USING *PSEUDOMONAS FLUORESCENS* AS MICROBIAL BIOCONTROL AGENT AGAINST THE SPIDER MITE,*TETRANYCHUS CUCURBITACEARUM* (SAYED)(ACARI: TETRANYCHIDAE)

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Abstract

This study investigated *Pseudomonas fluorescens* as a potential biological control agent of *Tetranychus cucurbitacearum* by using two different methods; spraying and dipping. The earliest death occurred within the first day after the treatments. The highest mortality (100%) and the lowest 68.75% for the spraying bacterial treatment and 58.75% and 16.25% for the dipping. The longevity was shorter than control in both methods. Female longevity averaged 10.32 and 17.04 days for the spraying and the dipping while, 22.5 and 24.19 days in control. The bacterial treatment reduced the total deposited eggs in females to half approximately in the spraying method while, in the dipping method was nearly with the control. The chitinase enzyme present in the cell was confirmed by the positive result of chitin clearing zone.

Keywords: *Pseudomonas fluorescens*, *Tetranychus cucurbitacearum,* biological control, chitinase

INTRODUCTION

In Egypt, field and vegetables crops are considered of great economic important for local consumption and exportation. Plant parasitic mites tend to be serious agriculture pests worldwide Zaher (1986). Mites especially family tetranychidae are common pests in agriculture system causing in many cases greater economic losses than any other arthropods pests. *Tetranychus cucurbitacearum* (Sayed) is considered one of the major pests attacking different crops; field, vegetables, fruits and ornamental plants (Taha *et al*. 2001 and Magouz and Saadoon 2005). Chemical control is a commonly used management tactic against *T.cucurbitacearum* in several crops in Egypt. The intensive use of pesticides has caused a considerable reduction of its efficacy due to the evaluation of resistance that indicates a need to develop alternative integrate pest management strategies for suppressing its population Amin *et al.* (2009). One of the alternative managements is using *Pseudomonas fluorescens* as microbial biocontrol agent. *Pseudomonas putida* as a potential biological control agent of *Tetranychus urticae.* Bacterial application significantly reduced total egg numbers and egg hatching, compared to their

respective controls. Bacterial spraying was significantly more effective than dipping the spray application demonstrated 100% efficacy Aksoy *et al*. (2008). Bacterial chitinases have been reported to be effective in controlling the insects and mites by hydrolyzing chitinous exoskeleton Kramer and Muthukrishnan, (1997). Highest rate of reduction and effectiveness on spider mite 100% mortality was achieved after four days by using high concentration of *P. fluorescens* Al-Sohim and Fouly (2015).

MATERIALS AND METHODS

Isolation of *Pseudomonas*

The 10 cm rhizhosphere soil particles loosely adhering to the roots were gently teased out and the roots were cut into small pieces and mixed well. The soil thus obtained was shaken with 90 ml of sterile distilled water for 10-20 min. to obtain standard soil suspension. Isolation of *Pseudomonas* was made by following the serial dilutions and pour plate method using the specific King's B medium King *et al.*(1954). One ml of soil suspension from aliquot dilutions was aseptically added to sterile Petri plates containing twenty ml of sterile medium and incubated at 28±2 ºC for 48 h. After incubation, well separated individual colonies with yellow green and blue white pigments were marked and detected by viewing under UV light. The individual colonies were picked up with sterile loop and transferred to fresh King's B slants and the pure cultures so obtained were stored in refrigerator at 4ºC for further use. Morphological characterization of pure cultures of the selected isolates was streaked on King's B agar Petri plates separately for colony development. The individual colonies were examined for shape, size, structure of colonies and pigmentation.

Morphological and Biochemical characteristics of the isolated bacteria

The direct microscopic examination of stained smears of bacterial isolates was carried out for studying shape of the bacterial cells, gram staining, spore staining and motility. Biochemical testes of these cultures were also examined according to the key of Bergey's Manual of Systematic Bacteriology (Krieg and Holt 1984 and Sneath *et al.* 1986).

Stock culture of spider mite, *Tetranychus cucurbitacearum*

Population of *T.cucurbitacearum* collected from Cucumber plant (*Cucumissativus*) zagazig district. Samples that contained the mites were transferred immediately to the laboratory used as stock culture. From the stock adults and immature stages were transferred onto fresh mulberry leaves (*Morusalba* L.) placed on moistened cotton pads in plastic trays. Rearing trays were kept under controlled conditions of 27±2 ºCand 65±5 R.H. Withered and dry leaves were regularly replaced.

