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Bioefficacy of Bacterium, *Pseudomonas fluorescens* using Foliar Spray and Cultivated Soil Application against *Tetranychus urticae* on Cucumber Plants Comparing with an Acaricide

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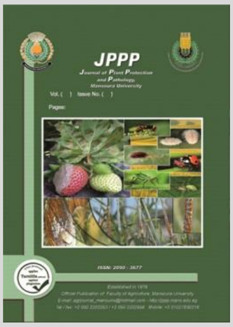
Dalia A. Waked [1*]; H. A. M. Mahgoub [2]; M. Eleawa [1] and A. Ali [1]

[1] Plant Protection Research Institute, ARC, Dokki, Giza, Egypt
[2] Botany and Microbiology Dep., Faculty of Science, AlAzhar University, Nasr City, Cairo, Egypt



ABSTRACT

The entomopthogenic bacterium , Pseudomonas fluorescens, was used alone as soil treatment and foliar application or mixed with *entomopthogenic fungus* , *Beauveria bassiana* for controlling the two spotted spider mite , *Tetranychus urticae* infesting Cucumber plants comparing with a standard synthetic acaricide ortus . *P. fluorescens* great effect as soil treatment and foliar spraying, recording reduction percentage of 75.21%. and no significant differences (df =6; P =<0.01) with the reduction caused by the acaricide used and this shows the importance of the role of biopesticides in reducing the population of *T. urticae* infesting Cucumber Plants . There was significant effect on the lipase activities by all treatments, while the activity of chitinase shows that its activity increased at a high rate in all the treatments except ortus treatment compared with control. The acaricide, ortus had strong effect on the activity of acetylcholinesterase (AchE) where the activity of this enzyme decreased significantly (df =5; P =<0.01) to the extent of inhibition. While, the activity of this enzyme increased in the other treatments, where the activity reached nearly the control . The treatments which caused high reduction percentage gave high yield amount. There were nonsignificant differences in yield amount in treatment with (ortus), and (soil and foliar application of *P. fluorescens*), that indicates the important role of *P. fluorescens* in IPM against mite.

Keywords: *Pseudomonas fluorescens*, *Tetranychus urticae*, Cucumber

INTRODUCTION

In Egypt, vegetable crops and fruit trees are considered of great economic importance for local consumption and exportation. Cucumber, *Cucumis sativus* L. considered one of the most important tasty nutrition vegetables which add lots of vitamins A and C to human diet. Phytophagous mites especially those belonging to families Tetranychidae, Tenuipalpidae and Eriophyidae constitute considerable agriculture pests, as they cause injury to decrease the quantity as well as the commercial quality of these crops. The tetranychid mite, *Tetranychus urticae* Koch is among the major pest occurring on many crops and fruits in different parts of the world, (Skorupska) (2004 a & b and Gencer *et al.* ,2005) . Two spotted spider mite, *Tetranychus urticae* is considered the main pest, which threaten the quantity and quality of the vegetable yield. Direct and indirect effects of mite feeding occur defoliation; lead burning and even plant death are examples of direct effects. Indirect effects, which may lead to other problems in the plant, include decreases in photosynthesis and transpiration. This combination of effects on the host plant often reduces the amount of yield for that crop, (Huffaker & McMurty ,1969). Therefore, the two spotted spider mite, *T. urticae* has been extensively studied. During the last two decades, biological control has received more attention with the increased consciousness in environment issues and integrated pest management methods, (Rao *et al.* ,1989; Catska *et al.* ,1989). Acaricides have been widely used for mite control in greenhouses, orchards and many other cropping systems, (Van *et al.* ,2005). On the other hand, biocides have low effect on the predatory mites. Microbial insecticides such as entomopathogenic bacteria and fungi can provide an alternative, more environmentally friendly option to control this insect pest. The entomopathogenic bacteria, *Pseudomonas fluorescens* and fungi, *Beauveria bassiana* are a promising and extensively biological control agents that can suppress a variety of economically important insect pests, (Coates *et al.* ,2002 ; McGuire *et al.*, 2005 ; Prasad and Syed, 2010 ; Hussein *et al.* ,(2010).

