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Virulence of Beauveria bassiana and Metarhizium anisopliae Isolates against the Oriental Fruit Fly Bactrocera dorsalis (Diptera: Tephritidae) Hendel under Laboratory Conditions

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ከኢትዮጵያ የተገኙ ሰባት የቢቬሪያ ባሲያና (Beauveria bassiana) እና ሲድስት የሜታሪሂዚየም አኒሶፓሌ (Metarhizium anisopliae) የፈንገስ ዓይነቶች በቤተ ሙከራ ዉስጥ ሶስት ጊዜ በመደጋገም የፍራፍሬ ዝንብ (Bactrocera dorsalis) ትል እና ሙሽሬ ላይ የመግደል ችሎታ ተሞክሯል። የጥናቱ ዓላማ የፍራፍሬ ዝንብን በተፈጥሯዊ መንገድ ለመቆጣጠርና IPM ዘዴ ዉስጥ ማካተት ይቻል እንደሆነ ለመመልከት ነዉ። በዚህም መሰረት ሁሉም የተሞከሩት የፈንገስ ኣይነቶች በ 1x10[8] conidia ml[-1] መጠን የፍራፍሬ ዝንብ ትልና ሙሽሬን መግደል ችለዋል። ነገር ግን የመግደል አቅማቸዉ ከ 40.8% እስከ 96.0% ነበር። ከመግደል አቅማቸዉ በመነሳት S-39, 34- GM and S-46 የተባለት የሜታሪሂዚየም ዓይነቶች እና S-13 የተባለት የቢቬሪያ ዓይነቶች ከፍተኛ (81.7 to 96%) የመግደል አቅም ነበራቸው። KF-3 and PPCR-29 የተባሉት የሜታሪሂዚየም ዓይነቶች እና S-39 የቢቬሪያ ዓይነት መካከለኛ (60-80%) የመግደል አቅም ሲኖራቸዉ አንድ የሜታሪሂዚየም የፈንገስ ኣይነት, GF-3 ፤ እና አምስት የቢቬሪያ ዓይነቶች (9609, 9604, S-46, S-10H and DLCO-41) ደካማ የመግደል አቅም ነበራቸዉ (<60%)። የመግደል አቅማቸዉ 81.7% እስከ 96% የሆኑ ከፍተኛ ገዳይ በመባል ሲፈረጁ ይህን አቅማቸዉ በተለያዩ 6 መጠኖች (1×10[4], 1×10[5], 1×10[6], 1×10[7], 1×10[8], and 1×10[9] ml[-1]0) በተጨማሪ ተገምግሟል። በውጤቱም ከፍ ባለ መጠን (1×10[9] Conidia ml[-1]) የተሞከሩት የሜታሪሂዚየም ዝሪያ የመግደል አቅማቸዉ በትንሽ መጠን (1×10[4] conidia ml[-1]) ከተሞከሩት በልጦ ታይቷል። በስድስት የተለያዩ መጠኖች በሙሽሬ ላይም የተሞከረ ሲሆን የሜታሪሂዚየም ዓይነቶች S-46 እና S-39 በከፍተኛ መጠን (1X 10[9]) ግማሽ (50%) የገደሉ ሲሆን የቢቬሪያ ዓይነት S -13 እና የሜታሪሂዚየም ዓይነት 34-GM ከግማሽ በታች ገድለዋል። S-39 የተባለዉ የሜታሪሂዚየም ፈንገስ ኣይነት ከሁሉም አነስተኛ LC_{50} (1.2×10[4]) ሲኖረው፤ 34-GM የተባለዉ ፈንገስ ሁለተኛው ደረጃ ዝቅተኛ LC_{50} (በ1.6×10[4])ነበረው። በዚህም መሰረት S-39, 34-GM, S-46 የሜታሪሂዚየም ዓይነቶች እና S-13 ቢቬሪያ ፈንገስ ዓይነት የፍራፍሬ ዝንብን በተፈጥሯዊ መንገድ ለመቆጣጠር ተስፋ ሰጪ ሆነዉ ተገኝተዋል።

Abstract

The Oriental fruit fly, Bactrocera dorsalis (Diptera: Tephritidae) Hendel, has become the major pest of fruits in tropical Africa. The objective of the study was to evaluate the virulence of Ethiopian origin entomopathogenic fungal isolates of Beauveria bassiana (Balsamo-Crivelli) Vuillemin and Metarhizium anisopliae (Metchnikoff) Sorokin against the larva and pupa of the oriental fruit fly. Thirteen isolates (seven B. bassiana and six M. anisopliae) were bio-assayed in the laboratory. All the tested isolates were pathogenic to the larvae of B. dorsalis at $1x10^8$ conidia ml^{-1} concentration with mortality range from 40.8% to 96%. Isolates S-39, 34-G and S-46 of Metarhizium and S-13 of Beauveria were categorized as highly virulent (81.7% to 96% mortality), isolate KF-3 and PPCR-29 of Metarhizium and S-39 of Beauveria moderately virulent (61.7%- 78% mortality), and isolates GF-

3 of Metarhizium and 9609, 9604, S-46, S-10H and DLCO-41 of Beauveria weakly virulent (40.8-53.3% mortality). Dose-response assay was undertaken on the four highly virulent isolates at six different doses (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, and $1\times10^9 ml^{-1}$) on larvae of the fruit fly. The isolates varied in virulence and showed direct relationship between mortality and concentrations. Isolate S-39 showed the least LC_{50} (1.2×10^4) followed by GM-34 (1.6×10^4), S-46 ($1.9x10^4$) and S-13 ($1.1x10^5$). The bio-assay on pupae of the fruit fly showed that Metharizium isolates S-46 and S-39 caused about 50% pupal mortality at the highest concentration of $1x10^9$, while the remaining two isolates, S-13 (Beauveria) and 34-GM (Metarhizium), caused below 50% pupal mortality at all concentrations. The four tested isolates are promising bio-agents against B. dorsalis and further field trials are recommended as a component of IPM program.

