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Pathogenicity of *Paecilomyces Lilicanus* Fungus Toward Sucking Insects Pests of Okra Crops and Their Predators

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ABSTRACT

The present work targeted to evaluate the role of entomopathogenic *P. lilicanus* fungus in overcoming the major okra sucking pests besides the side effect toward their predator. The targeted entomopathogenic fungus was cultured on Sabouraud dextrose yeast agar and prepared three Serial dilutions concentration suspensions to test their efficacy toward Aphids under laboratory and field conditions, while leafhopper was tested under field condition only.

The obtained results were cleared that the high concentration $1x10^8$ spore/ml, caused the best mortality percentage toward *Aphis gossypii* after 7 days of post-treatment**.** While under field conditions, the treatments were caused reduction ranged between 72.7% and 63.0 % toward *A. gossypi* population and 88.7%%, 86.7 % in leafhopper population, respectively. On the other side, the predators' population (F/coccinellidae) was also affected due to reducing the population of their prey.

INTRODUCTION

Okra, *Abelmoschus esculentus* L. Moench (Malvaceae) is a commonly grown green vegetable cultivated throughout the year and it is attacked by as many as 44 insect pests. Among them, sucking pests like leafhopper (*Amrasca biguttula* Ishida) and whitefly (*Bemisia tabaci* Gennadius) is a major threat, affecting okra production. Aphids and leafhoppers are important pests in the early stage of the crop which sucking sap of the plants, makes them weak and reduce the yield reach to 35.4 % to 96 % (Satpathy *et al.*, 2004 and Alak, *et al.*, 2017). In this regard, natural enemies occupy a central position in integrated pest management because of the safety of the cropping ecosystem (Telang *et al.*, 2004 and Sardana *et al*., 2005). The myco-insecticide based on *Beauveria bassiana* (Balsamo) Vaillemin, *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *Verticillium lecanii* (Zimm.) Viegas has been used to control various insect pests (Babu, *et al*. 2001, Sharma, 2004, Alter and Vandenberg 2000, Avery, *et al.* 2004, Butt, *et al.*, 2001)

The entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea Paecilomyces fumosoroseus* designated as *Isaria clade* have been used as myco-insecticides providing biological alternatives to chemical insecticides (Luangsa-Ard *et al*., 2005). Among their advantages are their reproductive potential and their persistence in the environment and they can play an important role in promoting integrated

pest management (Castrillo *et al.,* 2004; Faria and Wraight, 2001). The effectiveness of bio-pesticides against okra pests has been reported by Harischandra and Shekharappa (2009). Entomopathogenic fungi are natural enemies of various pests and are considered to be valuable bio-control agents in sustainable crop management (Alak, *et al* 2017). The present work targeted to test entomopathogenic *P. lilicanus* fungus toward major okra sucking pests and their predator.

MATERIALS AND METHODS

The experiments were carried out under laboratory and field conditions at National Research Center, Egypt during the summer season.

Source of Fungus Culture:

Paecilomyces lilicanus (Thom) was obtained from Mycological Center, Faculty of Science, Assiutt Univ. The isolate was cultured on Sabouraud dextrose yeast agar (SDYA) medium g/l, containing 40 g glucose, 20 g peptone, 20 g agar, 2 g Yeast extract and 1000 ml of distilled water in flasks, then autoclaved at 21 ^{0}C for 15 min (Sabouraud, 1892)

Preparation of Tested Bio-Agent:

Fungal culture was grown on Sabouraud dextrose yeast agar (SDYA) medium and incubated at 25±2 ºC in darkness for 14 days. Conidial suspensions were prepared by scraping cultures with sterile water containing 0.05% Tween 80 under a laminar flow chamber. The conidia were harvested by scraping the surface of the culture with an inoculation needle. The mixture was stirred for 10 minutes the hyphaldebris was removed by filtering the mixture through a fine-mesh sieve. The conidial concentration of the final suspension was determined by direct count using Haemocytometer. Serial dilutions were prepared at $1x10^6$, $1x10^7$ and $1x10^8$ concentrations and preserved at $5C^0$ until used.

Bioassay Treatments:

1. Under Laboratory Condition:

Normal fresh okra leaves symptoms (healthy) were collected, cleaned well by using wet cotton and placed in Petri-dishes 20cm. diameter provided by wet cotton for keeping okra leaves fresh. *Aphis gossypii* was collected from okra plants for artificial infestation (20 individuals/leave). The adjustable concentrations ($1x10^6$, $1x10^7$ and $1x10^8$spore / ml) were directly sprayed on the infested leaves. Three replicates per treatment were made all treatments were examined after three, five and seven days to calculate the percentage of mortality.

2. **Under Field Condition**:

The present study was carried out during the summer growing season at National Research farm, El Nobarya governorate in which cultivated of Okra crop, Baldy cv.

