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Efficacy of Entomopathogenic Fungi against Red Tef Worm, *Mentaxya Ignicollis* (Walker) (Lepdoptera: Noctuidae)

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Abstract

Red tef worm (M. ignicollis) is a serious pest of tef (Eragrostics tef (Zucc.), Trotter: Poaceae) grown on black soils. Hence the present study was conducted to identify the effective isolate and conidial concentration of the entomopathogenic fungi (Beauveria bassiana and Metarhizium anisopliea isolates) and to measure the larval mortality of M. ignicollis caused by the entomopathogenic fungi and, thereby controlling RTW to increase the production of tef on black soils. Laboratory studies were carried out in completely randomized design of with four fungal isolates at four conidial concentrations against 3rd instar larvae of red tef worm. The green house study were carried out in randomized complete block design with the four isolates at the concentration of $1x10^9$ spores/ml. Percent mortality increased from 46.67 to 83.33% and 43.33 to 73.33% when larvae were treated with M. anisopliae isolate, MM and PPRC-2, respectively, whereas, B. bassiana isolates PPRC-56 and PPRC-9609 caused mortality ranging from 43.33 to 66.67% and 43.33 to 50%, respectively when applied at the conidial concentration of $1x10^6$ to $1x10^9$spores/ml under laboratory condition. Percent mortality under greenhouse condition showed that MM (70%) was the most virulent isolate; however, PPRC-2, PPRC-56 and PPRC-9609 caused larval mortality of 60%, 53.33% and 46.67% respectively. It was concluded that all tested isolates can cause infection on 3rd instar larvae of red tef worm and able to cause delayed effect to the next stages after application; however, in both laboratory and greenhouse studies, isolate MM was the most effective at conidial concentration of $1x10^9$ spores/ml. In general, MM ($1x10^9$spores/ml) isolate was found to be effective and potency against 3rd instar larvae of red tef worm under laboratory and greenhouse conditions. Therefore, since this agent is safe to the environment and other beneficial organisms and it is recommended to be verified for usage under open and large field conditions for the control of red tef worm.

Key words: *Metarhizium anisopliae, Beauveria bassiana Entomopathogenic fungi, Red Tef Worm, Tef*

Introduction

Tef (*Eragrostics tef (*Zucc.*),* Trotter: Poaceae) is a staple food crop of Ethiopia where it originated and has diversified. Over 2.8 million hectares of land is covered with tef every year with its mean productivity at national level predicted 1228 kg ha^{-1} (CSA, 2011). Red tef worm (RTW) (*M. ignicollis)* is a serious pest of tef grown on black or heavy, deeply cracking clay soils. The status of the pest as a major pest of tef was reported in Shewa, Kefa, and Gojam and in some places in Tigray and Wollega. The loss from red tef worm was estimated to be l0-30% in the county; however, repeated crop losses have been observed in Becho area of Shewa. (IAR, 1986; Tadesse, 1987).

Control measures of RTW, including cultural, chemical and microbial methods have been attempted to some extent (Tadesse, 1987a, 1987b). Use of synthetic pesticides causes some unfortunate consequences, such as environmental pollution, pest resistance and toxicity to other non-target organisms. Although Entomopathogenic fungi (EPF) are among the first organisms to be used for the biological control of insect pests, there is an information gap regarding the use of EPF and other bio-rational methods to develop integrated pest management. Florez (2002) justified that entomopathogenic fungi are considered to be suitable for use as biological pesticides since they have a relatively narrow host range. The fungal isolate does not kill many different species and does not have a significantly negative impact on biodiversity or natural enemies.

Previously, no research has been done with the EPF to control RTW. Therefore, it is necessary to expand the choice of management options to minimize chemical control and thus minimizes the fear of adverse effect of chemical residues to human and animal health. Therefore, the present study was carried out under laboratory and greenhouse conditions with the following general and specific objectives:

- To identify the effective isolate and determine the conidial concentration of the entomopathogenic fungi (*B. bassiana* and *M. anisopliea* isolates) against the 3rd instar larvae of *M. ignicollis* under laboratory and greenhouse conditions.
- To measure larval mortality of *M. ignicollis* caused by the EPF and
- To determine the LT_{50}, LD_{50} and LC_{50} for the EPF
- To study the delayed effect of EPF on the next generation of treated larvae of RTW.

