

Potato Tuber Moth, *Phthorimaea Operculella* (Zeller) Management using Entomopathogenic Fungi on Seed Potato Tuber in West Showa, Ethiopia

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To cite this article:

Tekalign Zeleke, Bayeh Mulatu, Mulugeta Negeri. Potato Tuber Moth, *Phthorimaea Operculella* (Zeller) Management using Entomopathogenic Fungi on Seed Potato Tuber in West Showa, Ethiopia. *Journal of Plant Sciences*. Vol. 3, No. 4, 2015, pp. 207-211. doi: 10.11648/j.jps.20150304.16

Abstract: Potato Tuber Moth, *Phthorimaea operculella* is the major insect pest and causes damage both under field and seed storage of Potato, *Solanum tuberosum* in Ethiopia. For this major threat of productions the experiments were carried out in a simple CRD under laboratory and RCBD design under field condition having three replications to evaluate the Entomopathogenic fungi of *B. bassiana* (PPRC-56 isolate), *M. anisopliae* (MM isolate) and *V. lecani* each at 1x10⁴, 1x10⁵, 1x10⁶, 1x10⁷, 1x10⁸spore/ml (in laboratory) and 1x10⁶, 1x10⁷, 1x10⁸spore/ml (in field) for the management of Potato Tuber Moth (PTM). Laboratory investigation of *M. anisopliae* isolate significantly reduced the damage caused by PTM followed by *B. bassiana* isolate than the fungal type of *V. lecani*. The fungal species *B. bassiana* significantly reduced the infestation of PTM larvae both on the leaves and tubers followed by the *M. anisopliae* than the *V. lecani*. In general as the concentration levels of fungus increased, the number of larvae and the damage they caused on leaves were significantly decreased. From the presented studies for the effective management of PTM in potato using *B. bassiana* and *M. anisopliae* could be considered and using the higher fungal concentration also advantageous.

Keywords: Entomopathogenic Fungi, Potato Tuber Moth, Concentrations, *Phthorimaea operculella*

1. Introduction

Among the major insect pests were identified in Ethiopia by Bayeh and Tadesse (1992) Potato Tuber Moth, *Phthorimaea operculella*, (Lepidoptera: Gelechiidae) is the most economic important pest of potato and caused damage both under field and storage (Herman *et al.*, 2005). A study conducted at Alemaya, Eastern of Ethiopia in potato growing areas in 2001 revealed that, over 42% of potato tubers were infested by Potato Tuber Moth (PTM) and on the average, 8.7% of the potato tubers were lost due to field infestation by PTM. Field infested tubers are primary source of infestation in storage and later on in the field (CIP, 1988).

To reduce field damage and further infestation in storage, minimization of practices that increase exposure of tubers from one place to another, use of different management methods that are economical and selected appropriately

suitable to the environment, as well as which does not have significant adverse effect on non target organisms are strongly recommended (Sileshi and Teriessa, 2001).

PTM infestation and damaging of potato had been controlled using insecticides for the past years and has rapidly developed resistance to a wide variety of insecticides, hence, effective controlling foliage attack with current insecticides have not prevented occurrence of high level of tuber infestation (Clough *et al.*, 2008). Due to there are a limited work done on different management of PTM developing of Biological control methods for the pathogen agent to reduce the infestation of PTM, *P. operculella* in the field as well as under storage has thus become desirable to increase production and productivity of potato in the country.

Therefore, the objective of this study is to evaluate the effectiveness of different Entomopathogenic fungi and the concentration level in reducing infestation by PTM damage on potatoes.

2. Materials and Methods

2.1. Study Area

The experiment was carried out in insect science laboratory at Holeta Agricultural Research center (HARC) is located to the West Addis Ababa at 28km.

