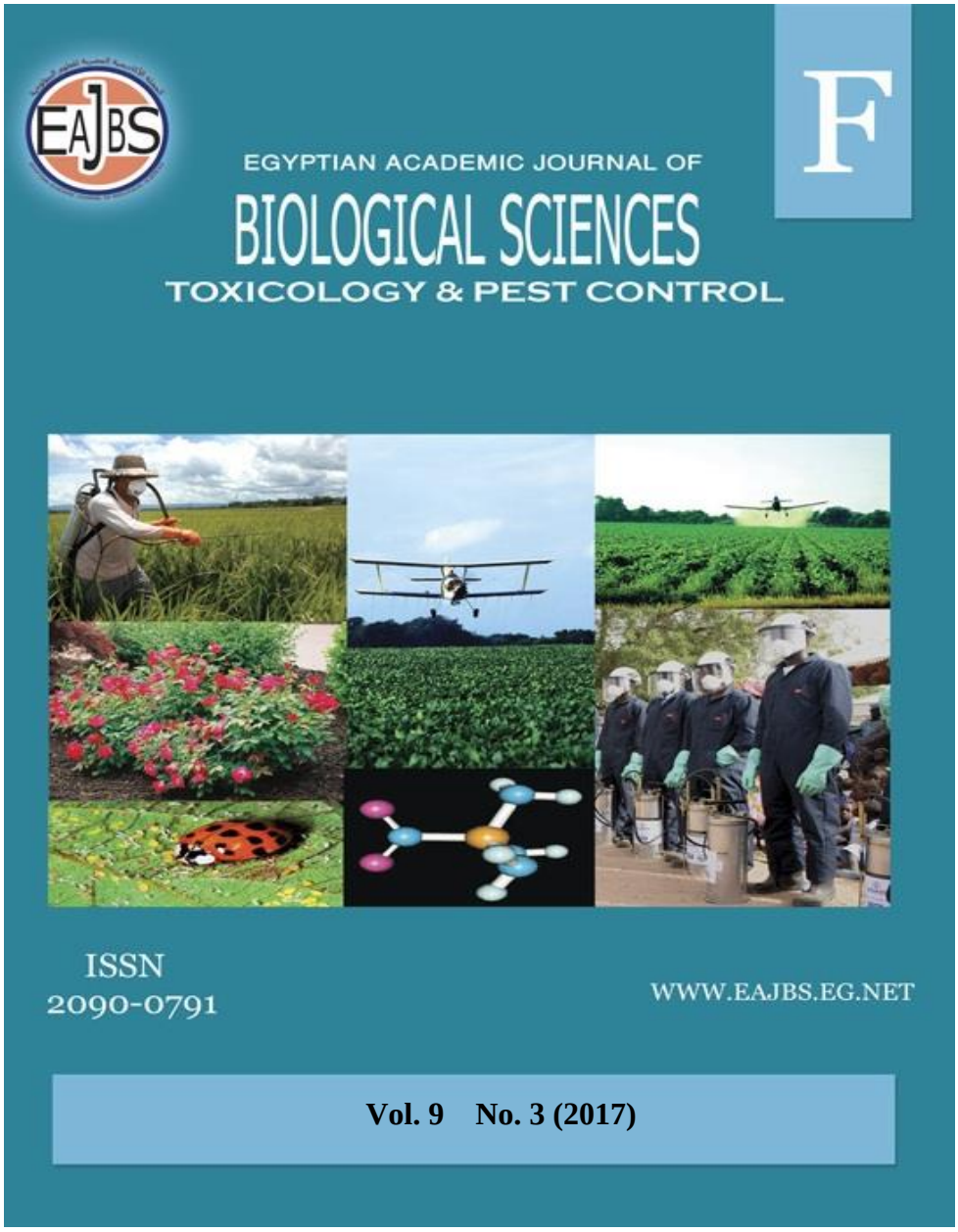

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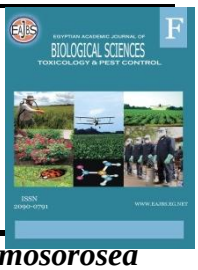

Laboratory Evaluation of an Entomopathogenic Fungus, *Isaria fumosorosea* wize pa208 against Two-spotted Spider Mite, *Tetranychus cucurbitacearum* (SAYED)

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ABSTRACT

The influence of the entomopathogenic fungus, *Isaria fumosorosea* Wize PA208 was evaluated against the two-spotted spider mite, *Tetranychus cucurbitacearum* (Sayed) under laboratory conditions at Plant Protection Research Institute, Sharkia branch, Egypt. Two different application methods; spray and dipping techniques of fungi spores suspension were tested at 25, 30±2˚C and 70±5 % R.H. Mortality percentages increased with an increase of spore concentration, exposure time and temperature degrees using spray compared with dipping technique. LC_{50} values were 2.14×10^6 and 1.70×10^4 spores/ml after four and seven days of spray application at 30 ˚C, respectively. On the other hand, LC_{50} values were 8.95×10^6 and 2.77×10^6 spores/ml after four and seven days of dipping technique at 30˚C, consecutively.

