

Research Article

Field level auto-inoculation of sorghum chafer, *Pachnoda interrupta* (Olivier) (Coleoptera: Scarabaedae) with *Metarhizium anisopliae* based microbial bio-control agents using locally affordable traps

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ABSTRACT: Sorghum chafer, *Pachnoda interrupta* (Olivier), is the most serious pest of sorghum in Ethiopia destroying the entire fields at the milk stage and causing up to 100% yield loss. Current control methods entirely depend on direct spraying and baiting with insecticides which does not provide long lasting control. Efficient biological control agents such as entomopathogenic fungi that can control the pest in the breeding sites need to be developed. Traps equipped with auto-inoculation devices are important alternative methods to spread entomopathogens into insect pest populations. Field studies on fungal auto-inoculation trap development from locally available materials conducted over three feeding and two mating seasons of *P. interrupta* resulted in two efficient auto-inoculation traps (AIT1 and AIT2) baited with a five compounds blend lure which were not significantly different in catch performance with the standard Japanese beetle trap. Two selected virulent isolates of *Metarhizium anisopliae* (PPRC51 and PPRC2) were tested for field efficacy using these two designs of locally affordable auto-inoculation traps loaded with 1gm of dry conidia. Using AIT1, PPRC51 and PPRC2 induced 41% and 40% field mortality respectively, on *P. interrupta* adults under high temperature and low relative humidity conditions, while highest field viability of the two isolates five days after application was 36 % and 40 % for PPRC51 and PPRC2, respectively. Based on the catch performance, field efficacy and viability data observed, the two AIT's are recommended for further development to be used with PPRC51 and PPRC2 for augmentation biological control in the pest's natural habitat as a component of integrated pest management against *P. interrupta*.

KEY WORDS: *Pachnoda interrupta*, biological control, *Metarhizium anisopliae*, spore viability, auto-inoculation

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INTRODUCTION

Sorghum chafer, *Pachnoda interrupta* (Olivier), is the most destructive pest of sorghum in Ethiopia destroying the entire fields at the milk stage (Tsedeke, 1988). During outbreaks, the pest can cause 70 –100% yield loss (Yitbarek and Hiwot, 2000). Current control methods of *P. interrupta* entirely depend on direct spraying and baiting with insecticides. However, since controlling of adult beetles through application of insecticides on scattered sorghum will not provide long lasting control, efficient biological control agents that can control the pest in the breeding sites need to be developed (Seneshaw and Mulugeta, 2000).

Traps equipped with auto-inoculation devices are important alternative methods to spread entomopathogens into insect pest populations. Auto-inoculation devices have been developed to infect coleopteran insects such as sap beetles (e.g. *Carpophilus lugubris* Murray) (Dowd and Vega, 2003), rhinoceros beetle (*Oryctes rhinoceros* (L.)) (Moslim *et al.*, 2011) and emerald ash borer (*Agrius planipennis* Fairmaire) (Lyons *et al.*, 2012) in a strategy that uses attraction, contamination and release of insect pests for biological control. Auto-inoculation traps (AIT) have also been used on non coleopteran insects like fruit flies (*Ceratitis cosyra* Walker, *C. fasciventris* Bezzi and

C. capitata (Weidemann)) (Dimbi *et al.*, 2003) and tsetse flies (*Glossina* spp.) (Maniana, 2002) offering new approaches for application of entomopathogenic fungi in the field for control of insects (Migiro *et al.*, 2010). Klein and Lacey (1999) have also demonstrated the possibility of fungal auto-dissemination to the Japanese beetle, *Popillia japonica* Newman, populations by modifying the standard Trece Catch Can Japanese beetle trap (JBT).

The use of auto-dissemination traps has been suggested for bio-control of several insect pests by many authors (Lacey *et al.*, 1994; Furlong *et al.*, 1995; Klein and Lacey, 1999; Dowd and Vega, 2003). In this technique, the target pest must be attracted to an auto-inoculation trap in substantial numbers and allowed to exit the trap after contamination with the fungal spores to horizontally transfer the inoculum to the populations elsewhere (Lyons *et al.*, 2012). Fungal auto-dissemination within a host population occurs as a result of activities and movements of the host (Scholte *et al.*, 2004). Auto-dissemination devices facilitate inoculation of insects with entomopathogens horizontally (within a generation) among con-specifics in the environment and vertically (between generations) within a species, between species and from a local scale on a single plant to a landscape after they have been attracted to contaminated chambers (Vega *et al.*, 2007; Jason *et al.*, 2010). Transmission which determines the rate of dissemination and potential of the pathogen (Steinkraus, 2006) can occur through direct contact between contaminated and uncontaminated individuals or indirectly via conidia that have been deposited on the substrate (Quesada-Moraga *et al.*, 2008).