Materials and Methods

Description of the Study Area

The experiment was carried out under laboratory condition at Ambo Plant Protection Research Center (APPRC). The center is located at Ambo district

of West Shoa Zone, Oromia regional state at 114km west of Addis Ababa having the altitude of 2115masl with longitude of $38^{0}7^{0}$E and latitude of $8^{0}57^{0}$ N. The temperature of the area ranges from 11.7^{0}C to 25.4^{0}C and mean annual rain fall is 1115mm.

Growing of Tef Plants on Pots

Tef seeds were sown following the standard greenhouse procedures. The size of the pots was 18x30cm. The pots were filled with mixture of soils: clay: compost: sand soil in the ratio of 1:2:1. The released tef variety (Kuncho) was sown on each pot at the recommended rate of 25 kg ha^{-1}

Rearing of Red Tef Worm

The larvae of red tef worm were collected from infested tef fields of South West Shoa Zone, Becho and Saden Sodo districts early in the morning on tef plants at grain filling stage. The collected larvae were transferred to plastic bowls which were quarter filled with mixture of fine sand and black soil and provided with fresh tef seedlings every 24 hours and kept under temperature of 26 ± 2^{o}C.

The larvae pupated in the plastic bowls at the depth of 3-9cm. The soil in the plastic bowls with pupae were wetted and kept undisturbed. On an average, 15 days after pupation adults started to emerge. To culture the adults, tef seedlings were grown on small pots and kept in the cage. Then emerged adults were carefully transferred to the cage with 3:1, female to male ratio and provided with 10% sugar solution (Tadesse and Matthews, 1986) by sprinkling on the tef seedlings, placing sugar solution soaked cotton wool in small cups in the cage as well as suspending cotton wool which was wetted with the solution. Every day, the sugar solution was sprinkled and the cotton in the cups was changed. As an alternative zigzag shaped paper were suspended on the corner for oviposition. Three days after emergence, adults started oviposition. The eggs were laid on the underside of tef leaves, on the suspended paper, and on the surface of cage (nylon cage). Ten to fourteen days after oviposition, eggs hatched and the larvae fed on the leaves of the seedlings.

Table 1 Origin and Sources of Fungal Isolates for the Experiment

Name of the fungal isolates	Isolates code	Location collected	Origin place
M. anisopliae	MM	Arbaminch	Soil
	PPRC-02	Ashan (N.shoa)	*Pacnoda interrupta*
B. bassiana	PPRC-56		
	9609	Mugando(Dilla road)	*Blosyrus rugulosus*

Source: Ambo Plant Protection Research Center

Efficacy of *B. bassiana* and *M. anisopliae* isolates against larvae of RTW under laboratory condition

Two isolates of *B. bassiana* (PPRC-56 and 9609) and two isolates of *M. anisopliae* (MM and PPRC-2) at four conidial concentrations ($1x10^6$, $1x10^7$, $1x10^8$ and $1x10^9$ spores/ml) were evaluated against 3rd instar RTW larvae under laboratory condition with untearted control included in a completely randomized design. All together there were seventeen treatments in three replications.

510 third instar RTW larvae were used in the experiment. To determine the differences between the larval instars, 20 larvae were randomly selected from a rearing cage and kept separately. Number of head capsules was counted through the instars. A total of 51 Petri dishes of each 12.5cm diameter were used for the experiment. Freshly chopped tef leaves were provided in each Petri dish as food. Ten third instar RTW larvae were introduced into each Petri dish. The fungal isolates at different conidial concentrations were applied using hand sprayer on the surface of the larvae (Hafez *et.al*., 1994). As a control, sterile distilled water with 0.05% Tween 80 was applied. New, fresh, leaves were changed by removing the old leaves every 24 hours to maintain the actual development of the larvae.

Larval mortality, pupal formation, deformed larvae and emerged adults were recorded for consecutive ten days. The dead larvae in each treatment were collected and submerged in 70% ethanol, 5% sodium hypochloride for two minutes and washed in steriled distilled water for three minutes (Odindo, 1994). The disinfected larval cadavers were dried for ten minutes on filter paper and kept on the Petri dishes containing damp filter paper and sealed with parafilm to maintain high humidity that promotes fungal growth and incubated at 27^0C for two days. Fungal growth observed on the larval cadavers confirmed that mortality was resulted from the fungal isolates applied (Seneshaw, 2002).

Efficacy of *B. bassiana* and *M. anisopliae* isolates against larvae of RTW under greenhouse

This experiment was conducted in APPRC, Entomology greenhouse conditions. The maximum and minimum temperature of the house was 34^oC and 10^oC, respectively. Released tef variety, Kuncho, was sown on 15 pots of 19cmx20cm size for the experiment.

