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In-vitro and *In-vivo* Evaluation of Entomopathogenic Fungi against the Maize Stem Borer (*Buseola fusca*)

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Abstract

The maize stem borer (*Buseola fusca*) is a devastating pest of maize causing up to 100% yield loss in Ethiopia. Biological control can be an important component of maize stem borer integrated pest management. Thirteen *Beauveria bassiana* and fourteen *Metarhizium anisopliae* isolates of native entomopathogenic fungi (EPF) were evaluated for their potential as biological control agents against the maize stem borer under laboratory and pot culture. In the first screening, a single dose of conidial suspension containing $1x10^8$ conidia per ml of each isolate was used to inoculate 10 *B. fusca* larvae. Selected isolates from both spp. were then tested for dose-response using three conidial concentrations (1×10^7, 1×10^8 and 1×10^9 conidia/ml). Furthermore, one isolate from each of the Metarhizium and Beauveria spp. which induced the highest mortality at the highest concentration and was tested in pot culture under lath house conditions. A completely randomized design and a randomized complete block design with three replications were used for the laboratory and pot experiments respectively. Three *M. anisopliae* isolates (PPRC-51, PPRC2, M1) and a *B. bassiana* isolate (B1) caused significantly higher ($p = 0.0001$) percentage mortality (87%, 70%, 87% and 87% respectively). In a dose-response test, isolates M1 and B1 showed significantly higher mortality (100%) within 72hrs at a concentration of 1x 109conidia/ml and were tested in the lath house pot experiment. Results in the first pot experiment showed that M1, B1 and Karte 5% EC caused 49%, 48%, 76% mortality in ten days significantly differing from the untreated check ($p = 0.004$) and in second experiment M1, B1 and Karte 5% EC caused 49%, 48%, 76% mortality in fifteen days significantly differing from the untreated check ($p = 0.037$) while being at par with each other. Further tests are recommended to verify the promising results under field conditions.

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Keywords

Stem borer, *Metarhizium anisopliae*, *Beauveria bassiana*, Biological control, Karte

Introduction

Maize is an important cereal crop grown both in the highlands and lowlands of Ethiopia ranking first in production with a total production of 5670 metric tons (CSA, 2014). The yield and quality of maize is affected by different biotic and abiotic factors.

Maize stem borer is one of the major biotic constraints in successful maize and sorghum production worldwide (Pingali, 2001; James, 2003), particularly in Asia and Africa (Siddiqui and Marwaha, 1993; Arabjafari and Jalali, 2007). Aberham *et al.*, (1998) reported up to 100% infestation level of maize stem borer on maize and

sorghum in most of the areas of Ethiopia. It has been reported to cause severe losses in maize crop throughout its geographical distribution including Ethiopia. Yield losses of 24-75% have been reported by the attack of this pest alone (Kumar and Mihm, 1995; Kumar, 2002; Khan, 1983). Moreover yield losses caused by stem borers in Africa could be as high as 80% for maize (Van den Berg 2009) and as much as 88% in sorghum (Seshu Reddy, 1988; Bhat and Baba, 2007).

Several chemical pesticides are used to control the stem borer but these chemicals are hazardous to the environment and humans besides being expensive to the subsistence farmers.

A key component of crop protection in modern agriculture is exploitation of Hyphomycetous fungi for the control of invertebrate pests and diseases. Fungal biological control is an exciting and rapidly developing research area with implications for plant productivity, human health and food production. Entomopathogenic fungi (EPF) and several taxa of the other fungi have demonstrated excellent suppression of insect pests in green house conditions (McCoy *et al*., 1988; Ferron *et al*., 1991; Inglis *et al*., 2001; Mascarin MG and Jaronski TS., 2016) and (Meyling and Eilenberg, 2006). *Beauveria bassiana* has recently been registered against array of greenhouse pests, including stem borers, aphids, thrips, white flies and spider mites (Shah and Goettel, 1999).

Use of entomopathogenic fungi (EPF) can be an important alternative to reduce the impact of pesticides on the environment while boosting maize production. Therefore, the objective of this study was to find a native entomopathogenic fungi bio-agent against maize stem borer, *B. fusca*

Materials and Methods

Mass rearing of *B. fusca*

Maize was planted both in glasshouse for the establishment of *B. fusca*. Larvae and pupae were collected from Ambo to Addis Alem areas and placed in rectangular cage of size having 30x30x30x30 cm, made from glass and have one side opening and the opening was covered by Muslim cloth: only inter one hand and putted maize stem borer. Mass reared at Ambo Agricultural Research Center, Plant Protection laboratory using chopped maize seedlings as a feed (Leul *et al*., 2009; Tesfaye *et al.*, 2012).