INTRODUCTION

Spider mites (Acari: Tetranychidae) are significant herbivorous pests and, the two-spotted spider mite, *Tetranychus cucurbitacearum* (Sayed) is considered one of the important ones, because its damage to many field, vegetable and horticultural crops in Egypt (Zaher, 1984). The wide use of acaricides caused many problems such as environmental pollution, destruction in the natural balance between the pest and its natural enemies develop resistance to acaricides and the severe restriction of chemical pesticides. In Egypt, climatic conditions are considered more suitable for fungal pathogens (Sewify, 1989). So, there is an increasing attention to biological control agents of phytophagous mites by developing microbial biopesticides against the spider mite as an alternative to broad-spectrum chemicals (Afifi *et al*., 2010; Zemek *et al*., 2016).

Entomopathogenic fungi play an important role in regulation of natural mite populations, and reduce populations of phytophagous mites (Van der Geest *et al*., 2000). The fungus, *I. fumosorosea* was known as *Paecilomyces fumosoroseus* for more than 30 years, which have a worldwide distribution and a relatively wide host range. The adult female of *Tetranychus urticae* Koch showed higher susceptibility to *I. fumosorosea* (92.1% mortality) compared to eggs (53.5% mortality) (Zemek *et al*., 2016).

This fungus was found to be highly virulent against several pests (Zemek *et al*., 2012; Hussein *et al*., 2013; Hussein *et al*., 2016) and therefore, considered to have an application potential. *Paecilomyces* spp., a common insect borne filamentous fungus, belong to Hyphomycetes of Deuteromycota and has been reported to cause diseases in a wide species of insects, occasionally resulting in natural epizootica (Nam *et al*., 2000).

Panyasiri *et al*., (2007) found five strains of *I. fumosorosea* isolated from a coleopteran, an unknown host and white flies, were highly active against thrips, *Ceratothripoides claratris* caused (80-93% mortality); moderate activity against *Bemisia tabaci* caused (37-77% mortality) and less active against mealybug, *Pseudococcus cyrptus* (10-43 % mortality).

The objective of this work was to study the pathogenicity of the entomopathogenic fungus *I. fumosorosea* PA208 on *T. cucurbitacearum* using two different methods of applications at 25 and 30 ˚C.

MATERIALS AND METHODS

Mite culture:

The initial culture of *T. cucurbitacearum* was collected from eggplants, *Solanum melongena* L. (Solanaceae) at Zagazig district, Sharkia Governorate, Egypt and established in the laboratory of Acarology, Plant Protection Research Institute (Sharkia Branch), Egypt. The spider mite was reared on mulberry leaves at 25, 30 ± 2˚C and 70± 5% R.H. The deutonymphs were collected from the mite culture using a fine camel hairbrush and placed on mulberry discs (3 cm in diameter). Two days later, newly emerging adult females of *T. cucurbitacearum* were excluded and used in the laboratory experiment according to Bugeme *et al*., (2008).

Isolation of fungus:

The fungus, *Isaria fumosorosea* was isolated from naturally infected *Ceroplastes floridensis* Comstock (Soft scale insect) collected from lemon trees at Sharkia Governorate, 2015 season. The insects showing signs of fungal infection or the living individuals inspection of the infection were collected and placed in containers in a standard conditions (25±2˚C and 90±5% R.H.) and daily examined for the appearance of mycosis symptoms. The insects with fungus symptoms were suspended in 10 ml saline solution (9 g NaCl in 1 L of H_2O) and shacked well. Serial dilutions were made by aseptically transferring 1ml. of the original suspension to 9 ml sterilized saline solution, shaked well to give 10^{-1} up to 10^{-6} dilutions. One ml of each concentration was transferred to sterilized Petri dish (9cm ×1.5 cm). Czapek-Dox's agar medium and potato dextrose agar medium were poured separately in the dishes aseptically. Rosebengal combined with streptomycin (1/50000 + 30 µg/ml, respectively) was used as bacteriostatic agent (Smith and Dawson, 1944 and Martin, 1950). The plates were horizontally shaken on the surface to spread the suspension and left until solidification of agar. They were incubated inverted in an incubator at 25±2˚C for 7 days. At the end of the incubation period (7 days), the plates were viewed for fungal growth. The formed colonies appeared were purified and identified by Prof. Ahmed Kamel Abd-Elsalam, Mycology Research and Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt.