Traps equipped with Auto-inoculation devices baited with attractant lures are important tools for application of entomopathogenic fungi in augmentation and inoculation pest management strategies and developing efficient auto inoculation trap (AIT) systems is a key in using these strategies for successful control especially in inaccessible breeding or overwintering larval and adult habitats (Klein and Lacey, 1999). Use of AIT to disseminate entomopathogenic fungi for pest control can also be regarded as a low-input approach in conditions where conventional control means raise economic feasibility questions as using chemical sprays can be costly (Dowd and Vega, 2003). Although fungal ecology in crop systems has been studied in attempts to assess their potential as myco-insecticides (Hesketh *et al.*, 2010), knowledge gaps still exist (Roy *et al.*, 2009). This necessitates assessment of the growth and virulence characteristics of candidate isolates under actual environmental conditions as a pre-requisite for successful development to myco-insecticides (Butt *et al.*, 2001; Kope *et al.*, 2008). Adults of *P. interrupta* are known to aggregate in plant hosts

such as sorghum and acacia trees but also aestivate in the soil in over wintering habitats (Welde-hawariat *et al.*, 2007; Bengtsson *et al.*, 2009). This behavior can be exploited for auto-dissemination of microbial bio-control agents such as entomopathogenic fungi to its natural breeding habitats in augmentation strategy for sustainable management of the pest. Use of auto-inoculation traps can facilitate the process of infection of beetles with virulent entomopathogenic fungi. The objectives of the experiments were therefore to develop an efficient trap equipped with fungus auto-inoculation device and to evaluate the field efficacy of selected *M. anisopliae* isolates in infecting adult *P. interrupta* beetles using the trap.

MATERIALS AND METHODS

Description of the study sites

The field experiments were conducted in two zones of the Amhara Regional State of Ethiopia. Two of the sites are located in Kewot district Rasa village at Lewtegn (09°57' N and 040°04' E) and at Ayele ager (09°55' N, 10°40' E) in North Showa zone of the Amhara Region, Ethiopia (Figure 1). These study sites are located 255 km northeast of Addis Ababa, at altitudinal range of 1300- 2600 m.a.s.l (meters above sea level). The area has a bimodal rainfall pattern with short rains observed between March and May and main rainy season between July and October. Unevenly distributed average annual rainfall of 500 to 700 mm and annual temperature range of 8°C to 40°C characterize the semi-arid ecological zone where the sites are located. Major crops grown in the area include: sorghum, teff, maize, mung beans and cowpea. Kewot district is one of the areas where *P. interrupta* is most prevalent. The third site is located in Bati district at Abuare village (10°57'N, 040°03'E; altitude 1383 m.a.s.l.) of the Oromia zone of the Amhara region with a distance of 355 km from Addis Ababa. These sites also have similar characteristics as the above mentioned sites. The distance between the two zones is approximately 150 km.

Lures

A blend of 5 compounds (Phenylacetaldehyde, 2,3-butanediol, Methylsalicylate, Eugenol, Isoamyl acetate) was used as an attractant during all the field experiments. One thousand micro-liters (1000µl) of each of the compounds was loaded on to a separate 4 ml glass vial (45 × 14.7 mm, clear, Skandinaviska GeneTec AB) dispenser with cotton roll (3.9 cm long and 0.9 cm in diameter, Top Dent®, Dental rolls) using micro pipette and mounted on one side of each of the traps (five vials per trap). The release rate of each of the compounds was adjusted to 0.5-1mg/hr or approximately 25mg/day and had longevity of at least 1 week.