B. bassiana (PPRC-56 and 9609 isolates) and *M. anisopliae* (MM and PPRC-2 isolates) at $1x10^9$ spores/ml was used against larvae of RTW. The experiment was carried out in randomized completely block design

with five treatments and replicated three times.

A total of ten 3rd instar larvae of RTW were placed into each pot with tef plants at grain filling stage. The total number of larvae used for this study was 150. Individual treatments were sprayed using hand sprayer early in the morning when the larvae were actively eating on the leaves and grain (milky stage) at $1x10^9$spores ml^{-1} conidial concentration. Sterilized distilled water with 0.01% Tween 80 was sprayed as control. Larval mortality was assessed every 24 hours after application for consecutive 10 days. The larval mortality that resulted from fungal infection was confirmed with the following procedure described for the laboratory experiment.

Data Analysis

Larval mortality under each treatment in both laboratory and greenhouse conditions was corrected using Abbott (1925) formula and the corrected mortality data in laboratory condition was analyzed by two way analysis and the data under greenhouse condition was analyzed using one way analysis SAS program (SAS, 2005). LD_{50} and LT_{50} were also calculated using SAS probit analysis. Pupal formation, deformed larvae and emerged adults were illustrated by graph presentation.

CM (%) = (T-C) / (100-C)*100

Where CM is corrected mortality; T is percent mortality in treated larvae of RTW;
C is percent mortality in untreated larvae of RTW

Results and Discussion

Efficacy of *B. bassiana* and *M. anisopliae* Isolates against 3rd Instar Larvae of RTW in Laboratory and Greenhouse Conditions

All tested fungal isolates were virulent against 3rd instar larvae of RTW when compared with the untreated control ten days after application both in laboratory and greenhouse conditions (Table 2 and 3, respectively). Significant differences at $P<0.05$ in terms of larval mortality were observed between control treatment and the fungal isolates as well as the different conidial concentrations.

Table 2: Percent mortality of 3rd instar Larvae of RTW when treated with *B. bassiana* and *M. anisopliae* isolates under laboratory condition.

	Mean(±SE) mortality due to different isolates at conidial concentrations			
EPF isolates	$1x10^6$ spores/ml	$1x10^7$ spores/ml	$1x10^8$ spores/ml	$1x10^9$ spores/ml
MM	46.67±3.33e	63.33±3.33bcd	66.67±3.33bc	83.33±3.33a
PPRC-2	43.33±3.33e	53.33±3.33cde	63.33±3.33bcd	73.33±3.33b
PPRC-56	43.33±3.33e	50±0.00de	56.67±3.33cde	63.33±3.33bcd
PPRC-9609	43.33±3.33e	43.33±3.33e	50±5.67de	50±0.00de
Untreated control	13.33±3.33f			

CV=10.82%

Means followed by the same letters are not significantly different by Student Newman Keuls(SNK) test (P<0.05)

Table3. Percent mortality of 3rd instar larvae of RTW when treated with *B. bassiana* and *M. anisopliae* isolates in greenhouse condition

Treatments	Mean(±SE) value
MM	70±0.00a
PPRC-2	60±0.00b
PPRC-56	53.33±3. 33bc
PPRC-9606	46.67±3.33c
Untreated control	13.33±3.33d

CV=8.79%, Means followed by the same letters are not significantly different by Student Newman Keuls (SNK) test (P<0.001)

When the larvae were treated with different concentrations of fungal isolates under laboratory condition, there was a linear correlation between percent mortality and concentrations applied. Percent mortality increased from 46.67 to 83.33% and 43.33 to 73.33% when *M. anisopliae* isolates, MM and PPRC-2, respectively were applied at the concentration of $1x10^6$ to $1x10^9$spores/ml. Similarly, *B. bassiana* isolates PPRC-56 and PPRC-9609 caused mortalities from 43.33 to 66.67% and 43.33 to 50%, respectively when applied at the concentration of $1x10^6$ to $1x10^9$ spores/ml.

The ANOVA results showed that there is no significant difference between fungal isolates against 3rd instar larvae of RTW at the conidial concentrations $1x10^6$, $1x10^7$and$1x10^8$ spores/ml; however, MM (66.67%) significantly differed from *M. anisopliae* isolates, PPRC-56 (56.67%) and PPRC-9609 (50%) at the concentration of $1x10^8$ spores/ml.