Preparation of fungal isolates

To produce inocula for experiments, slant cultures of different isolates from the collections was sub-cultured on to Sabouraud's dextrose agar with yeast extract (SDAY). Cultures were incubated at 27 ^{0}C and 75 % RH for ten days. The surface of ten-day old cultures was scrapped with a sterile scalpel and suspended in aqueous solution of 0.01% Tween 80. The fungal suspension was vortexes for one minute to break up the conidial chains or clumps and filtered through several layers of sterile cheesecloth to remove mycelia. The dose of conidia in the filtrate was estimated using haemocytometer under a light microscope (40 x magnifications).

Bioassay experiment

The isolate tested for screening experiment were PPRC-5, PPRC-67, PPRC-2, M2, DLCO-131 MEZ, PPRC-19, ICIPE-30, PPRC-27, M1, M3,AANK2B-5, AANK6B-1 and PPRC-60 from *Metarhizium* species and B1, ZG-1, ZG-2, ZG-3, 9609, F1115B, DLCO-73, F11161B, 9609, B-1, B-2, 9604 and PPRC-56 *Beauveria* species. All isolates used for this experiment were supplied by the Ethiopian Institute of Agricultural Research (EIAR), Ambo Agricultural Research Center Plant Protection Laboratory.

Each isolate was grown on SDYA and suspended in sterile distilled water having 0.01% Tween 80 to break up the conidial chains. The number of conidia was estimated with haemocytometer and adjusted to 1.0 x 10^8 concentrations per ml of conidial.

Dose experiment

Four potential Entomopathogenic fungi isolates, namely M1, PPRC-51 and PPRC-2 (*Metarhizium* species) B1 *Beauveria* species) were used for this experiment. The isolates were selected based on the observed potential in the screening experiment. For each isolate, aqueous suspension containing 1.0 x 10^7, 1.0 x 10^8 and 1.0 x 10^9 conidia ml^{-1} were used for inoculation and for the control group only sterilized distilled water was used. Ten 3rd instars of larvae of *B. fusca* were transferred to a Petridish with young chopped maize seedling. Each suspension was sprayed with half ml of suspension of isolates having a concentration of 1.0 x 10^7, 1.0 x 10^8 and 1.0 x 10^9 conidia ml^{-1}. Each treatment was replicated four times. After spraying, all larvae on Petridishes were incubated at 27 ^{0}C, 70 ± 5% RH, photoperiod of 12:12h day and night; and examined daily. Mortality data were collected, starting from 24h after inoculation.

Pot experiment

The experiment was conducted at Ambo Agricultural Research Center inside plant protection lath-house for two consecutive cropping seasons. For this experiment, *Jibat* cultivar of maize was used and five maize seeds were planted in each pot (21cm diameter and 19 cm height). The pots were initially filled with composition of black soil, compost and sand at a proportion of 2:1:1 and watered at three days interval. Urea at a rate of 0.52 g per pot will be applied at one and half months of age. Concentration was used the one which tested and selected in dose experiment. (i.e. each isolate at 1 X 10^8 ml^{-1} containing 0.01 % Tween 80). Controls will be treated with sterilized distilled water containing 0.01 % Tween 80 and second to third larval instars of *B. fusca* were inoculated in to the maize seedlings. After four hours of larval inoculation, treatments was applied using hand held sprayers. Each treated pot plants was placed in the lath-house in separate cages. The experiment was laid out in RCBD with four replications.

Results and Discussions

For bioassay experiment

Ten healthy larva of maize stem borer was used for each Petridis and bioassay test was made using 27 *Metarhizium* and *Beauveria* species by adjusting the concentration to $1x10^8$ conidia per ml. For each isolate an aqueous suspension containing 1.0 x 10^8 conidia ml^{-1} was prepared in 0.01% Tween 80. Sterile distilled water with 0.01% Tween 80 was used for the control treatment.

It was observed that there were significant variations in the percentage mortality among the 27 EPF isolates tested PPRC-51(86.64%), PPRC-2 (70%) M1 (86.64%) and B1 (86.64%) incurred highest mortality in ten days after treatment application, respectively, as compared to the others isolates. The other 23 isolates also kill 9.96 to 36.93 percent of MSB larvae. (Table 1)

Four *EPF* species (PPRC-2, PPRC-51, B1, and M1) with better performance were selected and further laboratory and greenhouse experiment was done.