Keywords: Biological control, entomopathogenic fungi, fruit fly IPM, microbial control.

Introduction

The oriental fruit fly, *Bactrocera dorsalis* Hendel, is one of the most destructive fruit fly species in the world (Kalloo, 2005). Losses in commercial orchards ranged between 10 and 25% while smallholders lose between 30 and 80% (Lux *et al*., 1998). In addition to the direct losses, producer countries may also lose potential markets due to stringent quarantine regulations to avoid entry and establishment of unwanted fruit flies. In most parts of Africa, smallholder farmers are responsible for producing the bulk of the fruits and rarely apply any control measures against fruit flies, which have resulted in large economic losses. Thus, application of appropriate control methods could enhance production of high quality fruits for domestic urban markets and help to observe the stringent quarantine regulations for export markets.

The persistent spread of *B. dorsalis* has threatened the commercial fruit industry, especially in the tropical and sub-tropical regions of the world, through higher costs of production and control and new quarantine restrictions (Aketarawong *et al*., 2014). *Bactrocera dorsalis* has high dispersal capacity and rapidly adapts to new environments and thus it is able to colonize new habitats (Wan et al., 2012). Moreover, international transportation, commercial trade and travel provide dispersal pathways that facilitate the movement of *B. dorsalis* (Kriticos *et al.*, 2013). More than 300 cultivated and wild fruit trees including *Annona*s spp., avocado, banana, bitter gourd, citrus, coffee, guava, macadamia, mango, papaya, passion fruit, pepper, persimmon are attacked by the oriental fruit fly (USDA, 2016: https://coffhi.cphst.org). Drew and Hancock (2004) collected 52 species of *Bactrocera* and eight of them were found to be economically important in Asia. *Bactrocera dorsalis* was trapped in Kenya in 2003, from where it rapidly expanded into East and West Africa, as well as central and southern parts of Africa (Manrakhan et al., 2015). Ekesi and Billah (2006) reported the presence of *B. dorsalis* in Ethiopia in 2005.

Fruit flies are managed by different methods including cultural, mechanical and physical, chemical and biological methods. Most of the published studies for fruit fly management focused on biological control, followed by chemical, behavioral control (including SIT) and quarantine treatments (Dias *et al.*, 2018). Cultural control method relies on farm sanitation and crop hygiene targeted at breaking the reproductive cycle of the pests. It

requires the prompt and periodic collection and destruction of all infested fruits found on the trees and all falling fruits containing fruit fly maggots and puparia. Cultural control is quite effective if the infested fruits are regularly collected and destroyed throughout the season, but the practice is time consuming and laborious.

Fruit flies are among the difficult pests to manage because the third-instar larvae leave decaying fruits and drop to the ground to pupate in the soil; consequently, both larvae and pupae in fruits and soils are protected from surface-applied insecticides (White and Elson-Harris, 1992; Heve *et al.,* 2016). Soil treatment with insecticides, like diazinon, beneath host trees to kill fruit fly larvae and puparia has been an important component of fruit fly suppression and eradication programs (Roessler, 1989; CDFA, 1993). Such soil treatments require repeated applications and could have a backlash of environmental contamination, adverse effects on non-target organisms and the development of resistance of pests.

The use of natural enemies for the suppression of fruit flies has always had a wide appeal because it is relatively safe, permanent and economical but it needs continuous applications until established in target areas. Entomopathogens, including *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycota: Hypocreales), have been studied as effective and safer alternatives to synthetic insecticides for the management of several fruit flies (Mochi et al., 2006; Ekesi *et al.* 2010). Their safety and selectivity to non-target beneficial organisms makes them ideal candidates for integration into various pest management programs (Ekesi et al., 1999).

Studies on Ethiopian origin entomopathogens in Ethiopia were conducted on *Sitophilus zea mais* and *Prostephanus truncates* (Kassa *et al.,* 2002), *Chilo partellus* (Tadele and Pringle, 2010), *Thrips tabacci* (Shiberu *et al.,* 2013), *Pachnoda interrupta* (Habtegebriel *et al.,* 2016) and *Tetranychus urtica*e (Negash *et al.,* 2017). However, studies on the management of fruit flies, specifically with the use of entompopathogens, in Ethiopia are limited. Thus, this study was undertaken to evaluate the efficacy of Ethiopian origin *B. bassiana* and *M. anisopliae* on larva and pupa of the Oriental fruit fly, *B. dorsalis*.