2.2. Rearing of PTM

The 3rd and 4th instars larvae of PTM, *P. operculella* were collected from potato field from leaves during irrigation time. The collected larvae were reared in transparent plastic cages in the Insect Science Laboratory at HARC, at ambient temperature and humidity. The 4th instars larvae that pupated on leaves and some larvae start to pupate moving from cages when from old leave to fresh leave at two days interval transferring was made. The pupae found on leave were collected regularly and kept carefully till adult emergence. The emerging adults were kept in ovipositor cages (in jars) to allow them lay eggs on tuber and leaves. As source of food they were provided with honey placed in all the ovipositor cages before adults started to emerge. At two days intervals fresh green leaves of potato were introduced and the old leave contain egg were transferred into another cages to allow the laid eggs to develop to 1st instars larvae. This routine activity was continued until the developing larvae were pupated. This was carried out in order to get sufficient population to conduct the trial.

Routine microbial protocols developed by (Fogalet *al.*, 1986) were followed to mass culturing and to determine the concentration of the spores of the pathogens and then were dipped in aqueous solutions prepared.

Potato tubers (n=25) were dipped in aqueous solutions of the EPF (*V. lecani*, *B. bassiana* (PPRC-56) and *M. anisoplae* (MM)), each at five concentrations level (1x10⁴, 1x05, 1x10⁶, 1x10⁷, 1x10⁸ spore/ml) and equal number of tubers dipped in Diazinon 60%EC were considered as a standard check and untreated tubers as control with sterilized distilled water having 0.01% Tween 80. Pair of adults (male and female) of PTM used for the experiment in per treatment introduced into transparent plastic cages on tubers. The experiment was set under room temperature in insect science laboratory at HARC. The cages were arranged again in a simple CRD design with three replications, therefore a total of 17 (seventeen) treatments were present in this trial. The tubers were checked for the level of infestation and the number eggs laid per tuber 3 days after a pair of adults PTM were introduced and the larvae present in tubers, damaged tubers and carved galleries present on damaged tubers were recorded after 30 days of introduction.

The abnormal and deformed PTMs were observed and the dead one was cultured on growth media of Sabouraud Dextrose Agar (SDAY) with extract Yeasts, which have prepared in a Petridis for checking of the fungal infection. Each Petri dish contained insects from the five concentrations and the three fungal species of isolate were kept separately. The fungi that subsequently started to grow on the insect

were observed and examined to their specific characteristics of the fungal species and the insect that had died from fungal infection.

2.3. Data Analysis

Collected data were subjected to the analysis of variance (ANOVA) with JMP IN (2000) version: 5.1 and SAS (2002) version 9.00 program software. Before to run ANOVA the different parameters were checked for normality and required transformation using logarithmic methods. Their differences among treatments were determined with a Tukey-kramer test at 5% probability level.

Natural mortality in the control was used to adjusted percent mortality of eggs, and then calculates the percent corrected mortality by using Abbott's formula (Abbott, 1925, cited by Agresti, 1990). Corrected Mortality (%) = $(\text{Mobs} - \text{Mcon.}) / 100 - \text{Mcon.}) * 100$. Where, Mobs represents % mortality of the observation and Mcon, % mortality in the untreated control. Then, run ANOVA for analysis of corrected egg mortality in treated tubers.

The LC50 of probit procedure outcomes of concentration on egg mortality of PTM and the correlation among variable was analyzed by using SAS system of version 9.00.

3. Result and Discussions

3.1. Oviposition Response

The effects of the three fungal isolates applied at different concentrations levels on tubers in preventing Oviposition by *P. operculella*, the number of eggs laid per tuber did not significantly influenced by the type of treatments applied.

The number of eggs laid on tubers treated by type of EPFs and level of concentration applied were not different significantly and also from untreated tubers. In this study doubting that potential of the EPFs was prevent the oviposition of PTM adult females released in treated tubers. The results were also supported by Hafezet *al.* (1997) that treated adult females of *P. operculella* with type of EPF; *B. bassiana* delayed effect on the egg production and also, feeding adult females of *P. operculella* with contaminated diet, resulted in a marked decrease in the deposited eggs. Khodadad *et al.* (2007) reported similar results of remarkable effects of *M. anisoplae*, *B. bassian* and *L. psalliotae* on the egg hatchability (%) and reproductive efficiency of *Rhipicephalus* (Boophilus) *annulatus* and the egg hatchability was suppressed in most of the treatments of *B. bassiana* against the Tobacco caterpillar, *Spodopteralitura* Fabricius (Noctuidae: Lepidoptera) in laboratory evaluation of the entomopathogenic fungi by Malarvannan (2010).

