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Evaluations of Two Isolated *Paecilomyces* Against *Phthorimaea operculella* (Lepidoptera:Gelechiidae) Under Laboratory and Field Conditions

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Abstract: ***Under laboratory conditions, the LC50 recorded 122X10^4 and 106X10^4 conidia/ml for the fungi, Paecilomyces fumosoroseus and Paecilomyces lilaceous, respectively. Under semi field conditions the corresponding LC50 136 X10^4 and 119 X104conidia/mlP. fumosoroseus caused a significant decrease in infestation reached to 21± 9.45 and 20± 8.12after 90 days of the post applications as compared to 89±13.03 and 79 ± 11.12 individuals in the control during season 2012 and 2013 ., respectively.The weight of the potato recorded during 2012 were, 5879± 8.28 and 5667± 7.18 kg/ feddan in the plots treated with P. lilaceous and P. fumosoroseus respectively as compared to 3398± 4.29 kg/ feddan in the control during season 2012. During season 2013 the highest weight scored 5992± 8.21kg/feddan after treatment with P. lilaceous.***

Keywords: Paecilomyces lilacinus,Paecilomyces fumosoroseus, Phthorimaea operculella

1. Introduction

Potato tuber moth *Phthorimaea operculella* PTM attacks solanaceous crops with potato being favored. Foliar injury is due to the larvae (tubeworm) mining into leaflets, causing them to form transparent blisters, then move into stem tissue causing death. Tubers are marred when larvae reach tubers by two major means. Upon hatching from eggs laid on leaves, larvae can drop to the ground and burrow through cracks in the soil to a tuber, entering it through the eye. This is common after vine desiccation. Another common way is that the female PTM lays its eggs directly on exposed tubers at or near the eye. When the larvae hatch, they just enter the tuber through the eye making a slender tunnel along the surface or deep into the tuber (pictured). A tunnel can be detected by mounds of worm excrement (frass) appearing black at the entrance (pictured). Tunnels do not heal and are entryways for diseases most notably soft rot and dry rot.In Egypt it is of the most economic important pests. Larvae cause severe damage to vegetable crops of family Solanaceae (Sarhan 2004; Soliman et al., 2008; Abul-Nasr et al., 1971& 1972; Sabbour, 2002). The entomopathogenic *P. carneus* is found on a wide range of material, and especially in soil. It is sometimes isolated from insects, though it appears to be a weak insect pathogen. Some isolates produce several metabolites of the antibiotic group cephalosporins. *P. farinosus* is also commonly isolated from soil. It is a well-known insect pathogen, and there has been interest in its use as an agent of biological control. Sabbourand Abdel-Rahman,2013, Sabbour et al,2011, Sabbour, 2002, Sahab et al, 2014 control the corn porwers by different entomopathogenic fungi under laboratory and field conditions. Entomopathogenic fungi are found worldwide associated to insects and phytophagous mite populations, contributing to biological control of these arthropods on several economically important crops (Sabbour and Sahab, 2007). Commercial products have been developed with entomopathogenic fungi (Alves and Pereira, 1998). Quintela and McCoy (1998) reported that fungal concentrations of 10^6 and 10^7 conidia/ml of *B. bassiana* and *N. rileyi* affected the larval development, movement and mobility of corn borers larvae during the seedlings and vegetative stages of corn plant under laboratory; greenhouse and field conditions. Success of a pest control program using *B. bassiana* however depends on conidia survival in the field environment (Benz, 1987). Conidia survival may be affected either by environmental factors (Furlong and Pell, 1997) or chemical products used to protect plants (Anderson and Roberts, 1983). Abdel-Rahman, *et al*. (2006) controlled the cereal aphids with the fungus *B. bassiana* and found that the infestation was reduced after fungal applications under laboratory and field conditions.

The present study aims to evaluate the pathogenicity of the two soil isolates of entomopathogenic fungus, *Paecilomyces lilacinus* and *Paecilomyces fumosoroseus* against potato tuber moth.

2. Materials and Methods

2.1 Tested Insects

Standard laboratory colony of the potato tuber moth *P. operculella* was reared on potato tubers *Solanumtuberosum* as a natural host plant under controlled conditions (26±2°C and 70±5% R.H). Eggs were obtainedfrom the stock culture and kept in Petri-dishes till larval hatch. The rearing technique by El-Sherif (1966) wasadopted. Pupae were individually kept in specimen tubes (1×3cm) till adult emergence. Adult moth were kept inoviposition cages that consist of chimney glass (8cm in diameter and 16cm height), the lower rim of whichrested on the bottom of a Petri-dish lined with a disk of filter paper (Watman) and the upper rim covered withmuslin. Each cage was provided with a small piece of cotton soaked in 5% honey solution as food supply. Thedeposited eggs were collected and kept in Petri-dishes till larval hatching. Groups of newly hatched

larvae were transferred into Petri-dishes containing fresh pieces of potato. Larval development was allowed to continue untilthe adult emergence