Materials and Methods

Fungal isolates

Thirteen different isolates of *B. bassiana* and *M. anisopliae* were supplied by Ambo Agricultural Research Center, Ethiopia. The isolates originated from various arthropods and crop fields in different agro-ecological zones of Ethiopia (Table 1). The isolates were kept in Ambo Agricultural Research Center laboratory as conidia in culture form at 4°C.

Table 1. Fungal isolates tested against the oriental fruit fly, *Bacterocera dorsalis*

S/N	Species	Isolate	Origin/Host	Locations collected	Altitude	Longitude	Latitude	Soil type
1	*B. bassiana*	S-13	Mango farm	East Wollega	1887	035.42,787	09.11,176	Loam
2	*B. bassiana*	9609	*Blosyrus rugulosus*	Dilla	1581	038.18,607	06· 26, 232	Clay
3	*B. bassiana*	S-39	Grazing land	Dawuro	2401	037.08,599	07.03,865	Clay loam
4	*B. bassiana*	S-65	Enset farm	Wolaita	1864	037. 44,216	06. 59,684	Loma
5	*B. bassiana*	S-10H	Banana farm	East Wollega	1792	035.40,907	09.16,254	Loam
6	*B. bassiana*	DLCO-141	Grasshopper	Wolaitta	1850	037.41,152	07.08,081	Loam
7	*B. bassiana*	9604	*Aceraea acerata*	Arbaminch	1180	037.36,730	06. 06,858	Loam
8	*M. anisopliae*	S-39	Grazing land	Dawuro	2401	037.08,599	07.03,865	Clay loam
9	*M. anisopliae*	S-46	Enset farm	Wolaita	1762	037.23,467	06. 48,644	Clay loam
10	*M. anisopliae*	PPRC-27	*Pachnoda interrupta*	North Shoa	2436	038.39,590	09.29,599	Loam
11	*M. anisopliae*	34-GM	Grazing land	Jimma zone	2029	036.77,559	07.73,054	Loam
12	*M. anisopliae*	KF3	Tomato farm	East shoa	1857	038.0 39,59	08. 00, 00	Loam
13	*M. anisopliae*	GF3	Tomato farm	West Shoa	2043	037.83,332	09.16,670	Loam

Insect culture

Larvae and pupae of *B. dorsalis* used in the bioassay study were obtained from *icipe* (International Center for Insect Physiology and Ecology) laboratory Addis Ababa, Ethiopia. Infested mango fruits collected from farmer's field in Gamo zone, Southern Ethiopia, were incubated in the laboratory and hatched larvae were collected and fed with larval diet (mixture of 18.27g sugar, 22.95g of yeast 2240, 7.65g of yeast LS65, 9g of citric acid, 0.3g of sodium benzoate, 0.3g of methyl 4 hydroxyl benzoate, 0.225g of streptomycin, 150ml of distilled water, and 1.5 ml of wheat germ oil) (Hooper, 1987). The mature larvae were allowed to pupate in plastic trays (6 by 20 by 30 cm) that contained a 2-cm-deep layer of moist (5-8% water) and sterilized sand (Onsongo *et al.,* 2019). Emerging adult flies were maintained on a mixture of sugar (3 parts) and commercial enzymatic yeast hydrolysate (1 part) based artificial diet.

Preparation of entomopathogenic fungal isolates

The fungal isolates were cultured on Sabouraud Dextrose Agar with Yeast extract (SDAY) media (10 g peptone, 40 g dextrose and 15 g agar per liter of water, pH 5.6, supplemented with 0.1% yeast extract) and incubated at 25°C for 2–3 weeks (Inglis *et al.,* 2012). Conidia were harvested into 1 ml of sterile aqueous solution of 0.01% Tween 80 and mixed vigorously until homogeneous conidial suspensions were obtained. The conidia were quantified with an improved neubour haemocytometer under a light microscope at 400x magnification. The conidial viability of each isolate was determined by germinating conidia on SDAY media (Inglis *et al.,* 2012). The percentage germination of conidia was determined from 100 spore counts under cover slips at 400x magnification. A conidium was designated as germinated if the length of its germ-tube is twice the diameter of the conidial propagule (Tadele and Pringle, 2004).

Evaluation of the virulence of fungal isolates on larvae of *B. dorsalis*

The virulence of seven isolates of *B. bassiana* (9604, 9609, S-13, S-39, S-65, S-10H, DLCO-141) and six isolates of *M. anisopliae* (PPCR-27, KF-3, 34-GM, GF-3, S-46, S-39) were evaluated in the laboratory in March 2019. Suspensions of the isolates were adjusted to final concentration of 1×10^8 ml^{-1}through serial dilutions with aqueous solution of 0.01% Tween 80.Twenty matured larvae of *B. dorsalis* that were ready to pupate were directly exposed to the spores of the isolates by immersion in 1-ml of conidia of fungal isolates for 20 seconds. The control groups were treated with aqueous solution of 0.01% Tween 80, and the *Metarhizium anisopliae* commercial product *Icipe*- 69 was used as standard check. The treated larvae were placed in sterile petri-dishes of 5.5 cm x 1.5 cm diameter with wet filter paper inside and incubated at 25±2°C. The treatments were arranged in were recorded each day until all larvae changed to pupa, and the pupa to adult. The

emerged adults were kept in insect rearing cages. The number of mycosed larvae, puparia and adults were recorded. Cadavers of larvae, pupae and adults without mycoses were surface sterilized with 1% sodium hypochlorite followed by three rinses with sterile distilled water for 20 seconds. They were transferred to Petri-dishes lined with moist filter paper and kept at 25±2 °C and inspected for the presence of external growth of the fungi.

