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Short communication

EFFECT OF DRY CONIDIA FORMULATIONS OF *METARHIZIUM ANISOPLIAE* ON *SITOPHILUS ZEAMAIS* (COLEOPTERA: CURCULIONIDAE) MORTALITY, PROGENY EMMERGENCE AND MAIZE GRAIN DAMAGE

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ABSTRACT: The efficacy of dry conidia formulation of *Metharhizium anisopliae* (PPRC-2) produced on locally available grains was assessed against *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. Conidial suspension was inoculated into glass jars containing 200 grams of maize, rice, wheat and sorghum grains sterilized and were incubated at 27°C and 70 ± 5% RH for 21days. Each grain substrate was air-dried and grounded to powder using a sterile coffee grinder. The powdered dry conidia were applied to the maize grain infested with *S. zeamais*. High mortality to the maize weevil was recorded 7-days after the fungus grown on rice (91%) and maize (84%) substrates were applied. The number of F1 progeny emerged was 0, 0.5 and 1, for primiphos methyl, rice and maize, respectively. Percentage grain protection ranged from 41% to 83% for fungal treatments. The number of grain damaged and percent grain weight loss were reduced in dry conidia treated maize grain than in the control. Therefore, the use of *M. anisopliae* holds promising prospect in managing *S. zeamais*.

Keywords: Exposure method, formulation, maize, *Metarhizium anisopliae*, *Sitophilus zeamais*

INTRODUCTION

Post-harvest losses due to storage pests have been recognized as an increasingly important problem in Africa (Markham *et al.*, 1994). The maize weevil, *Sitophilus zeamais* Motsch, is one of the most serious cosmopolitan pests of stored cereal grains, especially of maize (*Zea mays* L.), in tropical and sub-tropical regions (Throne, 1994). Infestation by this weevil commences in the field (Girma Demissie, 2006), but most damage is done during storage. Damaged grain has reduced nutritional value, low percent germination, reduced weight and market value. Alternative locally available and effective methods for reducing *S. zeamais* damage are needed (Danho *et al.*, 2002).

Synthetic insecticides have been widely used for the control of stored grain pests, particularly *S. zeamais*. The widespread use of such insecticides is of global concern with respect to environmental hazards, insecticide resistance development, chemical residues in foodstuffs, side effects on non-target organisms and the associated high costs (Cherry *et al.*, 2005). However, the increased public awareness and concern for environmental safety has directed research to the development of alternative control strategies such as the use of entomopathogenic fungi. In preliminary studies, *S. zeamais* adults sprayed with *M. anisopliae* conidial suspension resulted in more than 95% mortality (Addis Teshome and Tadele Tefera, 2009). However, the wide scale utilization of entomopathogens requires cost effective mass production and standardization. Mass production of dry conidia of *Beauveria bassiana* and *M. anisopliae* on cereal grains was reported effective in managing insect pests (Lord, 2001, Mamuye Haddis and Dawit Abate, 2002). Many fungi have the advantage of relatively easy mass production on inexpensive natural substrate in systems that are highly appropriate to small scale farmers making their commercialization and wider application easier. However, numerous factors influence the virulence of a pathogen against insect pests including genetics and growth substrates (Holder and Keyhani, 2005).

Hence, this study was conducted to determine the effect of dry conidia formulation of *M. anisopliae* from different grain substrates on *S. zeamais* mortality, F_1 progeny emergence and maize grain damage.

MATERIALS AND METHODS

Rearing of maize weevil

Unsexed adults of *S. zeamais* were reared on dried whole maize kernels, variety BH-540. The initial grain moisture content and seed viability were 12.5% and 100%, respectively. Grains were stored at –20°C for 21 days to eliminate natural unwanted infestations. After two weeks acclimatization the grain was used for experiment. 1500 adults of *S. zeamais* of mixed sex and different ages were introduced in 10 five-liter plastic containers each containing 4 kg of maize and were maintained under the laboratory conditions (27±2°C, 70 ± 5% RH). The top of each plastic container was covered with nylon mesh fastened tightly with elastic bands. The original adults were allowed for two-week oviposition periods before all adults were removed and transferred to fresh maize grain in another plastic container. Each plastic container, where the adult maize weevils oviposited, was kept for progeny emergence. After thirty-five days, progeny weevils emerged were removed daily and those emerged on the same day were transferred to fresh grain in plastic containers until sufficient number of such weevils were obtained. These newly emerged adults (males and females) of the same age were used in the experiment.