Bioassay

The effect of *P. fluorescens* was evaluated against adults of spider mite adopting two different methods, spraying and dipping methods of bacterial suspension at the concentrations of 100, 75 and 50%. Spray method was applied with Eighty adult females of *T.cucurbitacearum* transferred means of camel· s hair brush bio-assayed in 8 replicates of 10 adult females each to mulberry leaf discs each about 1.5 cm diameter were gently sprayed separately in serial concentrations of *P. fluorescens* and putted in petri-dishes, 10 cm. diameters. Dipping method was bio-assayed with the same number of adult females of *T.cucurbitacearum* from the above method. Mulberry leaves were dipped in each concentration for 10 seconds then left to dry that putted in petri-dishes that adult females of *T.cucurbitacearum* were transferred. Bioassay included untreated check in which leaves were sprayed and dipped in water only. All petri-dishes were held at the same conditions of 27±2 ºC and 65±5 R.H. All mites responded to touching with camel's hair brush was considered alive one percentages of accumulated mortality was collected according to Abbott's formula.

Latent effect of LC_{50} of *P. fluorescens*on some biological aspects of spider mite,*T. cucurbitacearum*

Eight adult individuals of *T.cucurbitacearum* were transferred to mulberry leaf discs about 2 cm. diameter each replicate 10 times. The spray and dipping methods was applied as mention above. The alive individuals were observed then the longevity and fecundity in addition to egg hatching of *T.cucurbitacearum* females and deterrent index %were calculated. Deterrent index % based on the number of eggs on control and tested leaf discs Lundgren (1975)formula as follow:

$$\text{Deterrent index \%} = \frac{B-A}{B+A} \times 100$$

A: Number of eggs in treatment, B: Number of eggs in control.

Statistical analysis

Data were subjected to statistical analysis using one way analysis of variance, ANOVA Duncan (1955).

RESULTS AND DISCUSSION

The toxicity of *Pseudomonas fluorescens* on spider mite, *Tetranychus cucurbitacearum*

The susceptibility of the mite females of *T. cucurbitacearum* to *P. fluorescens* was evaluated by using two different methods; spraying and dipping. The earliest deaths occurred within the first day after the treatments. All the mites in the spraying bacterial treatment died within 48 hours, so statistical analysis for mortality and efficacy were done using the data on all days. There were highly significant differences in adult mortality among the treatments Table (1). The highest mortality (100%) and the lowest 68.75% for the spraying bacterial treatment and 58.75% and 16.25% for the dipping. Bacterial dipping caused mite mortality, significantly lower than the effect of bacterial spraying. The results of this study revealed the potential of *P. fluorescens* as a microbial biocontrol agent by causing significant mortality of *T. cucurbitacearum*. These results agree with (De Flaun *et al*., 1994 and Mastropaolo *et al*. 2012) revealed that bacterial mortality may be one of the key elements regarding mite killing as acaricidal function. Al-Sohim and Fouly (2015) indicated that *Pseudomonas fluorescens* caused higher mortality reached 74.84% against mite, *Oligonychus afrasiaticus*.

Table 1. Effect of *Pseudomonas fluorescens* culture on spider mite, *Tetranychus cucurbitacearum* (Sayed)

Treatments technique		% Mortality after			
		24h.	48h.	72h.	96h.
100%	Spraying	96.25±3.12^{a}	100^{a}	100^{a}	100^{a}
	dipping	55.00±2.16^{b}	55.00±2.64^{b}	58.75±3.01^{b}	58.75±3.52^{b}
75%	Spraying	78.75±2.73^{b}	85.00±2.35^{b}	88.75±2.89^{b}	93.75±3.17^{b}
	dipping	33.75±2.34^{b}	37. 5±1.80^{c}	38.75±1.87^{c}	38.75±2.65^{c}
50%	Spraying	68.75±1.98^{c}	71.25±1.92^{c}	76.25±1.76^{c}	78.75±2.35^{c}
	dipping	16.25±1.85^{d}	16.25±1.78^{d}	25.00±1.09^{d}	27.50±1.89^{d}
Control	Spraying	5.00±0.65^{e}	6.25±0.97^{e}	6.25±0.64^{e}	6.25±0.34^{e}
	dipping	2.5±0.11^{e}	2.5±0.41^{e}	2.5±0.21^{e}	2.5±0.45^{e}