The ANOVA indicated that *M. anisopliae* isolate, MM at $1x10^9$ spores/ml caused the highest mortality 83.33%, which was significantly different from all treatments. *B. bassiana,* isolate PPRC-9609 at the concentration of $1x10^9$spores/ml resulted in the lowest mortality of the larvae (50%) when compared with the other isolates at the same conidial concentration.

Percentage commutative mortality of RTW larvae obtained as results of *M. anisopliae* and *B. bassiana* isolates application at conidial concentration $1x10^9$ spores/ml in greenhouse are presented in Table 3. All the fungal isolates caused mortality in the population of RTW larvae. It is

however important to note that, the individual fungal isolates at the different levels of conidial concentration tested showed variability in their effect against the insect.

The statistical analysis of the percentage commutative mortality showed that MM at $1x10^9$ spores/ml (70%) was the most virulent isolate and differed significantly from all the other fungal isolates tested. However, PPRC-2 (60%) and PPRC-56 (53.33%) did not differ significantly ($P<0.001$) among each others. But PPRC-2 is significantly different from PPRC-9609 (46.67%) which caused in the lowest percent mortality in the insect.

In line with this results, a number of laboratory bioassays have been conducted with suspected fungal entomopathogens (Zhioua *et al*. 1997, Monteiro *et al*. 1998, Samish *et al*. 2001, Gindin *et al*. 2002), and several of these studies have identified *M. anisopliae* as the most pathogenic fungus among the different species tested (Zhioua *et al*. 1997, Monteiro *et al*. 1998, Samish *et al*. 2001). El-Hawary and Abd El-Salam (2009) reported that *B. bassiana* cause 100% mortality of 3rd instar larvae of *Agrotis ipsilon* within 5.3 days at concentration of $1x10^9$ spores/ml. Nguyen (2007) also recorded 68.1 and 100% mortality of *H. armigera* within 4.3 and 6.5 LT50 when treated with *B. bassiana* and *M. anisopliae,* respectively. Samish *et al*. (2001) observed 93.33% mortality in III and IV instars of *H. armigera* in 96 h after the treated with *M. anisopliae.* Nahar *et al*. (2004) reported the pathogenicity of *M. anisopliae* which caused 66.74% mortality in III instar of *H. armigera*.

Generally, mortality occurred slowly four days after treatment application, as the larvae infected by fungal spores and their skin almost disintegrated. The external white mycelial growth from the cadavers was observed to appear within 24-48 hours after placement on moist filter paper. Conidial growth over the treated larval cadavers demonstrated that the insects died due to the infection of applied EPF (Fig.1). Apart from mortality, various abnormalities in treated larvae were also observed. When larvae were infested with different isolates of fungi, larval cadavers were found to be heavily contaminated with inclusion bodies. Arti *et al*., (2010) indicated that these bodies are fungal bodies that ultimately oozed out from the ruptured area. Generally, larval growth was ultimately restricted and thereby forming short and shrunken larvae, mycelial growth was observed on larval cadavers, larvae stopped feeding, blackening of body parts occurs and larvae die at larval-pupal intermediate stages. These abnormality observations were also observed by Arti *et al*.(2010), when *B. bassiana* tested against *Helicoverpa armigera*, caused these abnormalities which basically targeted chitin and the treated larvae died at larval-pupal

intermediate stages indicating that *B. bassiana* causes improper moulting.

The infected larvae showed the formation of weak and fragile cuticle that generally ruptured at mid gut. This indicated that EPF cause mortality not only through depleting the nutrients from the infected larvae but also due to crude toxic protein extraction. In line with this, Quesada *et al.*,(2006) reported that the death of *Spodoptera littoralies* larval instars by fungal species of *B. bassiana* and *M. anisopliae* were due to crude toxic proteins extracted by fungi and that death was EPF concentration dependent. Not only toxic proteins extracts but also they observed a progressive bleeding of the mid gut epithelium into the gut lumen with lysis of the epithelium layer.

Over all , the results indicated that all the fungal isolates caused mortality in the larvae of the insect compared with the untreated treatment (Fig.1). In addition to this, *M. anisopliae,* isolates MM, caused higher percentage mortality of the insect larvae when compared with the other isolates, thus providing greater protection to tef against the RTW. Finally, *M. anisopliae,* isolate MM and the other tested isolates at the highest conidial concentration ($1x10^9$ spores/ml), was considered in *M. ignicollis* management program due to their effectiveness, non hazardous and environmental friend nature.