3.2. PTM Larvae in Tubers

The number of larvae counted in tubers from eggs laid on the treated tubers were affected by the EPFs and its five level of concentrations significantly (F 16, 32 = 51.9, P<.0001). In the tubers treated a number of larvae counted were increased for each type of EPFs as their concentration increased. In the

tubers treated with *V. lecani* the highest number of larvae was recorded at concentration levels of 1x104spore/ml and 1x105spore/ml and in *B. bassiana* at concentration of 1x104spore/ml than the other level treatments, but the lowest numbers of larvae were observed in *B. bassiana* a 1x108 and *M. anisopliae* at 1x107, 1x108spore/ml than the other treatments and as well as the standard check and untreated control significantly (Table 1).

3.3. PTM Damages

Effects of different EPFs at different concentrations were evaluated on tuber damage by PTM in laboratory. The percent of tuber damaged significantly affected by the treatments of the type of EPFs at their different level of (F 16, 32 = 21.9, P<.0001). Tuber damaged were recorded in the most lower of concentration for each type of funguses and no more tuber damage observed in the highest level of concentration significantly. In *V. lecani* at 1x104, 1x105 the highest percent tuber damage observed (86, 85%) and next by *B. bassiana* at 1x104 and 1x105spore/ml as well as in *M. anisopliae* at 1x104 and 1x105 spore/ml than the other level of treatments and this also not differ from the untreated control. The minimum damage tubers were also in *M. anisopliae* at 1x106, 1x107 and 1x108spore/ml and next by *B.*

bassiana at 1x107 and 1x108 spore/ml than the other treatments as well as than the standard check and untreated control (Table 1).

3.4. PTM Carved Galleries

The effect of the different EPFs and its concentration levels used on the number of PTM larvae carved galleries recorded on the treated tubers. The type treatments applied of EPFs at different level of concentrations were significantly affect number of galleries on tubers carved by PTM larvae (F 16, 32 = 94.2, P<.0001). The number galleries carved in tubers treated with *V. lecani* at the first two lowest concentrations were not significantly different, as well as the untreated control, but significantly different from the other level treatments applied and from the standard check. The 1x106, 1x107 and 1x108 spore /ml levels in each those three types of EPFs were significantly different from each lowest level in EPFs applied and as well as from the standard check and untreated control on number of galleries carved per tuber. The tubers treated with *M. anisopliae* at the highest level was also significantly reduced when compared to all level of treatments and again the standard check as well as untreated control (Table 1).

Table 1. Effect of EPF and their concentration levels on PTM larvae, damage tubers, galleries per tuber and mortality of eggs.