Dose response bio-assay

Dose-mortality relationship of one *B. bassiana* and three *M. anisopliae* isolates (highly virulent isolates) were studied at six conidial concentrations: 1×10^4, 10^5, 10^6, 10^7, 10^8 and 10^9conidia ml^{-1}. Sixty oriental fruit fly larvae that were ready to pupate within the next 24h and 45 healthy pupae were dipped in 20ml of each fungal concentration of the isolates for 20 seconds and transferred into Petri-dish lined with moisten filter paper. The *Metarhizium anisopliae* commercial product *Icipe*-69 and aqueous solution of 0.01% Tween 80 were used as checks. Petri-dishes were maintained at25±2°C. Three replicates of 60 larvae and 45 pupae were used for each concentration of the isolates and arranged in complete randomized design. Deaths due to mycoses were determined following similar procedures like above.

Statistical analysis

The mortality data were corrected using the formula proposed by Schneider-Orelli (1947), arcsine transformed and subjected to analysis of variance and means were separated using Tukey's Honestly Significant Difference (HSD). The dose-response relationship between conidial concentration of the four highly virulent entomopathogenic fungi isolates and larval mortality, and LC_{50} were estimated with probit analysis by using SPSS software (SPSS, 2015).

Results

Pathogenicity test of *Beauveria and Metarhizium* isolates on *B. dorsalis* larvae

All the 13 isolates tested were able to infect and kill the larvae of *B. dorsalis* as confirmed by fungal growth (mycosis) on surface of cadavers. The virulence among the isolates was highly variable (Table 2). The isolates *Beauveria* S-13, and *Metarhizium*S-39, and S-46 caused significantly higher mortality on the *B. dorsalis* larva, while strains 9609, 9604, S-10H, S-65, DLCO-4, GF-3 caused significantly lower mortality. The isolates were separated in to three virulence categories based on levels of mortality: high (>80%), moderate (60 to 80%) and weak (< 60%) (Roza et al., 2017). Isolates *Beauveria* S-13, and *Metarhizium*S-39, S-46 and 34-GM were highly virulent, *Metarhizium* KF-3 and PPRC-29 and *Beauveria* S-39 moderately virulent, and *Metarhizium* KF-3 and *Beauveria* 9609, 9604, S-65, S-10H and DLCO-41 weak in virulence (Table 2).

Table 2. Percent corrected mortality (±SE) of *Bactrocera dorsalis* larvae treated with isolates of *B. bassiana* and *M. anisopliae* at the rate of $1x10^8$ conidia/ml.

Isolates	Species	Percent mortality	Virulence category
S-13	*B. bassiana*	95.0±0.00 a	High
S-46	*M. anisopliae*	96.7±1.67 a	High
S-39	*M. anisopliae*	93.3±1.67 ab	High
34-GM	*M. anisopliae*	81.7±5.00 bc	High
S-39	*B. bassiana*	75.0±1.67 cd	Moderate
PPCR-29	*M. anisopliae*	78.3±3.33 cd	Moderate
KF-3	*M. anisopliae*	61.7±1.67 de	Moderate
9609	*B. bassiana*	53.3±1.57 ef	Low
9604	*B. bassiana*	43.3±4.41 ef	Low
S-10H	*B. bassiana*	41.7±3.33 f	Low
S-65	*B. bassiana*	41.7±3.33 f	Low
DLCO-41	*B. bassiana*	40.0±5.00 f	Low
GF-3	*M. anisopliae*	50.0±0.00 ef	Low

Means with similar letters in the same column are not significantly different from each other according to Tukey's HSD test at α=0.05.

The relationship between fungal inoculum concentration and larvae mortality was highlighted by the regression analysis for each isolate using the Probit procedure (Table 4). The LC_{50} values ranged from 2.3×10^3 to 9.5×10^4 conidia ml^{-1} depending on the isolate. *Metarhizium anisopliae* isolates had lower LC_{50} values than *B. bassiana* isolate. The lowest LC_{50} was recorded for the *M. anisopliae* isolate S-39 (2.3×10^3 conidia/ml).