LT_{50} data

Based on, the cumulative mortality over ten days it can be seen that four of the isolates of *Metarhizium* and *Beauveria* species gave more than 50% control of the larval population of *B. fusca.* The highest virulence was recorded from fungal isolates M-1, B-1, PPRC-51 and PPRC-2. Though all the isolates induced mortality, the single dose time mortality experiment indicated significant differences in LT_{50} values among isolates. The mortality rates of the isolates were broadly correlated with LT_{50}. The isolates which had the least LT_{50} values showed the highest mortality rates and vice versa. The LT_{50} values ranged from 1.68 to 5.1 days. The most, effective and virulent isolates had LT_{50} values M-1(1.68 days), B-1(2.00 days), PPRC-2(3.2days) and PPRC-51(5.1days), respectively (Table-2). This was clearly shown that in addition to mortality, LT_{50} values can effectively measure the virulence of the isolates. The activity of the immune system of the host and the fungal response are likely to be important factors determining virulence (Moorehouse *et al*., 1993). The development of fungal pathogen within hosts can be influenced not only by immune reaction of the host but also indirectly by the hosts diet (Tadele, 2003).

Dose experiment

The experiment was conducted using four potential fungal isolates, namely PPRC-51, PPRC-2, M1 and B1. The isolates were selected based on the observed potential in the screening or bioassay experiment above and using three different conidial concentration levels ($1x10^7$, $1x10^8$ and $1x10^9$ conidia per ml). For each isolate an aqueous suspension containing $1x10^7$, $1x10^8$ and $1x10^9$ conidia ml^{-1} was prepared in 0.01% Tween 80. Sterile distilled water with 0.01% Tween 80 was used for the control treatment.

All the tested EPF isolates caused significantly higher larval mortality as their concentration and duration increased. The EPF isolate M1 and B1 produced significantly superior mortality at the higher concentration (1 x 10^8 and 1 x 10^9 conidia ml^{-1}) in 24, 48 and 72hrs after treatment applications. (Table-3)

Two *EPF* species (B1 and M1) with better performance were selected for further greenhouse experiment is underway.

Pot experiment

The experiment was conducted using the two most virulent EPF isolates, namely M1, B1, Karate (Standard chemical) and including untreated check indicated that there was no significant difference in mortality among isolates inoculated according to the statics but when we compared with control there is significant differences at ten and fifteen days after treatment application (Table 4).The least mortality was recorded in the untreated control even ten days after treatment application.

Table.1 Percentage mortality of the *B. fusca* larvae treated with different fungal isolates of *Beauveria* and *Metarhizium* spp at the rate of $1x10^8$ conidia ml^{-1}

No	Isolates	%Mortality10 DAT ±SE	No	Isolates	%Mortality 10 DAT ±SE
1	M-1	86.64 ±1.28a	15	ICIPE-30	31.93±0.85bc
2	B-1	86.64 ±1.28a	16	PPRC-19	31.93±0.85bc
3	PPRC-51	86.64 ±1.28a	17	AANK2B-5	30.55±1.69bc
4	PPRC-2	70±1.10ab	18	ZG-2	27.71±0.47c
5	ZG-1	36.93±1.02bc	19	9604	27.71±0.47c
6	PPRC-67	36.93 ±1.02bc	20	DLCO#131	27.71±1.66c
7	PPRC-60	36.93±1.02bc	21	PPRC-56	22.48±1.46c
8	Dlco#73	36.93±1.02bc	22	9609	22.48±1.46c
9	F1115B	36.15 ±0.77bc	23	B-2	19.41±1.31e
10	M-3	36.15 ±0.77bc	24	F11161B	18.26± 1.26e
11	ZG-3	32.71 ±1.10bc	25	PPRC-27	18.02±1.78e
12	MZE	32.47±1.93bc	26	AANK6B-1	14.18±1.55f
13	B-3	31.93±0.85bc	27	AANK6A-2	9.96±1.26f
14	M-2	31.93±0.85bc			

Table.1 LT_{50} of *B. fusca larvae* treated with 5 fungal isolates of *Beauveria* and *Metarhizium* species at the rate of $1x\ 10^8$ conidia ml^{-1}