EPF	Conc.(Spore/ml)	PTM recorded (Mean ± SE)			
		Larvae	% DT	GPT	% CEM
<i>V. lecani</i>	1x10 ⁴	1.5±0.07 ^b	86.3±3.2 ^a	4±0.1 ^a	6.8±0.9 ^{ji}
	1x10 ⁵	1.4±0.06 ^b	85.3±2.9 ^a	3.9±0.07 ^a	12±0.3 ⁱ
	1x10 ⁶	0.9±0.1 ^{dc}	63.3±3 ^{cbd}	3±0.1 ^c	36±2.03 ^f
	1x10 ⁷	0.7±0.06 ^{fe}	51.7±5.9 ^{efid}	2±0.06 ^e	53±2.3 ^d
	1x10 ⁸	0.7±0.03 ^{fe}	51.7±4.4 ^{efid}	1.3±0.2 ^g	52.9±1.4 ^d
<i>M. anisopliae</i>	1x10 ⁴	0.9±0.1 ^{dc}	61.3±1.8 ^{cbd}	2.5±0.2 ^d	42.7±2.2 ^e
	1x10 ⁵	0.9±0.1 ^{dc}	58.7±2.2 ^{ced}	2.2±0.1 ^{ed}	42.2±1.7 ^e
	1x10 ⁶	0.6±0.3 ^{fg}	32. ±1.2 ^{gh}	1.2±0.1 ^{hg}	63.4±1.6 ^c
	1x10 ⁷	0.3±0.5 ^h	23.3±1.3 ^h	0.9±0.06 ^{hi}	80.7±2.3 ^a
	1x10 ⁸	0.3±0.4 ^h	20.7±1.2 ^h	0.5±0.13 ^j	83.3±2 ^a
<i>B. bassiana</i>	1x10 ⁴	1.4±0.1 ^b	71.7±2.8 ^{cb}	3.5±0.13 ^b	18.4±1.2 ^h
	1x10 ⁵	1.1±0.04 ^c	72.7±1.9 ^b	3.2±0.08 ^{cb}	30.6±2.4 ^g
	1x10 ⁶	0.8±0.1 ^{de}	46.7±2.4 ^{ef}	2.4±0.1 ^{ed}	47.8±0.4 ^d
	1x10 ⁷	0.6±0.07 ^{fg}	38.3±1.2 ^{gf}	1.8±0.08 ^e	63.1±2.5 ^c
	1x10 ⁸	0.4±0.07 ^{hg}	38.7±0.9 ^{gf}	0.9±0.13 ^{hi}	71.9±2.7 ^b
Diazinon 60% EC		1±0.1 ^c	46±6.7 ^{gh}	2.3±0.1 ^d	1.9±0.2 ^{jk}
Untreated		1.5±0.3 ^a	85±11.5 ^{cedb}	4±0.4 ^f	0.0±0.0 ^k
F-ratio		51.90	21.9	94.2	228.6
P-value		<.0001	<.0001	<.0001	<.0001

*Means ± SE followed by the same letter (s) within column (lower case letter) are not significantly different from each other at P< 0.05.

3.5. Eggs Mortality

The effects of EPF at different concentrations on mortality of eggs were recorded on potato tuber in laboratory. The type of EPFs at different level of concentrations applied was significantly affecting the mortality of eggs (F 16, 32 = 228.6, P<.0001). The mortality of eggs recorded was ranged from 6.8% in *V. lecani* at 1x104spore/ml to 83.3% in *M. anisopliae* at 1x108spore/ml under laboratory conditions. Again in the tuber treated with *V. lecani* at the two lowest level of concentration there were recorded the minimum of egg

mortality percent as compared to the other level of treatments. *M. anisopliae* at 1x108spore/ml and 1x107spore/ml there were none significant differences observed from each other on egg mortality, and next by *B. bassiana* at 1x107, 1x108spore/ml than the other its lower concentrations and in other type of EPFs concentration when they compared to the standard check and untreated control (Table 1).

Larvae present in tubers, percent damaged of tubers and carved galleries on tuber damaged were reduced in all the level of concentration and in types of EPFs applied. Number of larvae, percent of damage tuber and number of carved

galleries decreased as the level of concentration were raised from 1×10^4 to 1×10^8 spore/ml, in tubers treated with *M. anisopliae* of concentrations were significantly different than *V. lecani* and followed by *B. bassiana* at all concentration levels. In general from the studies the effective of EPF in reducing damage by PTM on tubers in laboratory help for storing potato seed tubers that was treated/dipped seed tuber after harvested and foliar application in field keeping to a period of time. The previous finding also stated by Foot (1976) that a number of foliar insecticides are used to against the PTM but, control of foliage infestation does not always prevent tuber infestation. In the absence of pesticides and for the merit of sustainable management of crops, natural controls constitute an important mortality factor of PTM and help to prevent tuber infestation as long as in storage. The results of studies were similar to the previous laboratory findings that demonstrated high virulence of *B. bassiana* against *H. armigera* larvae by (Sandhuet al. 2001) and the study of the results *M. anisopliae* infected larvae of *Ocinara varians* were not able to survive and resulting approximately 100% mortality, indicating that potential as a biological control agent against of this Lepidoptera insects as reported by (Abdiet al. 2009).

3.6. Dose Mortality against PTM Eggs

Results were presented in Table (2) that, *M. anisopliae* was more virulent to *P. operculella* eggs, where the LC₅₀ value was 1.3×10^7 and *B. bassiana* recorded the LC₅₀ of 1.4×10^7 and the *V. lecani* showed less virulence to PTM eggs as their LC₅₀ was 1.8×10^7 . The concentration dependent mortality value of PTM eggs were presented as dosage mortality obtained through probit analysis. The *M. anisopliae* dose mortality showed high number of egg mortality at the lowest level of concentration applied than the other two types of EPF.