Conidia production

M. anisopliae isolate (PPRC-2) was isolated from its host sorghum chafer (*Pachnoda interrupta*). Initial cultures were stored at 4°C and sub-culturing was made for the present work. Conidia of PPRC-2 were mass-produced on cracked wheat, cracked maize, rice and sorghum grains. Water was added at a ratio of 1:0.5 (grain: water). Two hundred grams of each grain was sterilized in autoclavable polythene bags for 1hour at 121°C. The grain substrates were then transferred to 1L glass jars and separately inoculated with 20 ml of conidial suspension containing 1×10^8 conidia/ml. After twenty-one days, the substrates were removed and air-dried on a clean bench with a continuous airflow at room temperature. The substrates were then grounded to powder and the content was sieved through 50-µm mesh and the resulting dustable powder (DP) was stored in glass bottle at 4°C. The number of conidia in a gram of DP was determined by using a haemocytometer. The numbers of conidia produced were 2.55×10^8, 3.17×10^8, 2.35×10^8, and 4.2×10^8 conidia g^{-1} for cracked wheat, cracked maize, sorghum and rice, respectively.

Efficacy test

Two hundred and fifty grams of cleaned and disinfested maize grains were dispensed into twenty four glass jars (250 ml capacity). The moisture content were adjusted to 12.5%. Two grams of each DP formulation were mixed with the maize grains by gently shaking for 5 minutes. Thirty randomly picked maize weevils were then introduced into each jar. The jars were covered with perforated lid and maintained at 27°C and 70 ± 5% RH. Control insects were treated with sterile distilled water and Primiphos methyl 2% dust. The treatments were arranged in a randomized complete block design with four replicates.

Data collected

Mortality: 48 hours after treatment application, dead insects were counted and removed for seven days. Cadavers were washed three times with sterile distilled water and placed in a humidity chamber and incubated at 27°C. All live insects were removed after 7 days.

F_1 progeny emergence, grain damage and weight loss: After removing both live and dead insects, the treated grains were kept for 45 days. Emerging F_1 progenies were then removed and counted. The effectiveness (percent protection) of the treatment against maize weevils was calculated using the formula (El-Ghar *et al.*, 1987),

$$\%\,protection = \frac{(Total\ F1\ progeny\ in\ control - Total\ F1\ progeny\ in\ treatment)}{(Total\ F1\ progeny\ in\ control)} * 100$$

The number and weight of damaged and undamaged grains were recorded. Percentage grain weight loss was calculated using the count and weigh method (Boxall, 1986).

$$Grain\ weight\ loss\ (\%) = \frac{[(WU * ND) - (WD * NU)]}{[WU * (ND + NU)]} \times 100$$

where:

WU = weight of undamaged grains,
ND = number of damaged grains,
WD = weight of damaged grains, and
NU = number of undamaged grains.

Seed germination: seed germination was tested using 100 randomly picked seeds from undamaged grains. The seeds were placed on a moistened filter paper in plastic Petri dishes and the number of germinated seeds was recorded after ten days. The treatments were arranged in completely randomized design with three replications.

Data analysis

Weevil mortality was adjusted for control mortality (Abbot, 1925) and subjected to analysis of variance. Data on grain damage, weight loss, and seed germination were expressed as a percentage of the total number of grains in each replicate. All data were arcsine transformed before subjected to analysis of variance (ANOVA) (SAS, 1999) in order to stabilize the variance. Significant differences between treatment means were compared at P<0.05 using least significance difference (LSD) test.

RESULTS

There were significant differences (F=331.185, df=5, P<0.001) between the conidia grown on different grain substrates in causing mortality to the maize weevil (Table 1). In all the grain substrates mortality occurred four days after treatment application and mortality increased over time. Conidia grown on rice and maize substrates caused 91.6 and 84.1% mortality, respectively, seven days after treatment application. However, conidia grown on wheat and sorghum induced 34.1% and 38.3% mortality, respectively. Primiphos methyl caused the highest mortality (100%), two-days after treatment application. Significant differences (F = 9.67, df = 5, P <0.001) were also observed among the treatments in number of F_1 progeny emerged (Table 2). The number of F_1 progeny emerged was very low for Primiphos methyl, rice and maize treatments than for the remaining treatments. The number of F_1 progeny emerged was 0, 0.5 and 1, for primiphos methyl, rice and maize, respectively. Percentage grain protection ranged from 41% to 83% for fungal treatments while it was 100% for primiphos methyl (Table 2). Significant differences were also observed among the treatments in number of damaged grains (F = 16.365, df = 5, P < 0.001) and percent grain weight loss (F = 4.059, df = 5, P < 0.05) (Table 2). The highest proportion of damaged grains (8.25%) was recorded in the untreated check. Percent grain damage ranged from 1.25% to 3.5% while no damage was recorded for promiphos methyl treated grains. Weight losses of 0%, 0.73% and 0.71% were recorded for Primiphos methyl, rice and maize substrates treated grains, respectively (Table 2). The treatments did not affect seed viability as there was 100% seed germination in all treatments.

Table 1. Mean (±SE) percent mortality of adult maize weevils exposed for seven days to *M. anisopliea* (isolate PRC-2) grown on different cereal grains.