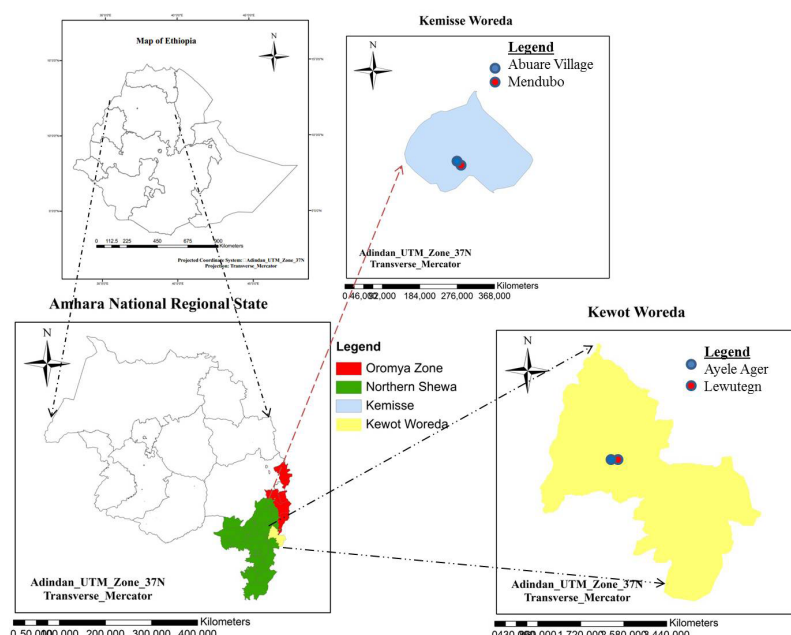


Fig. 1. Location of the study sites on the map of Amhara Regional State of Ethiopia.

* Woreda refers to a district in a zone (Map adapted from Getnet, 2014)

Evaluation of traps

Evaluation of traps for catch performance was conducted in October, 2012, October, 2013 and July, 2014. In October 2012, two designs of auto-inoculation traps (AITa and AITb) were developed from a big (5 liters) plastic water bottle with cut top (open plastic bucket trap). The designs of the auto-inoculation devices were developed from small (1/2 liters) plastic water bottles (Figure 2). The diameter of the canister opening was 15cm and had a height of 21cm. The two traps and the JBT were used as three treatments. In 2013 experiments also, three types of traps were used as treatments. The first one was a modified auto-inoculation trap (hereafter called AIT1) made of one 5 liter and two 1 liter plastic water containers fitted together at right angle. The second one was the same trap without the auto-inoculation device but fitted with a 2 liter plastic canister at the bottom. The modified traps were painted yellow and green as in the standard Japanese beetle trap (JBT). The third trap was the JBT which was used as a standard control trap. In July 2014, AIT1 and a further modified version (AIT2)

(Figure 3) were used as treatments with the JBT as a standard control trap. The pest population load in the trial areas was not at outbreak level. However, beetles were appearing in sufficient numbers in sorghum fields in the trial areas.

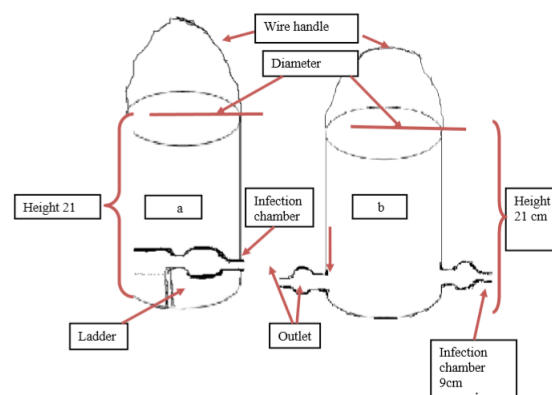


Fig. 2. Schematic sketch of the two auto-dissemination traps; single outlet (a); two outlets (b) used in the October 2012 experiments at Burka.

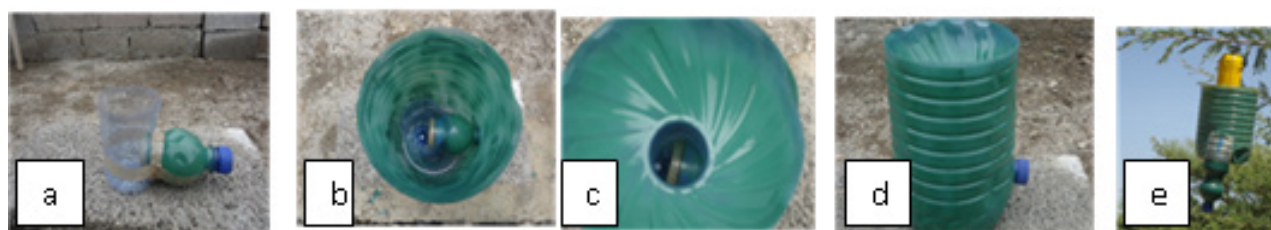


Fig. 3. Detailed parts of AIT2 a) Auto-inoculation chamber, b) Chamber fitted into canister, c) funnel put on top of canister, d) canister with protruding outlet, and e) trap with small water bottle suspended on top of canister and hang on a twig of an acacia tree.