Table 3. Mean mortality (±SE) of Oriental fruit fly larvae treated with different concentrations of fungal isolates under laboratory conditions

Treatments	Concentration	Treated larva (n)	Mortality (%)
S-13 (*B. bassiana*)	$1x10^9$	60	95.0±0.00 ab
	$1x10^8$	60	91.7±6.01 abcd
	$1x10^7$	60	95.0±2.89 ab
	$1x10^6$	60	75.0±7.64 cdefg
	$1x10^5$	60	48.2±4.41 ghi
	$1x10^4$	60	20.8±2.89 ij
S-46 (*M. anisopliae*)	$1x10^9$	60	100.0±0.00 a
	$1x10^8$	60	100.0±0.00 a
	$1x10^7$	60	93.3±6.67 ab
	$1x10^6$	60	85.3±0.00 bcde
	$1x10^5$	60	62.0±1.67 efgh
	$1x10^4$	60	51.7±3.33 gh
34-GM (*M. anisopliae*)	$1x10^9$	60	98.3±1.67 ab
	$1x10^8$	60	93.3±3.33 abcd
	$1x10^7$	60	93.3±1.33 abc
	$1x10^6$	60	73.3±1.67 defg
	$1x10^5$	60	65.0±0.00 efgh
	$1x10^4$	60	48.3±4.41 ghi
S-39 (*M. anisopliae*)	$1x10^9$	60	86.6±3.28 abcde
	$1x10^8$	60	83.5±0.67bcdef
	$1x10^7$	60	83.3±6.01 bcdef
	$1x10^6$	60	71.7±3.33 efgh
	$1x10^5$	60	56.7±6.01 fgh
	$1x10^4$	60	41.7±3.33 hi
Icipe-69	15%	60	70.0±2.89 efgh
Control	Aqueous solution	60	6.7±1.67 j

Means with similar letters are not significantly different from each other according to Tukey's HSD test at α=0.05.

Table 4. LC_{50} of *B. bassiana* (S-13) and *M. anisopliae* (S-46, S-39 & GM-34) isolates against larvae of *B. dorsalis*

Isolate	LC_{50}	95% Fiducial Limit	R^2	Slope(±Se)	P-value
S-13	9.5×10^4	4.54×10^3-1.99×10^5	0.8691	0.497±0.16	0.007
S-46	7.9×10^3	6.69×10^3- 9.44×10^3	0.9584	0.45 ± 0.04	0.0007
34-GM	1.6×10^4	$1.25x\ 10^4$- 2.25×10^4	0.9271	0.44 ± 0.06	0.0020
S-39	2.3×10^3	$6.52x\ 10^2$- 8.77×10^3	0.3384	0.18 ± 0.29	0.2258

Percent mortality of the Oriental fruit fly pupae treated with fungal isolates under laboratory conditions

The dose-dependent mortalities of *B. dorsalis* pupae by the four isolates varied considerably (Table 5). Significantly higher mortalities were recorded for isolates S-39 (*M. anisopliae*) at $1x10^5$ to $1x10^9$, S-46 (*M. anisopliae*) at $1x10^7$ to $1x10^9$, S-13 (*B. bassiana*) at $1x10^9$, and 34-GM (*M. anisopliae*) at $1x10^9$ conidia/ml (Table 5). *Metharizium* isolates S-46, and S-39 caused the highest mortalities of 55.6% and 53.0%, respectively, both at the concentration of $1x10^9$ conidia/ml, which

were followed by S-39 (*Metarhizium*) at concentration of $1x10^8$ and $1x10^7$ conidia/ml (48.9 and 44.4%). The remaining two isolates, S-13 (*Beauveria*) and 34-GM (*Metarhizium*) caused below 50% pupal mortality at all concentrations. On the other hand, the commercial product Metharizium *icipe*-69 caused 28.9% mortality on pupae, when applied at the recommended concentration of 15% aqueous suspension (Table 5). Probit analysis for pupae mortality was not performed because it was only on two occasions that mortalities were slightly above 50%.

Table 5. Mean mortality (±SE) of Oriental fruit fly pupae treated with *Beauveria* and *Metarhizium* isolates under laboratory conditions (N=45) at different concentrations

Isolates	Concentration	% Mortality
	$1x10^9$	38.3 ± 1.67 abc
	$1x10^8$	31.7 ± 3.33 bcde
	$1x10^7$	31.7 ± 1.67 bcde
	$1x10^6$	28.3 ± 4.41 bcde
	$1x10^5$	18.3 ± 1.67 def
S-13 (*B. bassiana*)	$1x10^4$	8.33 ± 1.67 fg
	$1x10^9$	55.0 ± 2.89 a
	$1x10^8$	43.3 ± 1.67 ab
	$1x10^7$	36.7 ± 4.41 abcd
	$1x10^6$	31.7 ± 1.67 bcde
	$1x10^5$	28.3 ± 1.67 bcde
S-46 (*M. anisopliae*)	$1x10^4$	15.0 ± 2.89 ef
	$1x10^9$	41.7 ± 3.33 ab
	$1x10^8$	31.7 ± 1.67 bcde
	$1x10^7$	20.0 ± 0.00 cdef
	$1x10^6$	18.33 ± 1.67 def
	$1x10^5$	16.40 ± 03 ef
34-GM (*M. anisopliae*)	$1x10^4$	11.67 ± 3.33 fg
	$1x10^9$	53.3 ± 1.67 a
	$1x10^8$	48.3 ± 4.41 ab
	$1x10^7$	45.0 ± 2.89 ab
	$1x10^6$	36.7 ± 1.67 abcd
	$1x10^5$	28.3 ±1.67 bcde
S-39 (*M. anisopliae*)	$1x10^4$	8.33 ± 1.67 g
Icipe-69	15%	28.3 ± 1.67 bcde
Control	Aqueous solution	3.33±1.67 g

Means with similar letters in the same column are not significantly different from each other according to Tukey's HSD test at α=0.05.