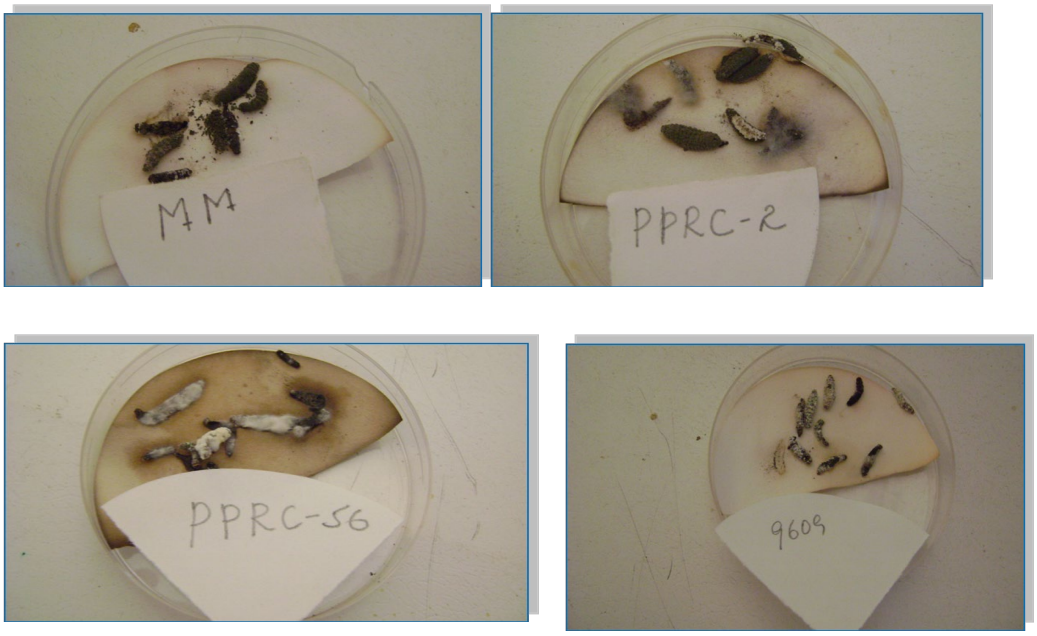

Fig. 1. Larval mortality with *M. anisopliae* isolates MM and PPRC-2 and *B. bassiana* isolates PPRC-56 and PPRC-9609

Concentration-mortality effect of *M. anisopliae* and *B. bassiana* isolates against 3rd instar larvae of RTW

The comparative virulence of *M. anisopliae* (MM and PPRC-20 and *B. bassiana* (PPRC-56 and PPRC-9609) on the 3rd instar larvae of RTW was determined in laboratory condition. The estimated LC_{50} and LC_{90} values based on the mortality trends across dosage and relative potency are presented in table 4.

Table 4. LC_{50} and LC_{90} of *M. anisopliae* and *B. bassiana* isolates (spores/ml) to 3rd Instar Larvae of RTW

Treatments	LC_{50} (95% CI)[a]	LC_{90}(95% CI)	Relative potency[b]	
			LC_{50}	LC_{90}
MM	$1.64x10^6$($2.31x10^5$-$4.93x10^6$)	$1.70x10^{10}$($2.42x10^9$ - $9.62x10^{11}$)	1	1
PPRC-2	$4.61x10^6$($6.41x10^5$-$1.44x10^7$)	$3.50x10^{11}$($1.75x10^{10}$ -$5.47x10^{14}$)	2.81	20.59
PPRC-56	$9.94x10^6$($3.20x10^5$-$5.60x10^7$)	$3.67x10^{14}$($5.03x10^{11}$ -$1.41x10^{28}$)	6.06	$2.16x10^4$
PPRC-9606	$5.61x10^8$	$6.59x10^{27}$	$3.42x10^2$	$3.88x10^{17}$

[a] LC_{50} or LC_{90} and 95% fiducial limits (CLs) are given in spores/ml

[b] Relative potency is calculated as LC_{50} or LC_{90} of the tested EPF isolates/LC_{50} or LC_{90} of the most effective isolates

The results obtained in the dose-mortality relationship studies further indicated that there were differences among the four tested isolates. *M .anisopliae* isolate MM showed the highest virulence with the lowest LC_{50} ($1.64x10^6$ spores/ml) and LC_{90} ($1.70x10^{10}$ spores/ml), whereas, *B. bassiana* isolate PPRC-9609 showed the lowest virulence with the highest LC_{50} ($5.61x10^8$ spores/ml) and LC_{90} ($6.59x10^{27}$ spores/ml) compared to the other isolates. Investigations carried out by Adane *et al*. (1996) on *S. zeamais* indicated that very virulent isolates could cause high mortality at lower concentrations. The relative potency values indicated that *M. anisopliae* isolate MM was the most effective than PPRC-2, PPRC-56 and PPRC-9609 with 2.81, 6.06 and $3.42x10^2$ times the highest potency at the LC_{50} and 20.59, $2.16x10^4$ and $3.88x10^{17}$ times great highest potency at the LC_{90} level, respectively.