Field efficacy of *Metarhizium anisopliae* isolates

Fungal isolates and application of fungal spores to traps

Evaluation of the field efficacy of the isolates was conducted in October 2012, October 2013 and July 2014. In 2012, the *M. anisopliae* isolate (PPRC51) was used. Pure spores of the isolate were harvested from three weeks old culture grown on quarter strength Sabouraud Dextrose Agar Yeast (SDAY) medium using sterile metal spatula and dried at 30° C in Petri-dishes for 12hrs to remove excess moisture (Lacey *et al.* 1994). The spores were then sealed in plastic bags and transported to the field in an ice box. Approximately 0.6gm of the spores was applied on the inner walls of each auto-inoculation device using sterile metal spatula and dispersed with a clean camel's hair brush. In the case of the trap with two auto-inoculation devices, 0.3gm of spores was applied to each device. Traps not treated with the fungus and the Japanese beetle trap (JBT) served as untreated and standard controls, respectively. In the 2013 experiments treatments consisted of spores of *M. anisopliae* isolate PPRC51 and sterilized wheat bran flour as a carrier at a ratio of 1: 2.5gm and 1:5gm in a pouch of muslin cloth. Auto-inoculation trap1 (AIT1) was used. The control treatment consisted of 5gm of carrier and trap with a pouch of muslin cloth only. In 2014, three *M. anisopliae* isolates (PPRC51, PPRC2 and IC69) were used. One gram of pure spores of each of the isolates mass produced on rice was applied to the auto-inoculation devices of each trap. The control AIT treatments were loaded with killed spores.

Collection bags were tied to the outlets of the traps to collect beetles which cross the inoculation devices. Traps were emptied everyday and collected beetles were separately kept in labeled Petri-dishes containing filter papers, fed with small slices of pilled banana and observed for mortality for 15 days. Dead beetles were surface sterilized with 70% alcohol and rinsed with sterile water. The beetles were then transferred to sterile Petri-dishes containing wet filter paper and kept at room temperature to check for mycosis. Only the beetles which showed visible signs of fungal growth after incubation were included for percentage mortality analysis.

Treatments and experimental designs

In the October 2012 feeding season there were seven treatments with five replications. The treatments were: T1=AITa +fungus + 50ml of water, T2= AITa +fungus only, T3= AITa with no fungus, T4= Japanese beetle trap (JBT), T5=AITb +fungus + 50ml of water, T6= AITb + fungus only, T7= AITb with no fungus. Traps were hung in sorghum fields on sorghum stalks with the head of the sorghum removed. In the October 2013 feeding season, there

were three treatments and ten replications (Tables 1and 2). For the July 2014 mating season, there were 8 treatments and 5 replications (Table 3). All treatments were laid out in randomized complete block design with a 50m and 10m spacing between blocks and traps respectively. This gives about 20 traps per 450m² area. Factorial arrangement was used in the October 2013 and July 2014 experiments.

Table 1. Treatments used for auto-inoculation experiment at Dohhada (Lewetegn) site around Rassa in October 2013

Code	Treatments	Remarks
T1	1:2.5 gm (Fungi :wheat bran flour)	Modified Auto-inoculation trap(AIT1)
T2	1:5 gm (Fungi :wheat bran flour)	Modified Auto-inoculation trap(AIT1)
T3	5 gm of wheat bran flour only(control)	Modified Auto-inoculation trap(AIT1)

Table 2. Treatments used for catch performance experiment at the Dohhada (Lewetegn) site around Rassa in October 2013

Code	Treatments	Remarks
T1	AIT1	Modified Auto-inoculation trap
T2	JBT (control)	Standard Japanese beetle trap
T3	LAT	Locally affordable trap with no auto-inoculation device

Table 3. Combinations of *Metarhizium anisopliae* isolates and auto-inoculation traps used for determination of the field viability of spores in October and July, 2014

Treatment	Isolate	Trap	Remarks
T1	PPRC51	AIT1	Auto-inoculation device from outside
T2	PPRC51	AIT2	Auto-inoculation device from inside
T3	PPRC2	AIT1	Auto-inoculation device from outside
T4	PPRC2	AIT2	Auto-inoculation device from inside
T5	ICIPE69	AIT1	Auto-inoculation device from outside
T6	ICIPE69	AIT2	Auto-inoculation device from inside
T7	All isolates	AIT1	Control (killed spores)
T8	All isolates	AIT2	Control (killed spores)