Discussions

In this study, the 13 fungal isolates bio-assayed were found to be pathogenic to the Oriental fruit fly larvae and pupae. When applied at conidial concentrations of 1 x 10^8, the *M. anisopliae* isolates caused 60-96% mortality, except for one isolate, which only killed 50% of the treated larvae. Out of the seven tested *B. bassiana* isolates, the two caused 75% and 95% mortality on *B. dorsalis* larvae, while the remaining five isolates caused 40 to 53 % mortality. Many studies have shown

that *B. bassiana* and *M. anisopliae* are among the most virulent entomopathogens used for fruit fly control (Castillo *et al.,* 2000; Ekesi *et al.,* 2007; Daniel and Wyss, 2009; Garrido-Jurado *et al.,* 2011).

Many studies reported variations in virulence of different entomopathogenic fungal species and their isolates on different species and stages of fruit flies. Ekesi *et al.* (2002) evaluated the pathogenicity of 13 isolates of *M. anisopliae* and two isolates of *B. bassiana* to *C. capitata* and *C.* var. *rosa fasciventris* and reported significant fluctuations in the mortality rates among larvae. Onsongo *et al.* (2019) reported mortality between 16.3% and 100% on *Zeugodacus cucurbitae* by the three *M. anisopliae* isolates; ICIPE 18, ICIPE 30, and ICIPE 69.

All the tested Ethiopian origin entomopathogenic fungi isolates were pathogenic to the pupae of *B. dorsalis*, although the levels of virulence were variable among isolates and conidial concentrations. The *Beauveria bassiana* and *M. anisopliae* isolates caused less than 55% mortality on *B. dorsalis* pupa even at the highest conidial concentration of $1x10^9$ spore ml^{-1}. The levels of virulence on pupa were much lower than on larvae for the same isolates and concentrations. Other studies have also reported variations in virulence of entmopathogenic fungi on pupae of *B. dorsalis*, other fruit flies and insects. Mar and Lumyong (2012) tested six entomopathogenic fungal isolates collected from naturally infected insects against pupa of *Bactrocera* spp. *in vitro* with different conidial concentrations and found that all tested isolates were pathogenic to pupa of *B. dorsalis* which varied from 25.9% to 100% in *M. flavoviride*, 22.2 to 100% in *Paecilomyces lilacinus* and 29.7% to 100% in *B. bassiana.*

Many studies have investigated the pathogenicity and virulence of entomopathogenic fungi on fruit fly species, besides *B. dorsalis*. De la Rosa et al. (2002) reported that *B. bassiana* caused lower mortality on immature stage of the Mexican fruit fly, *Anastrepha ludens*, which was 2-8% on larvae and 0% on pupae but up to 100% mortality on adults. Imoulan and Elmeziane (2014) also reported that *B. bassiana* isolates, namely, TAM6.2 and ERS4.16 caused mortality of 95% and 90%, respectively, on *Ceratitis capitata* pupa. Sookar et al. (2008) evaluated the pathogenicity of seven isolates of *M. anisopliae*, five isolates of *B. bassiana* and two isolates of *P. fumosoroseus* towards the adults of *Bactrocera zonata* and *Bactrocera cucurbitae* by topical application of conidial suspension of 1×10^6 conidia/ml. All the isolates tested were pathogenic to the two fruit fly species and the mortality of *B. zonata* varied between 12.0 and 98.0% and between 2.0 and 94.0% in *B. cucurbitae* at 5 days post- treatment.

The virulence of *B. bassiana* and *M. anisopliae* have been demonstrated on various pests with varied levels of mortalities. Erler and Ates (2015) evaluated the effectiveness of the entomopathogenic *B. bassiana* strain PPRI 5339 and *M. anisopliae* strain F52 against the larvae of the June beetle (*Polyphylla fullo*) and

found that *B. bassiana* was more effective than *M. anisopliae* product, causing mortalities up to 79.8 and 71.6% in young and older larvae, respectively. Habtegebriel *et al.* (2017) reported range of mortality on Sorghum chafer (*P. interrupta*) from 14% for isolate *Beauveria* 9604 to 82% for isolate *Metarhizium* PPRC51, and two *Metarhizium* isolates, PPRC2 with LD_{50} of 0.62 mg/10 beetles and PPRC51 with LD_{50} of 0.55 mg/10 beetles LD_{50},were the most potent.

Mortality of oriental fruit fly larvae was dose-dependent and increased with the increase in concentrations of all the tested isolates. The dose-response bioassay experiment on the fruit fly larvae showed that concentrations greater than $1x10^5$ conidia/ml for both *B. bassiana* and *M. anisopliae* caused >50% mortality. Similar types of dose-dependent mortality by entomopathognic fungi were also observed on tsetse flies (*Glossina* spp.) by *B. bassiana* and *M. anisopliae* (Kaaya and Munyinyi, 1995) and *Scolytus scolytus* larvae by *B. bassiana* (Barson, 1977). Negash *et al.* (2017); Irigaray *et al.* (2002) and Wekesa *et al.* (2006) reported dose-dependent mortality on two spotted spider mite, when treated with the two fungi. Tadele and Pringle (2004) also reported that *B. bassiana* isolate BB-01 and *M. anisopliae* isolate PPRC-4 at conidia concentration of 1×10^8 ml^{-1}caused high mortality and reduction in food consumption by second and third instar larvae of *Chilo partellus* than the lower concentrations.