Means in columns followed by the same letter are not significantly different at 5%level (Duncan'smultiple range tests). ± Standard Error

The effect of *P.fluorescens*on the biology of spider mite, *T. cucurbitacearum*

Statistical analysis in Table (2) cleared that *T. cucurbitacearum* longevity affected significantly in both methods of *P.fluorescens* treatment. The longevity was shorter than control in both methods. Female longevity averaged 10.32 and 17.04 days for the spraying and the dipping while, 22.5 and 24.19 days in control. The bacterial treatment reduced the total deposited eggs in females to half approximately in the spraying method while, in the dipping method was nearly with the control. Almost all eggs hatched in the two controls, but egg hatching was reduced to 48.34% and 73.12% in the spraying and the dipping methods. *P.fluorescens* showed efficacy for

reduced total deposited eggs with deterrent index 49.24% and 13.25% for the spraying and the dipping methods. Aksoy *et al*. (2008) investigated that *Pseudomonas putida*bio type B as a potential biological control agent of *Tetranychus urticae*that reduced total egg numbers and egg hatching.

Table 2. Latent effect with$LC_{50}$of *Pseudomonas fluorescens* on some biological aspects of *Tetranychus cucurbitacearum*

Methods of treatment	Longevity (in days)	Total eggs/female	Egg hatching %	Deterrent index %
Spraying	10.32±1.14^{c}	23.13±1.16^{c}	48.34±3.26^{c}	49.24
Control	22.5±1.95^{a}	68.01±5.2^{a}	95.57±5.73^{a}	-
Dipping	17.04±1.18^{b}	54.18±3.25^{b}	73.12±3.22^{b}	13.25
Control	24.19±2.02^{a}	70.63±5.36^{a}	95.23±4.54^{a}	-

Means in columns followed by the same letter are not significantly different at5% level (Duncan's multiple range tests). ± Standard Error

Enzyme assay

The mortality due to affected with *P.fluorescens* due to the chitinase enzyme present in the cell which was confirmed by the positive result of chitin clearing zone study. Fig. (1). Bacterial chitinases have been reported to be effective in controlling the insects and mites by hydrolyzing chitinous exoskeleton Kramer and Muthukrishnan (1997).

Fig. 1. Chitin clearing zone of *P.fluorescens* effect.

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إستخدام بكتيريا *Pseudomonas fluorescens* كعامل مكافحة ميكروبية حيوية ضد العنكبوت الأحمر *Tetranychus cucurbitacearum* (Sayed)(Acari:Tetranychidae)

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أثبتت هذه الدراسة فعالية بكتيريا *Pseudomonas fluorescens* كأحد عوامل المكافحة الميكروبية للحلم العنكبوتى *Tetranychus cucurbitacearum* حيث أُستخدم فى هذه الدراسة طريقتين للمعاملة وهما طريقة الرش وطريقة غمر الورقة.

- أظهرت النتائج المتحصل عليها إلى حدوث نسبة موت فى إناث الحلم المعامل بهذه البكتيريا وذلك بعد اليوم الأول من المعاملة حيث بلغت نسبة الموت ٩٦,٢٥ % ووصلت إلى ١٠٠ % بعد اليوم الثانى من المعاملة وذلك عند إستخدام طريقة الرش بينما إنخفضت هذه النسبة إلى ٥٨,٧٥ % عند إستخدام طريقة الغمر للورقة.
- قصرت مدة معيشة الإناث معنوياً بعد معاملتها بالبكتيريا حيث بلغت ١٠,٣٢ يوماًمقارنةً بالإناث الغير معاملة (الكنترول) ٢٢,٥٠ يوماً وذلك بإستخدام طريقة الرش بينما كانت ١٧,٠٤ يوماً والكنترول ٢٤,١٩ يوماًبإستخدام طريقة الغمر للورقة.
- أدت المعاملة بالبكتيريا إلى خفض معنوى فى الكفاءة التناسلية للإناث المعاملة حيث بلغ متوسط ما تضعه الأنثى ٢٣,١٣ بيضة و ٥٤,١٨ بيضة عند المعاملة بالرش والغمر على التوالى بينما كانت ٦٨,٠١ و٧٠,٦٣ بيضة فى حالة الإناث الغير معاملة (الكنترول).