Determination of field viability of spores

The field level viability of the spores before loading on to the auto-inoculation device and every day thereafter was checked in July 2014 and October, 2014. Spores were picked up from the inoculation chambers of the AIT's (each replicate of a treatment) using clean cotton buds, put in 1.8 ml sterile cryovials and kept at 4 °C until processed. In the laboratory, 1ml of sterile 0.01% Tween 80 solution was added to the vials and vortex shaken for 2 minutes to dislodge the conidia from the cotton buds. Spores were counted us-

ing an improved neubaur haemocytometer and the concentrations adjusted to 1×10^6 conidia/ml. Finally 100 μ l of the spore suspension was transferred and spread on SDA media and incubated at room temperature for 24hrs. Viability of spores was determined by counting at least 300 spores and calculating the percentage of germinated spores. The mean temperature and relative humidity were 31.3°C and 23% (October 2012), 30.7°C and 42.83% (October 2013) and 36.5°C and 18.33% (July 2014) during experiments.

Quantification of spores picked up by individual beetles

Quantification of the amount of spores picked up from the auto-inoculation devices by individual beetles was done in the green house at Ambo Plant Protection Center (AP-PRC). Each of the two AIT's was loaded with 1gm of the respective fungal spores. *M. anisopliae* isolates (PPRC2, PPRC51 and IC-69) were used. There were three replications for each of the isolates and the traps. Ten field collected beetles were then manually put into the traps loaded with spores of the respective isolates one by one and collected from the exit of the traps and put individually in sterile test tubes. One beetle from each treatment in a block was randomly selected and separately kept in a glass vial containing 1ml of Tween 80 (0.01%) and vortex shaken for 2 minutes. Spores were then counted using an improved neubaur haemocytometer.

Statistical analysis

Mortality data were corrected for control mortality using Abbot's formula (Abbot, 1925) before transformation. Percent germination and mortality data were arcsine transformed before analysis. Count data on trap catch were square root ($\sqrt{x+0.5}$) transformed. Analysis of variance (ANOVA) followed by mean separation was conducted on all transformed data using SAS software version 9.2.

RESULTS AND DISCUSSION

Catch performance of traps

In October 2012, there was no significant difference ($P=0.40$, $F=1.08$, $df=6, 24$) in the mean catch of all the auto-inoculation traps and the JBT used as a control (Figure 4). In October 2013 the JBT caught significantly higher number of beetles ($P=0.016$, $F=5.21$, $df=2, 18$) than AIT1 and the LAT which did not significantly differ from each other (Figure 5). In July 2014 the catch performance of the two locally affordable AIT's as compared to the JBT was not significantly different ($P=0.1141$, $df=2, n=5$) (Figure 6).

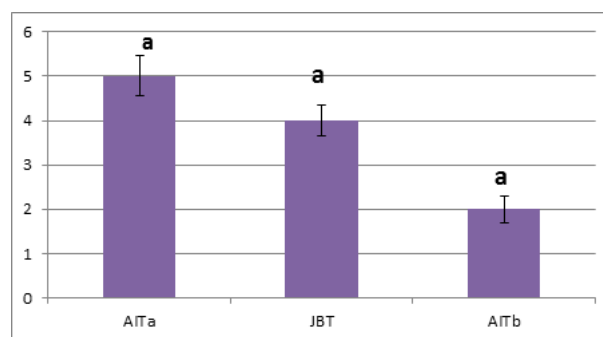


Fig. 4. Mean catch of traps with one outlet, the JBT and traps with two outlets in October, 2012 at Burka.

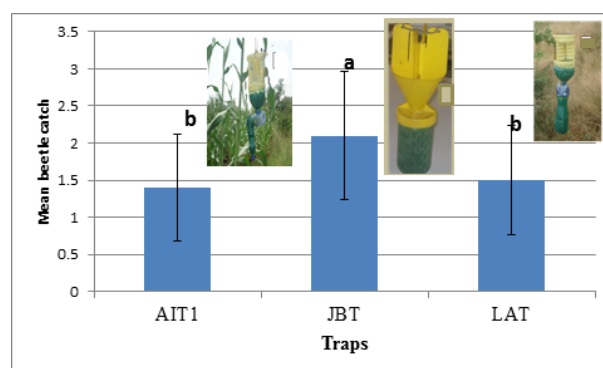


Fig. 5. Mean catch per trap in 3 days in the October, 2013 field Auto-inoculation trials at Dowhada (Rassa area).