2.2 Entomopathogenic Fungi

The fungus, *Paecilomyces lilacinus*, and *Paecilomyces fumosoroseus* isolated from the Egyptian soil from Ismailia governorate. They were reproduced on potato dextrose agar (PDA) plus 0.4% yeast extracts (PDAY) and poured onto sterilized Petri-dishes (Alves *et al.*, 1998). Plating was performed according to the full dish method. The conidia were transferred from the Eppendr of vial to dish containing medium by platinum loop and then streaked. Plates were incubated at 25°C with 12 hours photo phase for fungus growth and sporulation. After ten days, conidia were scraped and transferred to conical flasks (200 ml) containing 200 ml sterilized distilled water with 0.02% the speeder sticker (tween, 80). Conidial concentrations in the suspensions were quantified directly under the optical microscope with a haemacytometer. Then the suspensions were standardized until the direct concentration $1x10^7$ conidia/ ml was obtained.

2.3 Efficacy of Entomopathogenic Fungi against Pests Larvae

Spores of the entomopathogenic fungi; *Paecilomyces lilacinus* and *Paecilomyces fumosoroseus*,collected from the surface of mycelium growth and spore suspensions with 2 drops of tween 80 were prepared and adjusted at $1x10^7$ conidia/ ml. Conidial viability was determined by counting germ tubes produced on PDAY medium after 18 hrs, using light microscope at 400 x. Conidial viability was 95-100%. The surface of cultures was gently brushed in the presence of 20ml of sterilized water in order to free the spores and the suspension was filtered through muslin. Six concentrations of spore suspensions were prepared *i.e.*, 10^7, 10^6, 10^5, 10^4, 10^3, and 10^2 conidia/ml. Piece of corn leaves were dipped in the prepared suspensions and left for drying under laboratory conditions then placed in Petri-dishes (one/dish). For each concentration (4 replicates/ each), ten L3 larvae of each of the tested insects were transferred into each Petri-dish. Control larvae were fed on untreated castor leaves. Percentages of mortality were calculated according to Abbot, while LC_{50} was calculated throughout probit analysis. The experiment was carried out under laboratory conditions at 26°C± 2 and 60-70 % RH. Physiological and metabolic characteristics of *P. fumosoroseus*and *P.* ***lilacinus***

2.4 Semi-Field (green house) Trials

Potato plant as planted in the green house in 40 plots in each artificial infestation was made by spraying the plant with the bioinsecticides*Paecilomyces lilacinus* and *P. fumosoroseus* at the concentrations of 8.25 x 10^8 conidia/ml for each of the fungi. Control samples were sprayed by water only. The plants were examined every two days, the percentage of infestation was calculated until the end of the experiment. Each treatment was replicated 4 times. The percent mortality was counted and corrected according to Abott, 1925while Lc-50swere calculated through probit analysis after Finney 1964.

2.5 Field Trials

Field trials were carried out at Nobaria region (Behera Governorate), Egypt during the two successive corn seasons 2012 and 2013 to study the effectiveness of the tested fungi on corn borers. Corn (variety Giza 2) was cultivated by end of May during the two seasons in an area of about half feddan. Fungi were applied as single treatments in randomize plots. Regular agricultural practices were performed and no chemical control was used during the study period. Weeds were removed by hand. Five plots were sprayed with water as control. Samples from each treatment were collected weekly and transferred to the laboratory for investigation. Percentages of infection were estimated.

2.6 Yield Assessment:

Yield data in treated and untreated plots in the corn harvest seasons (2012and 2013), represented by weight in kgs were determined. Yield loss was estimated according to the following equation:

$$\text{Yield loss} = \frac{\text{Potential yield} - \text{Actual yield}}{\text{Potential yield}} \times 100$$

Potential yield is*Paecilomyces lilacinus* treatment (the best result among the tested pathogens) was considered the standard for comparison with the other ones (Actual yield).

3. Results and Discussion

Data in tabl1 show that under laboratory conditions , the LC50 recorded $122X10^4$ and $106X10^4$ conidia/ml for the fungi, *Paecilomyces fumosoroseus* and *Paecilomyces lilaceous* ., respectively. Under semifield conditions the corresponding LC50 136 $X10^4$ and119 X104conidia/ml (Table 2). These results agree with Sabbour &Shadia Abd El-Aziz, (2002 and 2010), proved that applications with bioinsecticides increased the yield and decreased the the infestation with insect pests.Similar results were obtained by Sabbour, (2003 and 2007), Sabbour and Sahab (2005) and 2007); Sabbour and Ismail (2001) and Ismail and Sabbour (2002). loss of the yield by Sabbour &Shadia Abd El-Aziz, (2002 and 2010), Shadia Abdel Aziz & Nofel (1998), proved that applications with bioinsecticides increased the yield and decreased the infestations. Sabbour and Sahab (2005 and 2007), reported that the different entomopathogenic fungi could reduce many insect pests under laboratory and field conditions and causing yield increase under field conditions. Sabbour, (1992 and 1995, 2003 and 2006), Sabbour and Sahab (2005 and 2007) reported that many insect pests could be controlled by the bioinsecticides under field conditions. The same obtain by Sabbour and Abdel-rahman 2007, who recorded LC_{50}s of the tested fungi against the *Phyllotretacruciferaem*, *Pegomyiahyoscami* and*Cassidavittata* ranged between $5.4X10^6$ and $1.43X10^7$ spores/ml.