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 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). The present study evaluated mite control on cucumber crop and the ability of products to prevent mite infestation using foliar spray and seed treatment of entomopathogenic bacteria, *Pseudomonas fluorescens* compared to a standard synthetic acaricide, ortus. The trial was conducted with entomopathogenic fungi, *Beauveria bassiana*, and untreated plants served as the control group.

*** Corresponding author.**
E-mail address: dr.dalia188@yahoo.com
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MATERIALS AND METHODS

Sowing practices

The experimental area was (350 m^2) for Cucumber plants that divided into 7 plots (treatments), each plot about 50 m^2 . Planting date was 1st of May. Seeds were sown in hills, on one side of the ridge of 80 cm in width. The treatments were, Cucumber seeds were planted and when the growth is completed wait until infected with *T. urticae* were sprayed with concentration (2.3×10^8 cfu/ml) of *P. fluorescens* using the motor back (T1), another Cucumber seeds were soaked in a concentration (2.3×10^8 cfu/ml) of *P. fluorescens* for 30 minutes then left to dry and planted in the soil (T2). Another group of Cucumber seeds were soaked in a concentration (2.3×10^8 cfu/ml) of *P. fluorescens* for 30 minutes then left to dry and were planted and when the growth is completed wait until infected with *T. urticae* were sprayed with *P. fluorescens* (T3). Untreated Cucumber seeds were planted in clean soil and after germination were followed until infected with *T. urticae* then sprayed with *P. fluorescens* and after one week plants were sprayed with concentration (1×10^8 spore/ml) of *Beauveria bassiana* (T4). The untreated Cucumber seeds were planted as previously mentioned and when infected with *T. urticae* sprayed with concentration (1×10^8 spore/ml) of *Beauveria bassiana* (T5). Cultivation of untreated Cucumber seeds as previously mentioned and when infected with *T. urticae* were sprayed with the acaricide ortus at the recommended concentration, 50ml/100litre water (T6). After planting Cucumber seeds and infected with *T. urticae*, they were left without as control and sprayed with water only (T7). Twenty leaves of each treatment were taken before spraying and 15 days after the first or seconed spray. The number of individuals in each treatment is recorded after 15 days of the second spray and reduction percentages were calculated in each treatment according to the formula, Hendrson and Tilton (1955). The reduction percentage in soil treatment is calculated according to Abbott's formula (1925). During the previous treatments and after the fruiting in the treatments fruits were taken day after day then weighted, take the average weight of the fruit, the number of fruits and plants in the experimental area can be attributed to the productivity of the feddan.

Biochemical effects of tested compounds on field strain of *T. urticae*

Some individuals from treated field strain of *T. urticae* were randomly taken after treatment with 24 hr. for acaricide, ortus and 5 days for *P. fluorescens* and *B. bassiana.* The activity of lipase of *T. urticae* was determined according to Tahoun and Abdel Ghaffar (1986). Acetyl cholinesterase (AchE) activity was measured according to the method of Simpson *et al*. (1964). using acetylcholine bromide (AchBr) as a substrate. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm. Chitinase activity was determined using 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexoamines liberated in chitin digestion according to the method described by Ishaaya and Casida (1974). The means were compared with Least Significant Difference according to Costat statistical software (2005).