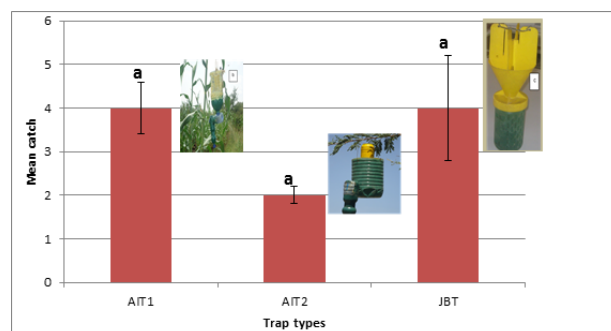


Fig. 6. Mean catch of two locally affordable auto-inoculation traps (AIT1 and AIT2) and the Japanese beetle trap in July, 2014 at Rassa.

Field efficacy of isolates

In October 2012, the mortality of the beetles varied significantly (Figure 7). Mortality ranged from 0 to 13.72% (~14%). The highest mortality was recorded from the *M. anisopliae* (PPRC51) treated bucket trap with one outlet containing 50ml of water (T1) and was significantly different ($P=0.017$, $F=3.25$, $df=6, 24$) from all the other treat-

ments. The rest of the treatments did not differ significantly from each other. In October 2013, there was significant difference ($P = 0.0001$, $F = 15.94$, $df = 2, 18$) in beetle mortality between the control (T3) and fungus treated AIT's with auto-inoculation device (T1 and T2) (Figure 8) although the later two did not significantly differ from each other. In July 2014, there were significant differences among the *M. anisopliae* treated and control traps ($P < 0.0018$, $F = 6.24$, $df = 4, n = 5$) in observed mortality of adults of *P. interrupta* (Figure 9). There was no significant difference between the two AIT's ($P = 0.06$, $F = 3.80$, $df = 1, 32$, $n = 5$).

The isolate PPRC51 was associated with the highest mortality (40.73%) followed by PPRC2 and IC69 39.91% and 26.16% respectively when applied using AIT1. Using AIT2, observed mortalities were 25.02%, (PPRC51), 26.69% (PPRC2) and 15.03% (IC69). The interaction between isolates and AIT's was not significant ($P = 1.00$, $F = 0.16$, $df = 3, 32$, $n = 5$).

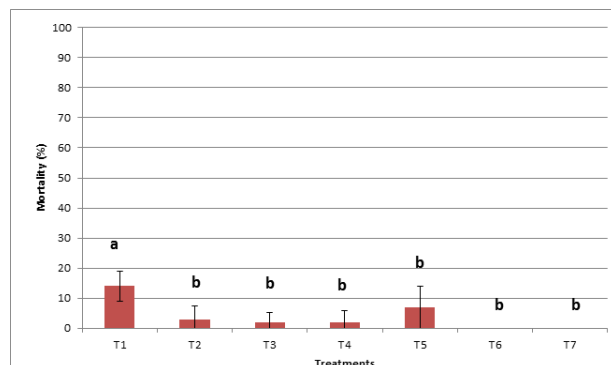


Fig. 7. Mean percent mortality of *Pachnoda interrupta* caught from *M. anisopliae* (PPRC51) treated and untreated traps over three days at Burka in October, 2012.

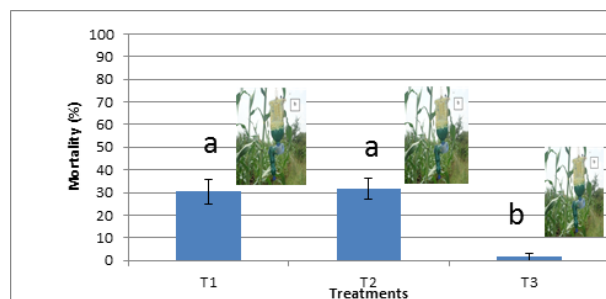


Fig. 8. Mean percent mortality of beetles collected from fungus treated and control AIT's during the October, 2013 field experiment at Lewtgen (Rassa area).

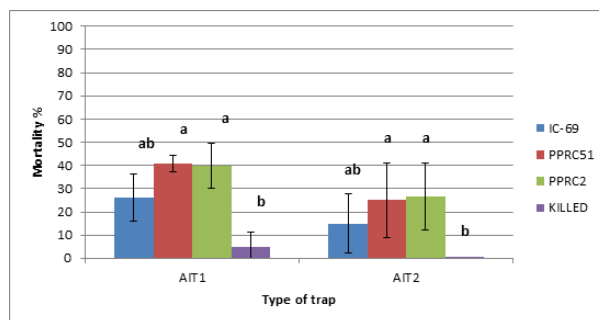


Fig. 9. Mean percent mortality of beetles collected from AIT1 and AIT2 treated with three isolates of *Metarhizium anisopliae* and killed spores in July, 2014 at Rassa.