Preparation of inocula:

Inoculum was prepared by agitating a slant of the fungal isolates (7 days old fungal culture) with 10 ml sterile distilled water using an inoculated needle. One ml of the spore suspension was then used as standard inocula.

Erlenmeyer conical flasks (250 ml capacity), containing 50 ml of fermented medium, were used. The PH of the medium adjusted to 6. Each flask, after being cooled, was inoculated with 1 ml spore suspension (standard inoculum) under aseptic conditions. The culture flasks were incubated at 25 ˚C.

Preparation of the fungus concentrations:

Spores of fungal isolates were harvested by rinsing with sterilized

0.005% Tween 80 from 7- day old culture (PDA media grown at 25±1˚C for *I. fumosorosea* isolates). The suspensions were filtered through cheese cloth to reduce mycelium clumping. The spores were counted in the suspensions using a haemocytometer. The concentrations were adjusted to 2×10^6, 3×10^6, 1.5×10^7, 5×10^7 and 1×10^8 spores/ml.

Bioassay procedure:

The pathogenicity of the fungus, *I. fumosorosea* on the adult females of *T. cucurbitacearum* was evaluated by two methods; leaf-dip and spray techniques at 25, 30 ±2˚C and 70± 5 % R.H. Treatments and the control were replicated five times, with 10 adult females of *T. cucurbitacearum* for each dipping and spray techniques. Control discs were prepared in both methods using mixture of water and 0.005 % Tween 80.

Adult females of *T. cucurbitacearum* were confined on the lower surface of mulberry leaf discs previously dipped in tested concentrations of each treatment for 10 seconds using the leaf-dip technique method as described by Dittrich (1962). In the second method, plant mulberry discs were treated by direct spray (spraying in the presence of the mite individuals) using a hand atomizer, then kept at 25 and 30ºC. The mortality percentages of the treated adult females were calculated 4 and 7 days post application, according to Abbott's formula (1925).

Statistical analysis:

Data were analyzed according to Finney (1971), LC_{50} and LC_{90} were calculated using Ldp line® software.

RESULTS AND DISCUSSION

The results cleared that the spray technique was the most active compared with dipping application at 25±2 and 30±2˚C. The higher fungus concentration caused the highest mortality on *T. cucurbitacearum* (Table 1).

Table 1: Mortality percentages of *Tetranychus cucurbitacearum* adult females after 4 and 7 days of spray and dipping applications with different concentrations of *Isaria fumosorosea* and LC_{50} and LC_{90} under laboratory conditions (25, 30 ± 2˚C and 70 ± 5 % R.H.).

Concentration Spores/ml	Mortality percentages of *T. cucurbitacearum* adult females							
	Spray technique				Dipping technique			
	25 ˚C		30 ˚C		25 ˚C		30 ˚C	
	4 days	7 days	4 days	7 days	4 days	7 days	4 days	7 days
2.0×10^6	27.03	37.84	54.05	72.97	20.00	32.50	30.77	43.59
3.0×10^6	27.50	52.50	47.37	73.68	29.73	40.54	35.00	50.00
1.5×10^7	67.50	70.00	55.26	78.95	34.21	47.37	59.46	78.38
5.0×10^7	72.50	75.00	58.97	82.05	55.00	72.50	75.00	87.50
1.0×10^8	60.00	86.67	65.00	87.50	69.23	84.62	73.33	88.24
LC_{50}	12.55×10^6	3.61×10^6	2.14×10^6	1.70×10^4	29.12×10^6	8.42×10^6	8.95×10^6	2.77×10^6
LC_{90}	10.63×10^8	2.21×10^8	8.57×10^{12}	5.72×10^8	19.93×10^8	3.69×10^8	5.02×10^8	8.37×10^7
Slope	0.665	0.717	0.194	0.283	0.698	0.781	0.733	0.866

Spray technique:

Data presented in Table (1) showed that fungal concentrations at 25±2˚C gave higher mortality after seven days compared with four days after applications. The LC_{50} and LC_{90} values were 12.55×10^6 and 10.63×10^8 spores/ml compared with 3.61×10^6 and 2.21×10^8 spores/ml after four and seven days of application, respectively.

The same trend was obtained at 30±2˚C and 70±5 % R.H. on adult females of *T. cucurbitacearum*. The LC_{50} and LC_{90} were recorded 2.14×10^6 and 8.57×10^{12} spores/ml compared to 1.70×10^4 and 5.72×10^8 spores/ml after four and seven days of application, consecutively.