Treatments	% Cumulative Mortality (days)			
	2	4	6	7
Cracked wheat	0.0[b]	22.5±1.59[c]	34.17±2.85[c]	34.17±2.85[c]
Cracked maize	0.0[b]	40.0±4.3[b]	84.17±0.833[b]	84.17±0.833[b]
Sorghum	0.0[b]	22.5±2.84[c]	38.33±7.39[c]	38.33±7.39[c]
Rice	0.0[b]	53.3±3.04[a]	91.67±3.19[ab]	91.67±3.19[ab]
Primiphos methyl	100.00[a]	**	**	**
Control	0.0[b]	0.0[d]	0.0[d]	0.0[d]

Means followed by the same letter in the same column do not differ significantly (P<0.05) using the Least Significance Difference (LSD) Test

Table 2. Mean (±SE) number of progeny emergence, proportion of grain damaged, percentage weight loss and seed germination in maize grains

Treatment (substrates)	No. of progeny emerged	% Protection	Proportion of grain damaged	% weight loss	% seed germination
Cracked wheat	1.75±0.47[b]	41.6	3.25±0.48[b]	0.80±0.31[bc]	100 [a]
Cracked maize	1.00±0.41[bc]	66.6	2.50±0.288[b]	0.71±0.32[bc]	100 [a]
Sorghum	1.75±0.25[b]	41.6	3.50±0.96[b]	2.19±0.81[b]	100 [a]
Rice	0.50±0.29[c]	83.3	1.50±0.65[bc]	0.73±0.44[bc]	100 [a]
Primiphos methyl	0.0[c]	100	0.00[c]	0.00[c]	100 [a]
Control	3±0.41[a]		8.25±1.1[a]	2.69±0.7[a]	100 [a]

Note: Notations are the same as in Table 1.

DISCUSSION

The present study demonstrated the variation existing between different substrates for growth and sporulation of *M. anisopliae* PPRC-2 and its subsequent effects on mortality and progeny emergence. Mamuye Haddis and Dawit Abate (2002) reported *M. anisopliae* isolates MM and EE grew best on rice giving a yield of 1.42 x10^9 and 1.62 x10^9 spore/gram, respectively. Higher mortality of maize weevils were recorded for conidia treated grains than the control. The result is also similar with Hidalgo *et al.* (1998) who observed 100% mortality of *S. zeamais* fourteen days after application of unformulated dry conidia to maize seeds at 4 g/ kg. Akbar *et al.* (2004) observed 62.7% mortality of *T. castaneum* with 2700 mg/ kg of wheat. Padin *et al.* (2002) also reported wheat grains infested with *S. oryzae* without *B. bassiana* were significantly more damaged by weevils than grains treated with the fungus.

The results show that application of *M. anisopliae* reduced progeny emergence in maize weevils. However, progeny emergence in the treated substrates was not completely inhibited, except in primiphos methyl. This might be due to adult females of *Sitophilus* spp. laying their eggs inside the kernel, where larval development occurs which in turn enables the larvae to remain unexposed to the presence of fungi until reaching the adult stage (Rice and Cogburn, 1999). Cherry *et al.* (2005) and Bourassa *et al.* (2001) also reported that *B. bassiana* did not prevent oviposition by *C. maculatus* and *P. trancatus* or subsequent emergence of F_1 generation. Thorne and Lord (2004) reported the presence of *B. bassiana* affects the number of progeny produced by saw-toothed grain beetle in stored oats. The reduction of progeny emergence in the treated substrate is considered equally or even more important than parental mortality. Progeny emergence is an important method to know how chemicals reduce the population built up through time (Adane Kassa *et al.*, 1996). Fungal infection might cause a decrease in the rate of oviposition and fertilization due to decline in the female physiological state related to fungal colonization of tissue, toxin production and/or depletion of resource needed for vital egg production (Carey *et al.*, 1998; Blay and Yuval, 1999; Quesada-Moraga *et al.*, 2006). In this study treatments which caused the highest mortality and least progeny emergence had also a low percentage of grain weight loss. Several studies have shown that *M. anisopliae*, and other entomopathgenic fungi, exhibit pre-lethal anti feedant properties probably due to the so called Destruxins (Scholte *et al.*, 2006).

All the treatments in this study invariably had no effect on seed germination. Thus, there was 100% seed germination in all treatments indicating that fungal treated seeds can be used for planting. It can be concluded that *M. anisopliea* (PPRC-2) induced high mortality to *S. zeamais* and subsequently reduced gain damage and weight loss. Although this is only an initial investigation into the use of locally available grain substrates, it seems that such techniques to produce fungal isolates for managing *S. zeamais* can be used as an effective alternative pathogen based protection system with a great potential benefit to the environment. However, additional studies are needed to focus on conditions that increase efficacy and to understand the mechanism of horizontal transmission in extending the periods of impact. Further consideration in production and commercialization of entmopathogens include methods of maintenance of stock culture, large scale production, substrate formulation and storage.

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