Field viability of isolates

In July 2014, the mean field viability (as measured by %germination) of spores of the three *M. anisopliae* isolates over five days significantly varied among the isolates ($P = 0.0001$, $df = 2, 24$ for days 0 to 4 and $P = 0.0015$, $df = 2, 24$ for day 5) (Figure 10). The mean initial viability (day 0) of PPRC51, PPRC2 and IC69 were 82.12%, 84.5% and 62.37% respectively. Viability decreased sharply especially for IC69 which dropped to 17.92% in the second day and plummeted to zero in day three. In contrast, the viability of PPRC51 and PPRC2 did not drop below 38% until after day 4.

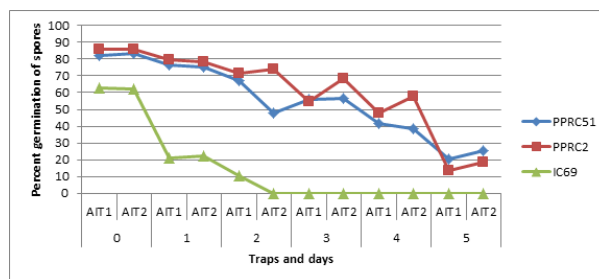


Fig. 10. Mean percent germination of three *Metarhizium anisopliae* isolates applied in two auto-inoculation traps over 5 days in July, 2014 at Rassa.

In October 2014, the field viability of spores of the three *M. anisopliae* isolates over five days showed a similar trend as that of the July, 2014 experiment (Figure 11). The viability of the isolates varied significantly and ranged from 78.3% (PPRC2) to 0% (IC69) over the five days ($P = 0.0001$, $df = 2, 24$ for days 0 to 5). The viability of isolates PPRC51 and PPRC2 did not significantly differ throughout the experiment days. However, both isolates significantly differed

from IC69. Whereas the viability of PPRC51 and PPRC2 did not drop below 35.6% and 40.3% respectively in day five, IC69 could not stay viable beyond day 2 particularly in AIT2. In contrast, in AIT1, the isolate stayed viable up to day 5 with mean viability of nearly 32% at day 5. There was a slight interaction effect between isolates and AIT's at day 3 ($P=0.0001$, $F=17.04$) and day 4 ($P=0.03$, $F=3.73$). Generally, the viability was relatively low in AIT2 than in AIT1.

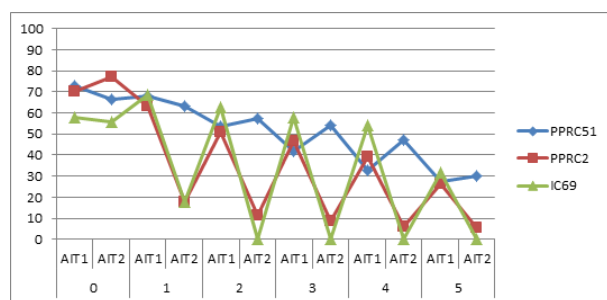


Fig. 11. Mean percent germination of three *Metarhizium anisopliae* isolates applied in two auto-inoculation traps over 5 days in October, 2014 at Rasa.

Spore pick-up by beetles

The mean number of spores picked up by a single beetle ranged from 3.14×10^7 (PPRC2) to 9.6×10^7 (PPRC51) in 2014 (Figure 12). Significant variations were observed in the number of spores picked from the isolates ($P<0.0001$, $df\ 2$, $n=10$). There was no significant difference between using AIT1 or AIT2 and interaction effects between AIT's and isolates were not significant.

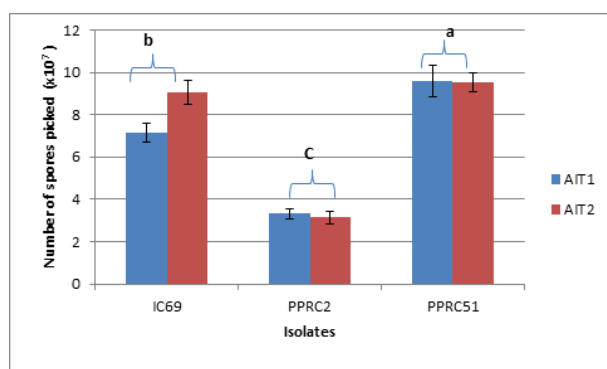


Fig. 12. Number of spores picked up by single beetle from AIT's loaded by three isolates of *Metarhizium anisopliae* in 2014.