Dipping technique:

Results in (Table 1) revealed that the pathogenicity of fungus concentrations caused higher mortality of *T. cucurbitacearum* females at 30 than

25±2°C. The LC_{50} and LC_{90} values at 25˚C were 29.12×10^6 and 19.93×10^8 spores/ml compared to 8.42×10^6 and 3.69×10^8 spores/ml after four and seven days of application, consecutively.

Whereas, LC_{50} and LC_{90} values at 30±2˚C recorded 8.95×10^6 and 5.02×10^8 and 2.77×10^6 and 8.37×10^7 spores/ml after four and seven days of application, respectively.

Mite control programes aim at maintaining two-spotted spider mite at low levels throughout the year, i.e. the populations are allowed to reach an acceptable level before the mites become difficult to control (Zhang, *et al*. 2016). Therefore, the important targets in integrated pest management (IPM) of the mites are the eggs and females. Some studies indicated that pathogenicity of many entomopathogenic fungi towards *Tetranychus urticae* Koch can be disruption of mite development through their penetration and subsequent nutrient taking (Zhang, *et al*. 2014 and Shi & Feng, 2009). The pathogenicity of the fungus *I. cateniannulata* (Liang) towards female of *T. urticae* gave 100% mortality of the female mites, and its sporogenous structure could be observed on the mite body 3-5 days after treatment (Zhang *et al*., 2013). The same authors recorded that a suspention of 2×10^7 submerged conidia ml^{-1} caused the highest mortalities of mite eggs, larvae and females were 100, 100 and 70 %, respectively at 100 % R.H. The temperature is considered one of the important factors affecting the natural activity of parasitic fungi. The rapidity of mycelial development and evaluation of infection depend on temperature. In general, the optimum growth and germination rates on artificial media vary around 25°C for *I. fumosorosea*. The mycelial growth of *I. fumosorosea* has been expedited gradually in proportion to the rise of temperature and was the most suitable at 25°C. Even though the mycelial growth of *I. fumosoroseus* was favorable at the range of 20-25°C and had been expedited in proportion to rise of temperature, the mycelial growth appeared to be suppressed at the temperature higher than 30°C (Macleod 1963; Kalvish 1974; Fang *et al.*, 1985; Moore *et al*., 2000 and Shim *et al*., 2003). Similarly, there was slow growth at 15 and 35°C. Hussein *et al.* (2014) studied the toxicity of *I. fumosorosea* spores suspension on citrus mealybug, *Planococcus citri*; the LC_{50} and LC_{90} values were 5×10^6 and 4×10^{13} spores/ml, respectively. Zemek *et al*. (2016) revealed that *Tetranychus urticae* Koch females showed higher susceptibility to the entomopathogenic fungus *I. fumosorosea* at concentration 4×10^7 spores/ ml (92.1 % mortality) compared to eggs (53.5% mortality).

According to the current results the fungus, *I. fumosorosea* may be a promising biological control agent of *T. cucurbitacearum,* especially that this entomopathogenic fungus is easily cultured on artificial media.

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ARABIC SUMMERY

التقييم المعملى للفطر الممرض للحشرات *Isaria fumosorosea* Wize PA208 ضد الحلم العنكبوتى ذو البقعتين *Tetranychus cucurbitacearum* (Sayed)

هانى محمد جلال القواص- حسن أحمد نبيل- فاطمة شحاتة قلموش- رنا حسين محمد حسين
معهد بحوث وقاية النباتات- مركز البحوث الزراعية – جيزة – مصر.

تم تقييم فعالية الفطر الممرض للحشرات *Isaria fumosorosea* Wize PA208 علي الحلم العنكبوتي ذو البقعتين *Tetranychus cucurbitacearum* (Sayed) معمليا بمعهد بحوث وقاية النباتات فرع الشرقية ، مصر.حيث تم إختبار طريقتين للمعاملة هما طريقتى الرش والغمر بمعلق جراثيم الفطر على درجتى حرارة ٢٥ و٣٠±٢ درجة مئوية و رطوبة نسبية ٧٠±٥%.

وجد أن النسبة المئوية للموت تزداد بزيادة تركيز معلق جراثيم الفطر المستخدم وزمن التعرض ودرجة الحرارة باستخدام طريقة الرش. حيث وجد أن قيم LC_{50} بعد ٤ و ٧ أيام من معاملة الاكاروس باستخدام طريقة الرش على درجة حرارة ٣٠م° كانت ٢,١٤ × ١٠^{٦} و ١,٧ × ١٠^{٤} جرثومة/ملل، على الترتيب. بينما كانت قيم LC_{50} هى ٨,٩٥ × ١٠^{٦} و ٢,٧٧ × ١٠^{٦} بعد ٤ و ٧ أيام من معاملة الاكاروس غمراً على درجة حرارة ٣٠م°، على التوالى.