal transmission, and sublethal reproductive effects of *Metarhizium anisopliae* (Anamorphic fungi) on the German cockroach (Blattodea: Blattellidae). Journal of Invertebrate Pathology. 87: 51-58.

Quesada-Moraga, E., I. Martin-Carballo, I. Garrido-Jurado, and C. Santiago-Álvarez. 2008. Horizontal transmission of *Metarhizium anisopliae* among laboratory populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Biological Control. 47: 115-124.

Shah, P. A., and J. K. Pell. 2003. Entomopathogenic fungi as biological control agents. Applied Microbiology and Biotechnology. 61: 413-423.

Shimazu, M., and J. Takatsuka. 2006. Factors affecting poor mycelial growth on cadavers of *Monochamus alternatus* infected with an entomopathogenic fungus, *Beauveria bassiana*. Bulletin of FFPRI. 5: 235–242.

Sookar, P., S. Bhagwant, and M. N. Allymamod. 2014. Effect of *Metarhizium anisopliae* on the fertility and fecundity of two species of fruit flies and horizontal transmission of mycotic infection. Journal of Insect Science. 14: 100.

Sookar, P., S. Bhagwant, and O. E. Awuor. 2008. Isolation of entomopathogenic fungi from the soil and their pathogenicity to two fruit fly species (Diptera: Tephritidae). Journal of Applied Entomology. 132: 778-788.

Stephens, A., D. Kriticos, and A. Leriche. 2007. The current and future potential geographical distribution of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). Bulletin of Entomological Research. 97: 369-378.

Toledo, J., S. E. Campos, S. Flores, P. F. Liedo, J. Barrera, A. Villaseñor, and P. Montoya. 2007. Horizontal transmission of *Beauveria bassiana* in *Anastrepha ludens* (Diptera: Tephritidae) under laboratory and field cage conditions. Journal of Economic. Entomology. 100: 291-297.

Vargas, R. I., J. C. Piñero, R. F. L. Mau, E. B. Jang, L. M. Klungness, and D. O. Mcinnis. 2010. Area-wide suppression of the Mediterranean fruit fly, *Ceratitis capitata*, and the oriental fruit fly, *Bactrocera dorsalis*, in Kamuela, Hawaii. Journal of Insect Science 10: 135.

Zenil, M., P. Liedo, T. Williams, J. Valle, J. Cancino, and P. Montoyab. 2004. Reproductive biology of *Fopius arisanus* (Hymenoptera: Braconidae) on *Ceratitis capitata* and *Anastrepha* spp. (Diptera: Tephritidae). Biological Control. 29: 169-178.