Isolates	**LT_{50} days**	**95% Confidence Limits**		**Slope**
		lower	**Upper**	
M-1	1.68b	1.12	2.16	3.84a
B-1	2.00b	0.91	2.55	4.58a
PPRC-51	5.1c	2.07	5.98	2.95ab
PPRC-2	3.2c	1.63	3.60	2.92ab
Control	39.27a	-	-	1.03b
CV	167.92			
LSD	28.95			

* Values followed by the same letter in the same column do not differ significantly

Table.2 Percentage mortality of maize stem borer treated with 4 EPF isolates in dose experiment

Treatment	Doses(IJ/ml)	Mean 3rd Instars larvae of DBM Mortality ±SD		
		24hrs	48hrs	72hrs
PPRC-2	1.0 x 10^9conidia ml^{-1}	22.5^{b}	66.00^{ab}	84.2^{b}
	1.0 x 10^8conidia ml^{-1}	26.33^{b}	45.22^{c}	$66.^{7cd}$
	1.0 x 10^7conidia ml^{-1}	10^{cd}	37.78^{d}	47.1^{e}
PPRC51	1.0 x 10^9conidia ml^{-1}	23.7^{b}	60.00^{b}	80.00^{b}
	1.0 x 10^8conidia ml^{-1}	22^{b}	44.4^{c}	73.6^{c}
	1.0 x 10^7conidia ml^{-1}	10^{cd}	36.6^{d}	48.6^{e}
B1	1.0 x 10^9conidia ml^{-1}	36.2^{a}	80.00^{a}	100.00^{a}
	1.0 x 10^8conidia ml^{-1}	36.2^{a}	63.5^{ab}	97.00^{a}
	1.0 x 10^7conidia ml^{-1}	22^{b}	50.00^{c}	54.3^{cde}
M1	1.0 x 10^9conidia ml^{-1}	38.2^{a}	76.2^{a}	100.00^{a}
	1.0 x 10^8conidia ml^{-1}	36.2^{a}	62.5^{ab}	100.00a
	1.0 x 10^7conidia ml^{-1}	22^{b}	46.00^{c}	$64.^{17cd}$
Control	0 (Control)	0^{d}	0^{e}	10^{f}
CV		8.15	8.57	6.1

Note: Values followed by the same letter in the same column do not differ significantly

Table.3 Mortality of *B. fusca* larvae treated with 2 fungal isolates at the rate of 1x 10^9 conidia ml^{-1} and Karate 5% EC in pot planted maize seedlings

Treatment	First Experiment Mortality 10DAT ± SE	Second Experiment Mortality 15DAT ± SE
Karate	76.22a	81.25 a
M-1	48.75ab	51.5 ab
B-1	47.50ab	44.56 ab
Untreated	0	10 b
CV	47.97	53.61
LSD	31.35	37.71
F-value	9.30	4.33
P-value	0.0041	0.0377

NB: DAT mean data after treatment application

Fig.1A and B Native *Beauveria* and *Metarhizium* isolates growing on Sabouraud Dextrose Agar (SDA)

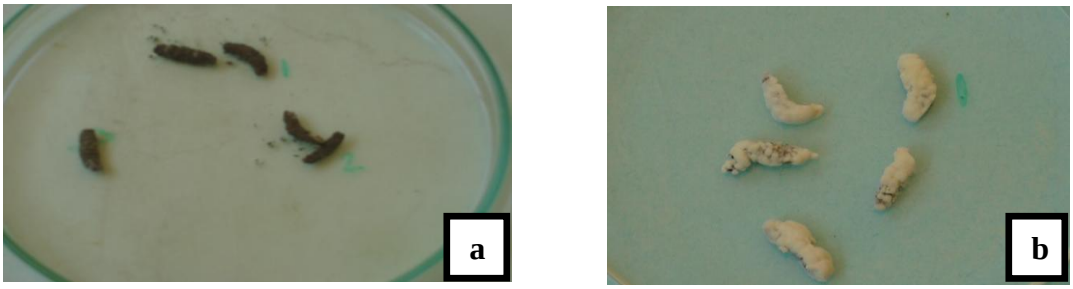

Fig.2 a and b *B. fusca* larvae killed by native *Beauveria* and *Metarhizium* isolates (a) M1 and (b) B1

Conclusions and Recommendation are as follows:

The study has showed that both EPF isolates have a potential isolates that may be exploited for the sustainable management of maize stem borer larva along with other suitable strategies in integrated pest management. Future research works experiments are to test the efficacy of the strains under field conditions and techniques for mass production, appropriate formulation to keep the quality, large scale application are needed.

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