RESULTS AND DISCUSSION

Results obtained show the effectiveness of *Pseudomonas fluorescens* with treating soil and foliar spray against the red spider, *T. urticae* in addition to its use one of the most important fungi, *B. basianna* used in control *T. urticae.* Comparing with one of the recommended chemical acaricide, ortus .Table (1) showed that there were significant differences (df =6; P =<0.01) between all treatments, ortus, (T6) was the most effective treatment, causing a reduction of more than 80%, followed by *P. fluorescens* on soil and foliar spraying treatment, (T3) where the reduction was 75.21%, and no significant differences between (T3&T6) this finding shows the importance of the role of *P. fluorescens* in reducing the population of this pest. The other treatments were arranged in descending order in terms of the reduction percentages as follows: treatment of foliar spray with *P. fluorescens* combined with *B. basianna*, (T4) followed by foliar spray treatment with *P. fluorescens*, (T1) and then foliar spray treatment with *B. basianna*, (T5) while, soil treatment with *P. fluorescens*, (T2) was the least effective treatments, since the reduction percentages were 71.51, 70.33, 63.12 and 58.83%, respectively. It is shown in the same table that the population of *T. urticae* individuals decreased after the first and second spray, where the interval between the two sprays was 15 days, where the average decreases in the population was calculated for two sprays and this was showed in all treatments, while the control treatment showed a fluctuating rate between high and low. Comparable results had been obtained by Al-Sohim, and Fouly (2015), who studied the effect of *P. fluorescens* against red spider mite in greenhouse where the reduction percentages reached to 76.63%. After application of *P. fluorescens* the mites slowed down their movements, stopped feeding and the body fluid began oozing out. In direct spraying method, 100% mortality was observed within 24 hr. after the treatment whereas in the dipping method, 100% mortality was achieved only after 3 days (Amsalingam, *et al.* ,2011). The occurrence of resistance to an insecticide in insects mainly due to the action of enzymes which are either insensitive to insecticide or able to degrade it to nontoxic metabolites. Enzyme of some activities in field strain of *T. urticae* were summarized in Table (2). There was significant effect (df =5; P =<0.05) on the lipase activity by all treatments . The acaricide ortus treatment increased the activity of lipase enzyme recording 16.23 and 22.23 (µg oleic acid liberated/min./g.b.wt.) after first and second spry respectively. While, the activity showed the highest decrease indicating 7.54 (µg oleic acid liberated/min./g.b.wt.) in (T3) after 1st spray compared with control 12.61 (µg oleic acid liberated/min./g.b.wt.)

while, after the 2nd spray was 5.26 (µg oleic acid liberated/min./g.b.wt.) in (T2) compared with control 15.56 (µg oleic acid liberated/min./g.b.wt.) but the activity of lipase decreased in the other treatments after both sprays, while the chitinase activities were increased at a high rate in all the treatments except ortus treatment compared with control, where the activity of this enzyme increased to 105.43 (µgNAGA/min/g.b.wt) in the treatment of bacteria (T2) followed by (T1) 95.18 (µgNAGA/min/g.b.wt) while the least effect on the activity of this enzyme was recorded in treatment (T5), which was near similar to control after the first spray and decreased the activity of this enzyme in all treatments after the second spray was recorded compared with ortus . The chemical pesticide, ortus had significant effect (df =5; P =<0.01) that cause inhibition of the acetylcholinesterase (AchE) activity compared to the other treatments, where some treatments increased or decreased the activity from the control with simple significant differences. The chemical pesticide ortus had strong effect on the activity of this enzyme where the activity of this enzyme decreased to the extent of inhibition, reaching 35.43 and 19.44 (µgAchBr/min/g.b.wt) after first and second spry respectively while the activity of this enzyme increased in the other treatments with the exception that T2, where the activity reached nearly the control. Bacterial chitinases have been reported to be effective in controlling the insects and mites by hydrolyzing chitinous exoskeleton, (Kramer and Muthukrishna 1997). It forms the major component of exoskeleton and gut linings of insects; hence chitin metabolism can be an excellent target for selective pest management strategy (Kramer *et al*. ,1997). Chitinolytic enzymes and their genes have gained attention in the recent years because of the importance of chitin and its metabolic enzymes in insect growth and development. (Vodovar *et al.* ,2006), reported that *Pseudomonas entomophilia* exhibited virulence against *Drosophila melanogaster* due to strong hemolytic activity, involving enzymes such as lipases, chitinases and/ or hydrolases.