The estimated LC₅₀ values based on the mortality eggs on three fungal type also the *M. anisopliae* showed the highest virulence with the lowest LC₅₀ value (1.3×10^7 spore/ml), and followed by *B. bassiana* (1.4×10^7 spore/ml) and *V. lecani* (1.8×10^7 spore/ml). This also supported by the reports made by (Hafez et al, 1997), which indicated that microbial control of PTM has been developed both in the field and storage, such as the fungi of *M. anisopliae* and *B. bassiana* which caused up to 80% infection and shortened larval life of PTM and lowered reproduction capacity of adults.

Table 2. Median lethal concentration (LC₅₀) of *M. anisopliae*, *B. bassiana* and *V. lecani* (conidia/ml) on mortality of PTM eggs under laboratory condition.

Fungal species	LC ₅₀ of EPF concentration on eggs mortality	
	LC ₅₀	95% Fiducial Limits
<i>V. lecani</i>	1.8×10^7	$2.1 \times 10^6 - 1.0 \times 10^8$
<i>M. anisopliae</i> (MM)	1.3×10^7	$2.4 \times 10^6 - 0.4 \times 10^8$
<i>B. bassiana</i> (PPRC-56)	1.4×10^7	$2.0 \times 10^6 - 1.0 \times 10^8$

3.7. Correlations

The concentration level applied in tubers treated with *M. anisopliae* were highly significantly and negative correlated with number of larvae, percent of tuber damaged and number of galleries, and highly positive correlated with percent of egg mortality.

The percent mortality of eggs with number of larvae, damaged tuber and number of galleries were very highly significantly and negatively correlated. Numbers of larvae and damage tuber, damage tuber and number galleries were also very highly positive correlated (Table 3).

Table 3. Correlation among parameter estimates during PTM damage management using EPF, *M. anisopliae*.

	CON.	LPT	EMPT	DT	GPT
CON.	1.00				
LPT	-0.95**	1.000	---	---	---
EMPT	0.95**	-0.99***	1.000	---	---
DT	-0.97**	0.98***	-0.98***	1.000	---
GPT	-0.97**	0.99***	-0.99***	0.99***	1.000

*LPT = larvae counted per tuber, EMPT = Egg mortality per tuber, DT = damage tuber, GPT = Galleries per tuber. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

4. Summary and Conclusions

In Ethiopia potato is an important crop in many part of the country and plays an important role as food source for many millions. PTM, *P. operculella* is a major threat to potato production worldwide as well as in Ethiopia because of its close relationship with its host, high adaptability of *P. operculella* has become a major pest of seed potatoes either in the field or in storage. The *P. operculella* (Lepidoptera: Gelechiidae) is pests potentially a cause of total crop loss in the form of discards or unfitness of tubers for seed. Therefore, it is important to find ways to manage PTM better by minimizing economic losses, by reducing harm to the environment or people working with the crop. In this study the tested fungal species isolates were found to be effective against potato tuber moth and resulted in causing high mortality and reduction of infestation.

There were liner relationships between concentrations and egg mortality, number of larvae, damage to tubers and number of galleries carved by PTM larvae. *M. anisopliae* (MM) had the highest virulence and may be potential to invade the PTM and by way of that reducing infestation in potato seed tubers followed by *B. bassiana* than by *V. lecani* in all the parameter estimated.

The findings presented here for effective control of *P. operculella* in potato with *M. anisopliae* and *B. bassiana* that could be developed as biological control and effective agent as an alternative to conventional synthetic insecticides in IMP program. From the present studies it is possible also to conclude that the uses of higher fungal concentration level of those fungi are advantageous for the better management of PTM. Interesting subjects for future studies could be the

testing of different fungal isolates of *B. bassiana* and *M. anisopliae* in storage management of PTM in seed potato tubers and to test different ways of using them, such as spraying, dipping or applying them in different traps.

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