An area (about 700 m^2) was divided into four equal plots. Each plot with ridges (3 replicates) of 5 meters length and 60 cm apart; all normal cultural practices of land preparation, thinning, irrigation, mechanical weed control were followed out and kept free from any insecticidal application. One month after plantation before treatment, Samples were picked randomly from three levels of the plant (10 leaves of okra plants from each replicate making a sum of 30 leaves for each treatment). After that spraying tested fungus was carried out by using a handling sprayer using 10L from prepared fungal concentration to cover one karat. The random samples were picked up at interval times after spraying. Each one was kept in a tightly closed paper bag and transferred to the laboratory to record the number of dead adults in both *Aphis gossypii* and leafhopper with the aid of the Stereo - binocular microscope.

3 - Statistical Analysis:

The percent reduction of infestation was statistically calculated according to the equation of (Henderson and Tilton). Mortality % = 100 (1- Ta x Cb / Tb xCa).

Where:

Ta = Post treatment insect counts.

Cb = Untreated insect count before treatment.

Tb = Pretreatment counts.

Ca = Untreated insect count after treatment.

RESULTS

1. Under Laboratory Conditions (*in vitro*):

Data in Figure (1) show that the mortality percentage of aphids increased with increasing fungus concentration and time of exposure to treatment. The treatment was caused mortality % to Aphids individual ranged between 37.1 to 98.7% at 10^6, 10^7 and 10^8 spore/ ml, after post 3, 5 and 7 days respectively. The symptoms of aphids mortality were cleared in Fig (2).

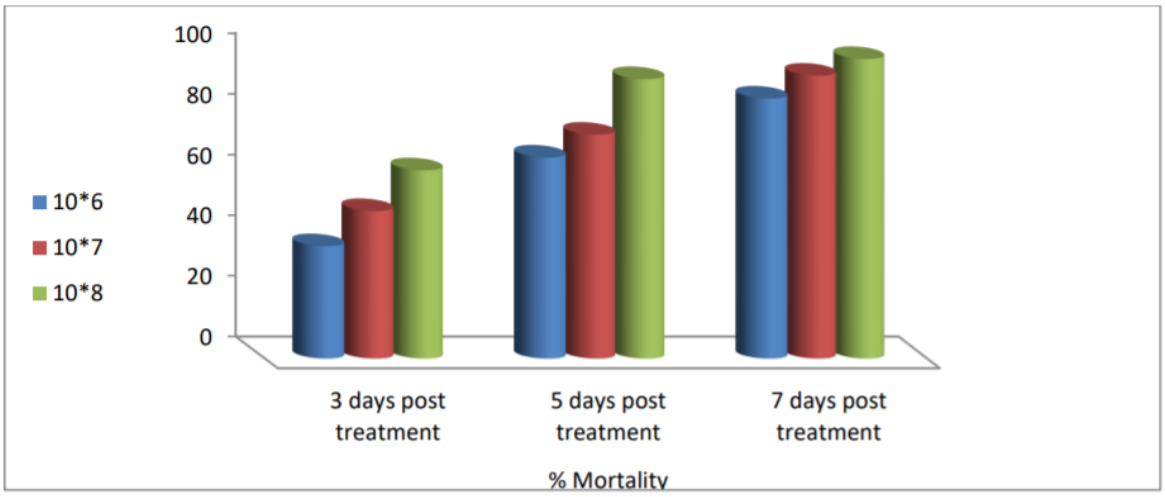

Fig.1: Effect of different concentrations of *Paecilomyces lilicanus* fungus on aphid mortality under laboratory condition**.**

Fig.2: Morality symptom of aphids after treatment with pathogenic fungus showing fungus growth on the outer surface of aphids

2- Under Field Conditions (*in vivo*):

2.a on an aphids population: Data in Table (1) described that *P. lilicanus* had a myco-insecticide effect which reduces significantly the population dynamic of aphids and extends their effect to 10 days recorded reduction reaches to 72.7and 63.0% after 5 and 10 days of treatment, respectively.

Table (1): Effect of *P. lilicanus* fungus against *A.gossypii* on okra under field condition

Tested materials	Before Treatment	After5 days	Reduction %	After 10 days	Reduction %
P. lilicanus	73.6±32.188	18.6±4.970	72.7%	31.6±2.33	63.0%
Control	74±7.234	68.6±19.18	--	86±9.53	--
Statistical analysis	**L.S.D 0.05=91.48 L.S.D 0.01=126.05**				

2.b- On Leafhopper Population: Data in Table (2) cleared that *P. lilicanus* had a bio-pesticide effect. It reduces significantly the population dynamic of leafhopper and their effect was extended to 10 days so, the reduction was reached to 88.7and 86.7% after 5 and 10 days of treatment, respectively.