The studies were contrary to the previous findings of Dhembare and Siddique (2004) who reported that the highest virulence in *B. bassiana* isolates compared with isolates of *M. anisopliae* against *H. armigera* under laboratory condition. Rodrigues and Pratissoli (1990) and Moino *et al*., (1998) also reported higher mortalities of stored grain pests inoculated with *B. bassiana* than with *M. anisopliae*. These contrary results occurred may be due to the variability of virulence between the isolates

In agreement with this study results, Kassa *et al*., (2002) reported that *M. anisopliae* isolate (PPRC-EE) is highly virulent than other isolates of *B. bassiana* on *S. zeamais*. And also clearly indicated that when either *B. bassiana*

or *M. anisopliae* were applied to *S. zeamais* at a lower concentration ($1x10^6$ conidia ml^{-1}), the latter caused a significantly higher mortality and shorter median survival time. Furthermore, selection of virulent isolates for the pest plays an essential part in the development of microbial control programmes. Based on the present study, *M. anisopliae* (MM) can be recommended as primary candidate for further research in order to develop a mycoinsecticide for RTW control in Ethiopia. *M anisopliae* isolate (PPRC-02) and *B. bassiana* isolates (PPRC-56) could be considered as alternatives. Taking into account further aspects, such as mass production, formulation, storage, spectrum of activity to the pest and safety to non-target organisms will help focus on a single isolate for product development.

Time-mortality response of *M anisopliae* and *B. bassiana* isolates to 3rd instar larvae of RTW

The median lethal time values of the tested EPF on the 3rd larval instar of RTW are shown in Table 5.

Table 5. LT_{50} of *M. anisopliae* and *B. bassiana* isolates to 3rd Instar Larvae of RTW under Laboratory Condition

Treatments	LT_{50}(95% fiducial limits)[a]			
	$1x10^6$ spores/ml	$1x10^7$spores/ml	$1x10^8$spores/ml	$1x10^9$ spores/ml
MM	8.21(7.77-8.73)	6.78(6.32 -7.31)	6.45(5.89-7.08)	4.42(4.08 -4.76)
PPRC-2	8.34(7.82 - 8.99)	7.84(7.31-8.47)	6.96(6.51-7.48)	5.66(5.24-6.14)
PPRC-56	7.92(7.38 -8.57)	6.99(6.50 -7.55)	6.86(6.24-7.59)	5.51(5.04-6.03)
PPRC-9609	8.59(8.15-9.14)	7.98(7.51-8.54)	8.05(7.57 -8.64)	7.77(7.25 -8.40)

[a] LT_{50} and 95% fiducial limits (FLs) are given in days

It is indicated that the time required for 50% death decreased with increasing concentrations in all tested isolates on 3rd larval instar. However, there is no considerable difference between concentrations $1x10^7$and $1x10^8$spores/ml in each isolate. At lower concentration ($1x10^6$ spores/ml), *B. bassiana* isolate PPRC-56 caused 50% death within shorter day (7.92) than the others isolates. *M. anisopliae* isolates MM exhibited more efficiency in killing, and killed 50% of the treated 3rd instar larvae faster (4.42days) at conidial concentration of $1x10^9$ spores/ml than the other treatments at the same concentration. *B. bassiana* isolate PPRC-9609 caused 50% death slower (7.77 days) than the others, whereas, no big differences between PPRC-2 (5.66 days) and PPRC-56 (5.51 days) to kill 50% of the larvae.

In line with these results, El-Hawary and Abd El-Salam (2009) indicated that the percentage mortality of larvae of *H. armigera* increased with increasing concentrations of both pathogens; however, the mean time to death decreased as the spores concentration increased. Dhembare

and Siddique (2004) also reported the same results when *B. bassiana* was evaluated against *H. armigera* under laboratory. Tounou *et al*., (2008) also found that when *M. anisopliae* was applied at $1x10^3$ and $1 x 10^4$ spores/ml on *Schistocerca gregaria* nymph the median survival time estimates for these treatments were 18.8 ± 0.6 and 12.3 ± 0.9 days, respectively. The long time in their results may be due to the low concentration and also the insect species.