Table 1. Field efficacy of *Pseudomonas fluorescens* against *T. urticae* on cucumber plants

Tr. No.	Treatments	Mean number of *T. urticae*/ leaf±SE				
		PTC	1st Spray	2nd spray	Mean	Reduction %
T1	Foliar application of *P. fluorescens*	32.29±2.53	17.12±1.50	9.52±0.98	13.32±1.23	70.33b
T2	Soil application of *P. fluorescens*	22.36±1.09	18.04±1.42	7.57±0.13	12.80±1.36	58.83c
T3	Soil and foliar application of *P.fluorescens*	20.51±1.21	09.34±0.59	4.81±0.10	07.07±1.11	75.21a
T4	Foliar application of *P. fluorescens* and *B. basianna*	23.80±1.63	12.86±0.76	6.00±0.12	09.43±1.15	71.51b
T5	Foliar application of *B. basianna*	26.35±1.66	20.44±1.73	8.73±0.93	14.58±1.38	63.12c
T6	Ortus	30.00±1.90	12.23±1.35	4.09±0.83	08.16±1.02	80.44a
T7	Untreated check	28.42±2.02	36.40±2.25	42.64±2.11	39.52±2.71	-
	df	-	6	6	-	6
	P					<0.001

In column, means followed by a common letter (s) are not significantly different (P = 0.05)
PTC: Pre-treatment count

Table 2. Enzymes activity in field strain of *T. urticae*

Tr. No.	Treatments	Enzyme activity ±SE					
		Lipase (µg oleic acid liberated/min./ g.b.wt.)		Chitinase (µgNAGA/min./g.b.wt)		Acetyl-cholinestrase (µgAchBr/min./g.b.wt)	
		1st spray	2nd spray	1st spray	2nd spray	1st spray	2nd spray
T1	Foliar application of *P. fluorescens*	9.25±0.9c	7.08±0.66d	95.18±6.59a	81.03±4.73a	124.15±5.31a	129.31±4.50a
T2	Soil and foliar application of *P. fluorescens*	8.68±0.92c	5.26±0.20d	105.43±9.13a	73.56±5.02a	107.30±6.14b	97.43±3.85b
T3	Foliar application of *P.fluorescens* and *B. basianna*	7.54±0.73c	9.31±0.78c	80.73±7.14b	60.2±3.39b	120.13±2.75a	112.35±4.11a
T4	Foliar application of *B. basianna*	12.49±1.05b	18.07±1.03b	73.98±7.35c	55.36±3.56b	117.21±1.36b	126.67±5.31a
T5	Ortus	16.23±1.40a	22.32±1.22a	30.39±3.50e	35.42±2.50c	35.43±1.50c	19.44±2.50c
T6	Untreated check	12.61±1.39b	15.56±1.63b	48.26±3.96d	40.67±2.16c	115±2.93b	102.43±3.12b
	df	5	5	5	5	5	5
	P	<0. 05	<0. 05	<0.01	<0.01	<0.01	<0.01

In column, means followed by a common letter (s) are not significantly different (P = 0.05)

Data in Table (3) showed that the treatment of cucumber plants with *tested* compounds used in the control of mite resulted in significant differences (df =6; P =<0.01) in the amount of crop produced during the growing season. Also data revealed the importance of these tested treatments in the control against major mite and harmful to the cucumber crop where the chemical pesticide had great effect in increasing the crop where the area treated with chemical pesticide (T6) has given 8.12 tons/fed. of cucumber followed by the treatment (T3) then (T4) where both treatments recorded 7.98 and 7.00 tons/fed., respectively, also the other three treatments recorded significant productivity compared to the control. Given the productivity and comparison with the control it is clear that the chemical pesticide (T6) achieved high productivity more than doubled from the control followed by the treatment (T3), which slightly less than the chemical pesticide. Weight of the fruit and the number of fruits are the determinant of the yield of the crop, knowing that the decline in productivity in bio-treatments is not of concern because the price of the biological product far exceeds the price of the product treated with chemical pesticide and this illustrates the importance of bio-compounds in the mite control. Vinoth *et al*. (2009) found that the highest fruit yield coupled with 12.22% yield increase over untreated control in *P. fluorescens* in brinjal.