Table (2): Effect of *P.lilicanus* fungus against leafhopper on okra under field condition**.**

Tested materials	Before Treatment	After5 days	Reduction %	After 10 days	Reduction %
P. lilicanus	15±4.1633	2.33±1.20	88.7%	3.0±2.08	86.7%
Control	20±5.13	27.6±1.3	-	30.3±4.4	-
Statistical analysis	**L.S.D0.05=22.85 L.S.D0.01= 31.48**				

3. On Predator: The results in Table (3) indicated that *P. lilicanus* caused a reduction in the population dynamics of a predator to reach 76.4%and 43.3% after 5 and 10 days of treatment, respectively. From investigation wasn't observed mortality **a**mong individuals of the predator by fungus so their effects were attributed to the reduction in the population of their prey.

Table (3): Effect of *P. lilicanus* fungus against Predator (F/Coccinellidae) on Okra under field condition

Tested materials	Before Treatment	After5 days	Reduction %	After 10 days	Reduction %
P. lilicanus	14.3±2.926	1.33±1.33	76.4%	5.33±1.201	43.3%
Control	7.6±3.92	3±0.577	-----	5±2.30	-------
Statistical analysis	**L.S.D 0.05 =16.717 L.S.D 0.01 =23.03**				

DISCUSSION

Our results showed that the high concentration 1x 10^8 of entom-pathogenic fungus was elicited effects toward aphids under laboratory test. While it showed the best effect toward reduction population of aphids for 10 days under field test. On the other side, leafhopper, *E. decipiens* were affected by tested materials. *P. lilicanus*the best effect toward reduction population of aphids for 10 days under field test. These results agree with (Ibrahim, *et al*., 2020) who showed that *M. anisopliae* (S1) caused the best impact on the population of aphids to extend to 10 days. Also, (Harischandra and Shekharappa,2009) studied the bio-efficacy of different entomopathogenic fungal formulations crude, wettable powder and oil-based formulation of *Beauveria bassiana, Metarhizium anisopliae* and *Verticillium lecanii* against sucking pests of okra during kharif 2007-2008. Oil-based formulation of *M. anisopli* recorded 5.25 leafhopper/3 leaves followed by. *bassiana* oil-based (6.88) and *V.lecanii* oil-based (7.75) after the second spray. (Moustafa *et al*., 2019) Showed that the toxicity of *P. lilicanus* was higher on *P. gossypiella* treatment. On the other hand, the effect of the *P. lilicanus* on *C. carnea* eggs was obviously effective, whereas, *C. septempunctata* was not affected after the same egg's treatment.

(Dar, *et al*.,2020) who showed that the most effective reduction percentage of bolls infestation *E. insulana* is *M. anisopliae, B. bassiana* either isolates or (W. P.) with Economy Micron ULVA (15 L./Fed.) followed by *Bacillus thuringiensis*. (Desoky *et al.*, 2020) showed that in this study *Aphis craccivora* nymph infected *P.lilacinum. P.lilacinum* has a high toxic effect than *M. anisopliae* in which the LC_{50} of fungal spore suspensions and culture filtrates after 7 days post-treatment was (7×10^2,1×10^5 spore/ml and 3.2, 4.7 %) for *P. lilacinum* and *M.anisopliae*, respectively. (Sadek, *et al*.,2021) *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces lilicanus* produced a moderate effect on *Thrips tabaci Metarhizium anisopliae* and *Paecilomyces lilicanus* recorded the same mortality 82% and 82% at 10^8 spores/ ml.

CONCLUSION

The obtained results in this study explore the pathogenicity of the entomopathogenic fungus, *P. lilicanus*as promising biological control agent alternative to chemical control against sucking pests.

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ARABIC SUMMARY

القدرة الإمراضية لفطر *Paecilomyces lilicanus* تجاه الحشرات الثاقبة الماصة ومفترساتها علي نبات البامية.

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استهدفت الدراسة الحالية تقييم دور فطر *Paecilomyces lilicanus* الممرض للحشرات في التغلب على آفات البامية الماصة بالإضافة إلى التأثيرات الجانبية للمفترس. تم زرع الفطر المستهدف الممرض للحشرات على Sabouraud dextrose وتحضير ثلاث تركيزت متسلسله لاختبار فعاليتها تجاه حشرات المن تحت الظروف المعملية والحقليه .تم توضيح النتائج التي تم الحصول عليها بأن التركيز العالي $1x10^8$ spore / ml تسبب في أفضل نسبة وفيات تجاه حشرات المن بعد 7 أيام من العلاج. في ظل الظروف الحقلية ، تسببت المعاملات في انخفاضات تراوحت بين 72.7٪ و 63.0٪ في عشيرة *A. gossypii* و 88.7 ٪ ، 86.7٪ في الأوراق على التوالي. على الجانب الآخر ، تأثرت أعداد المفترسات (*F / coccinellidae*) أيضًا بسبب تقليل عدد فرائسها.