Thus, the present study indicated that the pathogenecity of all isolates were almost the same at the lowest concentration ($1x10^6$spores/ml). However, as the concentration was increased, especially to $1x10^9$spores/ml, the isolates considerably showed pathogenecity differences. On the other hand, concentrations of $1x10^7$ and $1x10^8$spores/ml showed no pathogenecity differences within the same isolates. The results concluded that the time to kill 50% of the larvae decreased with increasing concentrations of all isolates. Hence, to get immediate response using these isolates one could increase the concentrations. *M. anisopliae* isolates MM showed faster response, thereby high pathogenecity, so preferred than others.

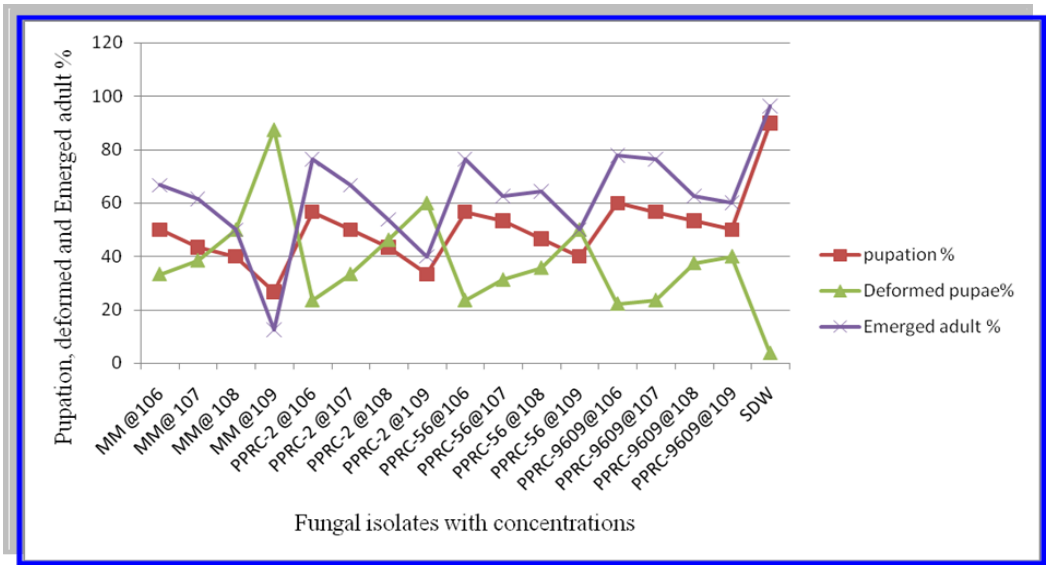

Fig.2. Delayed Effect of Concentration of Fungal Isolates on RTW

Delayed Effect of *M. anisopliae* and *B. bassiana* Isolates to the Larvae of RTW

The delayed effect of *M. anisopliae* and *B. bassiana* isolates on treated larvae of RTW was observed. The percent pupation when per-pupae of RTW treated with the EPF isolates decreased through the increasing of concentrations (Fig.2). This indicated that pupation percentage of RTW and conidial concentrations of the isolates were inversely correlated. Pupation percentage was decreased from 90 (control) to 26.67, 33.33, 40 and 50 % when larvae of RTW were treated with MM, PPRC-2, PPRC-56 and PPRC-9609 respectively at a concentration of $1x10^9$ spores/ml.

From this study, pupal deformation of RTW larvae treated with the EPF isolates was observed (Fig.3). Deformed percent of pupae were progressively increased with increased concentrations in all of the isolates; however, highly increased (33.33 to 87.5%) in MM isolates when the concentration increased from $1x10^6$ to $1x10^9$ spores/ml. In addition, variations were also observed between the isolates at the same conidial concentration. The percent deformity increased from 3.7 (untreated control) to 87.5, 60, 50 and 40 % when the larvae treated with MM, PPRC-2, PPRC-56 and PPRC-9609, respectively at the conidial concentration $1x10^9$ spores/ml. This indicated that the variation between the fungal isolates in causing delayed effect on the pupae when larvae of the RTW were treated with the isolates. The percentage of moth emergence showed a highly progressive decrease with the increase of concentration of the fungal isolates. Thus emergence decreased from 96.3% in the control to 12.5% in *M. anisopliae* isolate MM at $1x10^9$ spores/ml. High adult emergence occurred when the larvae were treated with *B. bassiana* isolate PPRC-9609.