Table 3. Effect of some treatments on the cucumber fruit yield

Tr. No.	Treatments	Yield(Ton/fed.)	% increase over control	Fruit weight (gm)
T1	Foliar application of *P. fluorescens*	5.80±0.12c	24.31	80.49±5.32
T2	Soil application of *P. fluorescens*	5.12±0.26c	14.25	75.34±6.11
T3	Soil and foliar application of *P. fluorescens*	7.98±0.55a	44.98	95.03±6.96
T4	Foliar application of *P. fluorescens* and *B. basianna*	7.00±0.11b	37.28	89.54±4.73
T5	Foliar application of *B. basianna*	5.22±0.90c	15.90	83.43±4.12
T6	Ortus	8.12±0.12a	45.93	97.45±5.66
T7	Untreated check	4.39±0.97d	-	76.43±4.29
	df	6		
	P	<0.01		

In column, means followed by a common letter (s) are not significantly different (P = 0.05)

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الكفاءه الحيويه لبكتريا *Pseudomonas fluorescens* مستخدماً الرش الورقي ومعاملة التربه المنزرعة ضد *Tetranychus urticae* علي نباتات الخيار

داليا عبد الرحمن احمد واكد[1]، هاني احمد مرغني محجوب[2]، محمد عليوه محمد سليمان[1] وعبد الرحمن صالح عبد الرحمن علي[1]

[1] معهد بحوث وقاية النباتات - مركز بحوث الزراعية – الدقي – الجيزه – مصر

[2] قسم النبات والميكروبيولوجي – كلية العلوم – جامعة الازهر – مدينة نصر – القاهرة – مصر

يعتبر الحلم العنكبوتي ذو البقعتين أحد الأفات الهامه التي تصيب نباتات الخيار ، ولذا كان من الضروري مكافحة هذه الأفة الهامه ، وتهدف هذه الدراسه الي استخدام أحد أنواع البكتريا الممرضه *Pseudomonas fluorescens* كأحد عوامل المكافحه الحيويه المستخدمه في مجال مكافحة الاكاروسات ، تم استخدام البكتريا *Pseudomonas fluorescens* علي نباتات الخيار تحت الظروف الحقليه عن طريق الرش الورقي ومعاملة التربه بالاضافه الي استخدام المعاملتين معاً ، سببت البكتريا عند معاملة الاوراق من الخيار نسبة خفض ٧٠,٣٣٪ ، كما بلغت نسبة الخفض ٥٨,٨٣٪ عند معاملة التربه ، وذادت نسبة الخفض حتي وصلت الي ٧٥,٢١٪ عند استخدام البكتريا رشاً علي الاوراق ومعاملة التربه ، وتم ايضاً استخدام أحد المبيدات الحيويه والكميائيه كنوع من أنواع التوافق بين هذه البكتريا والمركبات الأكثر شيوعاً في مكافحة الاكاروس ، كما سبب فطر *Beauveria bassiana* وبكتريا *Pseudomonas fluorescens* نسبة خفض ٧١,٥١٪ عند رشهما علي الاوراق ، وكما انخفضت نسبة الخفض الي ٦٣,١٢٪ عند معاملة الفطر منفرداً ، وسبب الاورتس كمبيد كيماوي أعلي نسبة حفض حيث وصلت الي ٨٠,٤٤٪ ، وقد تم رش هذه المركبات السابقة الذكر مرتين أثناء موسم النمو. من النتائج السابقة يتضح أهمية استخدام هذه البكتريا *Pseudomonas fluorescens* كأحد عوامل المكافحة الحيويه الفعاله في مكافحة الاكاروس بالاضافه الي امكانية استخدامها مع المبيد الفطري والمبيد الكيماوي علي حد سواء .