In the current study, the LC_{50} values observed were lower than those observed by Ekesi *et al.* (2002) indicating that they are more potent. *Metarhizium anisopliae* isolates S-39, GM-34 and S-46 which had lower LC_{50} values appeared to be promising and have the potential to be used in fruit fly control programs. Entomopathogenic fungi could be effective when applied under the canopy of trees (Lezama-Gutierrez *et al.,* 2000; Garrido-Jurado *et al.,* 2009). For instance, one soil treatment by bio-insecticide ‘‘BioGreen’’ based on *M. anisopliae* was sufficient for the suppression of the scarab beetle *Adoryphorus couloni* from the soil for a period of 5-10 years (Rath *et al.,* 1995). In the current study, probit analyses was not performed on *B. dorsalis* pupa due to the lower level of mortality, which indicates higher doses than used in the experiment are required to make the test.

Conclusion and Recommendations

The evaluated Ethiopian origin *B. bassiana* and *M. anisopliae* isolates were pathogenic to the larvae and pupae of *B. dorsalis*. The variations in virulence among those Ethiopian entomopathogenic isolates tested against the *B. dorsalis* larvae and pupae strongly suggests the possibility of obtaining diverse and more potent isolates from collections and screening conducted on different agro-ecological zones of the country and different insect species. The effective isolates

in this study need to be further evaluated under field conditions to be recommended as an integral part of fruit fly IPM in Ethiopia.

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References

Aketarawong N, Guglielmino CR, Karam N, Falchetto M, Manni M, Scolari F and Malacrida AR. 2014. The oriental fruit fly *Bactrocera dorsalis s.s.* in East Asia: Disentangling the different force promoting the invasion and shaping the genetic makeup of populations. *Genetica*142: 201–213.

Barson G. 1997. Laboratory evaluation of *Beauveria bassiana*, a pathogen of the larval stage of the large Elm bark beetle, *Scolytus scolytus*. *Journal of Invertebrate Pathology*. 29 (3): 361-366.

Castillo MA, Moya P, Hernandez E and Fera EPY. 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. *Biological Control* 19:274–282.

California Department of Food and Agriculture (CDFA). 1993. Final programmatic environmental impact report: the exotic fruit fly eradication program utilizing male annihilation and allied methods. State Clearinghouse No. 90021212, Sacramento, CA.

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

De la Rosa, López WFL and Liedo P. 2002. *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. *Journal of Economic Entomology* 95: 36-43.

Dias NP, Zotti MJ, Montoya P, Carvalho IR and Nava DE. 2018. Fruit fly management research: A systematic review of monitoring and control tactics in the world. *Crop Protection* 112: 187–200.

Drew RAI and Hancock DL. 2004. Biogeography and Speciation in the Dacini (Diptera: Tephritidae: Dacinae). *Bishop Museum Bulletin Entomology* 12: 165–178.

Ekesi S and Billah MK. 2006. A field guide to the management of economically important Tephritid fruit flies in Africa. ICIPE Science Press Nairobi, Kenya

Ekesi S, Dimbi S and Maniania NK. 2007. The role of entomopathogenic fungi in the integrated management of tephritid fruit flies (Diptera: Tephritidae) with emphasis on species occurring in Africa. In: Ekesi S, Maniania NK (eds) Use

of entomopathogenic fungi in biological pest management research. SignPost, Kerala, pp 239–274.

Ekesi S, Maniania NK, Ampong-Nyarko K. and Onu I. 1999. Effect of intercropping cowpea with maize on the performance of *Metarhizium anisopliae* against the legume flower thrips, *Megalurothrips sjostedti* and predators. *Environmental Entomology* 28: 1154 – 116.

Ekesi S, Maniania NK and Lux SA. 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: 7-17.

Ekesi S, Nderitu PW and Rwomushana I. 2006. Field infestation, life history and demographic parameters of the fruit fly *Bactrocera invadens* (Diptera: Tephritidae) in Africa. *Bulletin of Entomological Research* 96: 379-386.

Erler F and Ates AO. 2015. Potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. *Journal of Insect Science* 15(1): 44.

Garrido-Jurado I, Torrent J, Barron V, Corpas A, Quesada-Moraga E. 2011. Soil properties affect the availability, movement, and virulence of entomopathogenic fungi conidia against puparia of *Ceratitis capitata* (Diptera: Tephritidae). *Biological Control* 58: 277–285.

Habtegebriel B, Getu E, Dawd D, Seyoum E, Atnafu G, Khamis F, Hilbur Y, Ekesi S and Larsson MC. 2016. Molecular characterization and evaluation of indigenous entomopathogenic fungal isolates against Sorghum Chafer, *Pachnoda interrupta* (Olivier) in Ethiopia. *Journal of Entomology and Nematology* 8(5): 34-45.

Hooper GHS. 1987. Application of quality control procedures for large scale rearing of the Mediterranean fruit fly. *Entomologia Experimentalis et Applicata* 44:161- 16.

Imoulan A and Elmeziane A. 2014. Pathogenicity of *Beauveria bassiana* isolated from Moroccan Argan forests soil against larvae of *Ceratitis capitata* (Diptera: Tephritidae) in laboratory conditions. *World Journal of Microbiology and Biotechnol*gy 30: 959–965.