The Japanese beetle trap is often used to trap *P. interrupta* in Ethiopia. But, use of standard commercial traps is costly and difficult to obtain for the subsistence farmers in Ethiopia. This study attempted to develop cheap auto-inoculation traps from locally available plastic water bottles to infect *P. interrupta* adults with *M. anisopliae*. In a similar

low cost approach, auto-inoculation device made of locally available plastic water bottle materials was used to contaminate tsetse flies (Maniania, 1998). Although the JBT was reported as a more efficient trap for *P. interrupta* (Weldehariat *et al.*, 2007), subsequent improvements in this study eventually resulted in AIT's as efficient as the JBT.

The mortality of *P. interrupta* associated with isolate PPRC51 increased from 14% in October 2012 to 41% in July 2014. This may be attributed to the modifications done on the auto-inoculation devices which increased the protection of spores in the devices. It can also be attributed to combinations of environmental and device improvement factors. However, the percentage mortality observed is relatively high considering the hot climatic conditions of the breeding areas of *P. interrupta*. The field efficacy of entomopathogenic fungi is influenced by strain genetic characteristics and high conidial viability (Soetopo, 2004), the behavior of the target pest in its natural habitat (Gindin *et al.*, 2006) and the environmental factors such as solar radiation (UV light), temperature and low relative humidity (Inglis *et al.*, 2001; Wraight *et al.*, 2007). In contrast, existence of favorable microenvironment in the insect host's body surface and high humidity leaf zones may facilitate conidial germination after contact with the cuticle (Inglis *et al.*, 2001; Shipp *et al.*, 2003). In this study use of either of 1:5 or 1:2.5 ratio (spore: wheat bran) in 2013 did not cause significant variation in mortality of beetles. Klein and Lacey (1999) also obtained similar non significant effect of spore to wheat bran ratio on mortality of *P. japonica* adults using a modified auto-inoculation trap.

As depicted in Figure 10, the viability of PPRC51 in AIT1 dropped to 20.39% by day 5 from initial viability of 85.7% at the start of the experiment (day 0) which is a four-fold decrease. In a similar study, Klein and Lacey (1999), found that the viability of spores used in auto-inoculation traps for Japanese beetle dropped by about 50% (to less than 34.5% in six days from initial viability of 73%). This condition might be attributed to the high temperature and low relative humidity observed in the experiment sites. In the current study, the mean temperature and relative humidity were 31.3°C and 23% (October 2012), 30.7°C and 42.83% (October 2013) and 36.5°C and 18.33% (July 2014). The temperature of the target eco-system heavily influences growth and pathogenicity (Yeo *et al.*, 2003) as well as speed of germination and kill (Migiro *et al.*, 2010) of a fungal entomopathogen. In agreement with this study, Jaronski (2010) found that conidial germination was adversely affected by and rapidly slowed in temperatures above 30°C. Similarly, Dimbi *et al.* (2003) also reported limited development of entomopathogenic fungi below 15

°C or above 35 °C. Conidia are generally instable at high temperatures (Fernandes *et al.*, 2007). This necessitates the selection of isolates tolerant to the temperature range of the target ecosystem (Ferron *et al.*, 1991) and appropriate formulation (Forgues *et al.*, 1997) as a means to overcome the problem. Niassy *et al.* (2012) were able to demonstrate that kairomone LUREM-TR used for thrips monitoring and auto-dissemination had detrimental effect on conidial viability of ICIPE 69. A similar condition might have occurred during these experiments too.

The number of spores picked up by individual beetles was generally greater than 3.14×10^7 for all isolates in the green house trial. The number of conidia acquired by an insect depends on the formulation of the pathogen, the trap design, duration of contact with the conidia and the size of the beetle (Kreutz *et al.*, 2004). The finding of this study is in agreement with the findings of Klein and Lacey (1999) who found up to 1.13×10^8 conidia per beetle in *P. Japonica* passing through an auto-inoculation trap.

In conclusion, this study has demonstrated a ground breaking development of auto-inoculation device for use in the management of *P. interrupta* in Ethiopia. The isolates PPRC51 and PPRC2 with field efficacy of 41% and 40%, respectively, are recommended as potential candidates for development of myco-pesticide against *P. interrupta* for integrated management of the pest. Additional field studies under high population conditions, assessment of the dissemination of the fungi to breeding areas and to cause epizootics in larvae and adults in the breeding areas and more research on mass production characteristics and storage of the isolates need to be carried out.

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