Table 3 show that inte field, the plots reated with *P. fumosoroseus*caused a significant decrease in infestation reached to 21± 9.45 and 20± 8.12after 90 days of the post applications as compared to 89±13.03 and 79 ± 11.12 individuals in the control during season 2012 and 2013 .,

respectively. The infestations with potato tuber mothwere significantly decreased to26 ± 10.11, 21 ± 9.12 and 20 ± 8.28 individuals after treatments with the fungus *P. lilacinus* after 20, 50 and 90 days .,respectively during season 2012. During season 2013 the corresponding number of infestation reached to 23 ± 12.11, 23 ±9.11 and 25 ± 9.71 individuals., respectively. The same results obtained by Sabbour 2003,(20014a&b), 2013.Magda Mahmoud Sabbour and Shadia El-Sayed Abd-El-Aziz. 2014, Magda Sabbour, 2001, Sabbour (2002 a &b), Magda Sabbour and Ismail2002, Sabbour and Sahab 2005 &2007, 20011.These results agree with Sabbour &ShadiaAbd El-Aziz, (2002 and 2010) Sabbour and Soliman (2014 a&b).

Table 4, show that the weight of the potato recorded during 2012 were, 5879± 8.28 and 5667± 7.18 kg/ feddan in the plots treated with *P. lilaceous* and *P.fumosoroseus*respectively as compared to 3398± 4.29 kg/ feddan in the control during season 2012 during season 2013 the highest weitsecorded 5992± 8.21kg/feddan after teament with *P. lilaceous* . (Sabbour &Sahab 2005, 2007, Tanda and Kaya, 1993 and Sahab and Sabbour 2011) found that the fungi *B. bassiana, M. anisopliae, Pacilomyces fumosoroseus Verticillium lecanii***;**reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. Sabbour and Abdel-Rahman 2013 found that, in all treatments the number of corn pests were significantly decreased. loss of the yield by Sabbour &Shadia Abd El-Aziz, (2002 and 2010), proved that applications with bioinsecticides increased the yield and decreased the infestations. They found that the infestation was reduced after fungi applications under laboratory and field conditions. Sabbour & Sahab (2005, 2007 and 2011) found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. These results agree with Sabbour&Shadia Abd El-Aziz, (2002 and 2010), proved that applications with bioinsecticides increased the yield and decreased the the infestation with insect pests.

4. Acknowledgment

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Table 1: Effect of the entomopathogenic fungi, against (PTM) the target insect pests larvae under laboratory conditions.

Insects	LC_{50}	slope	variance 95%	confidence limits
Paecilomyces fumosoroseus	$122X10^4$	0.1	1.01	99-166
Paecilomyces lilacinus	$106X10^4$	0.2	1.00	110-189

Table 2: Effect of the entomopathogenic fungi against the target insect pests larvae under semifield conditions.

Insects	LC_{50}	slope	variance 95%	confidence limits
Paecilomyces fumosoroseus	$136\ X10^4$	1.01	0.02	111-176
Paecilomyces lilacinus	$119\ X10^4$	0.1	1.01	134-187

Table 3: Effect of different treatments on the target insect pests under field conditions

Post 1st Application Date	Treatments	Number of infestation (means)±s.e during both two seasons *20122013*	
20	Control	52± 11.5	55± 7.89
50		53±9.33	54 ± 5.56
90		89±13.03	79 ± 11.12
20	*P. fumosoroseus*	31± 9.12	24 ± 8.23
50		28±11.20	27± 7.66
90		21± 9.45	20± 8.12
20	*P. lilacinus*	26 ± 10.11	23 ± 12.11
50		21 ± 9.12	23 ±9.11
90		20 ± 8.28	25 ± 9.71
F value		25.23	
Lsd%		.4141	

Table 4: Assessments of damage caused in potato field after the fungi treatment

Treatments	Season 2012 Wt of potato crop (kg/ feddan) yield loss%	Season 2013 Wt of potato crop (kg/ feddan) yield loss%
P. lilaceous	5879± 8.28-	5992± 8.21-
P. P.fumosoroseus	5667± 7.183	5887± 6.201
control	3398± 4.2942	3255± 3.2645
F value	28.6	26.8
Lsd5%=	15.1	14.1

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