Inglis, GD, Enkerli J, and Goettel MS. 2012. Laboratory techniques used for entomopathogenic fungi: hypocreales, pp. 189–253. *In* L.A. Lacey (ed.), Manual of techniques in invertebrate pathology. Academic Press, London, United Kingdom.

Irigaray FJS, Vicente M and Ignacio PM. 2002. The Entomopathogenic fungus *Beauveria bassiana* and its compatibility with Triflumuron: Effects on the two spotted spider mite, *Tetranychus urticae. Biological Control* 26 (2): 168-173.

Kaaya GP and Munyinyi DM. 1995. Biocontrol potential of the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* for Tsetse Flies

(*Glossina*spp.) at developmental sites. *Journal of Invertebrate Pathology* 66 (3): 237-241.

Kalloo G. 2005. Fruit fly research in India: Pest management in horticultural ecosystems. *Pakistan Journal of Biological Sciences* 10 (20): 3656-3661.

Kassa A, Zimmermann G, Stephan D and Vidal S. 2002. Susceptibility of *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to Entomopathogenic Fungi from Ethiopia. *Biocontrol Science and Technology* 12(6): 727-736.

Kriticos DJ, Leriche A, Palmer DJ, Cook DC, Brockerhoff EG, Stephens AEA and Watt MS. 2013. Linking climate suitability, spread rates and host- impact when estimating the potential costs of invasive pests. *PLoS ONE, 8*.

Lezama-Gutierrez R, Trujillo-De LA, Molina-Ocha J and Rebolledo-Dominguez O. 2000. Virulence of *Metarhizium anisopliae* on *Anastrepha ludens*: Laboratory and field trials. *Journal of Economic Entomology* 93: 1080–1084.

Lux SA, Overholt W and Kimani S. 1998. Economic role and distribution of fruit flies, in 1995-1997 ICIPE Annual Scientific Report, ICIPE Science Press, Nairobi pp. 29- 30.

Manrakhan A, Venter JH and Hattingh V. 2015. The progressive invasion of *Bactrocera dorsalis* (Diptera: Tephritidae) in South Africa. *Biological Invasions* 17: 2803–2809.

Mar TT and Lumyong S. 2012. Evaluation of effective entomopathogenic fungi to fruit fly pupa, *Bactrocera* spp. and their antimicrobial activity. *Chiang Mai Journal of Science* 39(3): 464-477.

Negash R, Dawd D and Azerefegne F. 2017. Efficacy of Ethiopian *Beauveria bassiana* and *Metarhizium anisopliae* Isolates on Spotted Spider Mites, *Tetranychus urticae* (Acari: Tetranychidae) under Laboratory Conditions. *Ethiopia Journal of Agricultural Science* 27(2): 61-71.

Onsongo SK, Gichimu BM, Akutse KS, Dubois T and Mohamed SA. 2019. Performance of three isolates of *Metarhizium anisopliae* and their virulence against *Zeugodacus cucurbitae* under different temperature regimes, with global extrapolation of their efficiency. *Insects* 10(9): 270. doi: 10.3390/insects10090270.

Rath AC, Koen TB, Anderson GC and Worledge D. 1995. Long term efficacy of the entomopathogenic fungus, *Metarhizium anisopliae* against the subterranean scarab, *Adoryphorus couloni*. *Australian Journal of Agricultural Research* 46: 429–440.

Roessler Y. 1989. Insecticidal bait and cover sprays, in fruit flies: their biology, natural enemies and control (Robinson, A.S. & Hoopher, G., Eds) . World Crop Pests. Elsevier, Amsterdam pp. 329 – 336.

Schneider-Orelli O. 1947. Entomologisches Praktikum. Sauerlander, Aarau, Switzerland.

Shiberu T, Negeri M and Selvaraj T. 2013. Evaluation of some botanicals and entomopathogenic fungi for the control of onion thrips (*Thrips tabaci* L.) in west Showa, Ethiopia. *Journal of Plant Pathology and Microbiology* 4 (1): 1-7.

Sookar P, Bhagwant S and Awuor OE. 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.

SPSS. 2015. IBM SPSS Statistics for Windows (Version 23.0). IBM Corp, Armonk Chicago, IL.

Tadele T and Pringle KL. 2004. Evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* for controlling *Chilo partellus* (Lepidoptera: Crambidae) in maize. *Biocontrol Science and Technology* 14(8): 849-853.

United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ). (https://coffhi.cphst.org)

Wan XW, Liu YH and Zhang B. 2012. Invasion history of the oriental fruit fly, *Bactrocera dorsalis*, in the Pacific- Asia region: Two main invasion routes. *PLoS ONE* 7(5): e36176. https://doi.org/10.1371/journal.pone.0036176.

Wekesa VM, Knapp NK, Maniania and HI Boga. 2006. Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on mortality, fecundity and egg fertility of *Tetranychus evansi. Journal of Applied Entomology* 130 (3): 155 – 159.

White IM and Elson-Harris MM. 1992. Fruit flies of economic importance: Their identifications and bionomics. CAB International, Wallingford, Oxon, 601pp.