In agreement with this study, El-Hawary *et al*.,(2009) reported delayed effect of *B. bassiana* on *Spodoptera littoralis.* The results showed that the percentage adults obtained decreased from 12.5% to 5.0% at $1.0x10^9$ spores/ml**.** Hafeze *et al*.,(1994) also reported that *B. bassiana* resulted in decreasing the number of emerged adults of *P. operculella* which showed a high percent of malformation. Resulted adult males and females lived shorter time and laid low number of eggs.

Generally, this study showed that all the tested fungal isolates caused delayed effect on the next genaration. Increased conidial concentration resulted in increasing infection of the next stage of the insect. In group, *M anisopliae* caused more delayed effect than *B. bassiana* on RTW. Specifically, MM isolate resulted in the most delayed effect compared to the other isolatess. It's also caused 83.8% pupae deformation at conidial concentration $1x10^9$ spores/ml than the untreated

control, whereas PPRC-2, PPRC-56 and PPRC-9609 caused 56.3, 46.3 and 36.3% deformity, respectively. Adult emergence was also highly affected by pre-pupal treated with the fungal isolates. Thus, it can be concluded that the tested EPF have delayed effect on RTW, as a result could be employed to reduce the population of RTW and as effective and environmentally safe management option.

A) Deformed pupae B) Healthy pupae

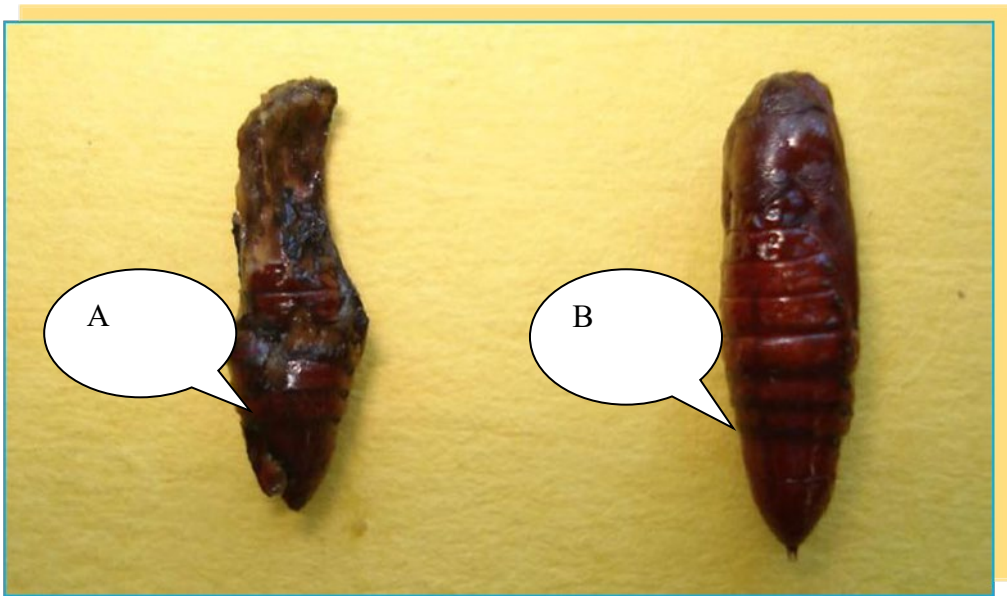

Fig.3 Deformed and Healthy Pupae of RTW when Treated with *B. bassiana* and *M. anisopliae* Isolates

Conclusions

The present study on the efficacy of *M. anisopliae* and *B. bassiana* isolates concluded that all tested isolates can cause infection on 3rd instar larvae of RTW and able to cause delayed effect to the next stages after application. Isolates MM is the most effective at conidial concentration of $1x10^9$ spores/ml to control larvae of *M. ignicollis* under both laboratory and greenhouse conditions. PPRC-2 and PPRC-56 can be used alternatively at concentration of $1x10^9$spors/ml. MM was the most toxic to RTW larvae because it caused mortality with the lowest concentration and showed faster response. Since lethal time and concentration levels were inversely related, to get immediate response using these isolates, one could increase the concentration. Further study with these EPF could be initiated mainly around the area of mass multiplication, formulation, and field persistence of these isolates.

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