# Bioefficacy of *Pseudomonas fluorescens*, foliar and cultivated soil application against *Tetranychus urticae* on Cucumber crop

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**ABSTRACT:** *Tetranychus urticae*, is a key pest of Cucumber, irrespective of the use of conventional or organic management. In organic systems, however, the use of synthetic insecticides is not allowed, increasing the difficulty of controlling this pest. *P. fluorescens* had an effective effect on soil treatment and foliar spraying, (T3) where the reduction was 75.21%. Where there were no significant differences between them and the reduction caused by the chemical pesticide and this shows the importance of the role of biopesticides in reducing the population of this serious pest. There is no significant effect on the protease by all treatments. While the chitinase enzyme shows that its activity increased at a high rate in all the treatments except ortus treatment compared with control. The chemical pesticide ortus had an effective and strong effect on the activity of acetylcholinesterase (AchE) where the activity of this enzyme decreased to the extent of inhibition, reaching 35.43 (µgAchBr/min/g.b.wt) while, the activity of this enzyme increased in the other treatments, where the activity reached nearly control or increase after the first spray. The treatments which caused high reduction percent gave high productivity. There were not any significant differences in yield amount in treatment with (ortus), (soil and foliar application of *P. fluorescens*) and (foliar application of *P. fluorescens* and *B. bassiana*).

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**Key words:** *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 pests, 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* is a promising and extensively biological control agent 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 insecticide. The trial was conducted with entomopathogenic fungi, *Beauveria bassiana* and untreated plants served as the control group.

## 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 on the 1st of May. Seeds were sown in hills, on one side of the ridge of 80 cm in width. The treatments were, Soil application of *P. fluorescens*. Cucumber seeds were treated with *P. fluorescens*, the seeds were soaked in a concentration ($2.3\times10^8$) of *P. fluorescens* for 30 minutes then left to dry and planted in the soil (T1). A group of seeds were soaked in a concentration ($2.3\times10^8$) 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* and sprayed with *P. fluorescens* (T2). Untreated seeds were planted in clean soil and after germination were followed until infected with *T. urticae* then sprayed with *P. fluorescens* using the motor back (T3). The seeds of the untreated cucumber were planted as previously mentioned and when infected with *T. urticae* sprayed with *P. fluorescens* and after a week is sprayed with concentration ($1\times10^8$ spore/ml) of *Beauveria bassiana* (T4). The untreated cucumber seeds were planted as previously mentioned and when infected with *T. urticae*, they were sprayed with the same concentration of *B. bassiana* as previously (T5). Cultivation of untreated cucumber seeds as previously mentioned and when infected with *T. urticae* was sprayed with chemical pesticide, ortus at the recommended concentration, 50cm/100litre water (T6). After planting cucumber seeds and infected with *T. urticae*, they were left without treated and sprayed with water (T7). Twenty leaves of each treatment are taken before spraying and 15 days after spraying. The number of individuals in each treatment is calculated with the second spray and after 15 days the number of individuals is calculated to calculate the reduction percent in each treatment according to the formula, Hendrson and Tilton (1955), The reduction percent in soil treatment is calculated according to Abbott's formula (1925). During the previous treatments and after the fruiting in the treatments was taken a group of fruits day after day then weighted, take the average weight of the fruit, the number of fruits and plants in the experimental area thus can be attributed to the productivity of the feddan.

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

Some individuals from field strain were randomly taken after treatment with 24 hr. for acaricide, ortus and 5 days for *P. fluorescens* and *B. bassiana.* The activity of protease and amylase of *T. urticae* were determined according to Birk, *et al*., (1962).

### Acetyl cholinesterase determination

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

The results obtained show the effectiveness of *Pseudomonas fluorescens* by treating soil and foliar spray against the red spider, *T. urticae* in addition to its use with one of the most important fungi, *B. basianna* used in control *T. urticae*. In addition to comparing it with one of the recommended chemical pesticides, Ortus. Table (1) shows that Ortus, (T6) was the most effective treatment, causing a reduction of more than 80%, while *P. fluorescens* had an effective effect on soil treatment and foliar spraying, (T3) where the reduction was 75.21%. Where there were no significant differences between them and the reduction caused by the chemical pesticide and this shows the importance of the role of biopesticides in reducing the population of this serious pest. The other of treatments were arranged in descending order in terms of the reduction percent 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 % 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 the second spray, where the interval between the two sprays was 15 days. Where the average decrease 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 percent reached 76.63%. After application of *P. fluorescens* the mites slowed down their movements and stopped feeding. Shortly, the body fluid began oozing out. In direct spraying method, 100% mortality was observed within 24 h after the treatment whereas in the dipping method, 100% mortality was achieved only after 3 days Babu *et al*., (2010).

**Table (1). Field Efficacy of *Pseudomonas fluorescens* against *T. urticae* on Cucumber crop**

| Tr. No. | Treatments | Mean number of *T. urticae*/ leaf | | | | |
|---|---|---|---|---|---|---|
| | | PTC | 1st spray | 2rd spray | Mean | Reduction % |
| **T1** | Foliar application of *P. fluorescens* | 32.29 | 17.12 | 9.52 | 13.32 | 70.33b |
| **T2** | Soil application of *P. fluorescens* | 22.36 | 18.04 | 7.57 | 12.80 | 58.83c |
| **T3** | Soil and Foliar application of *P. fluorescens* | 20.51 | 9.34 | 4.81 | 7.07 | 75.21a |
| **T4** | Foliar application of *P. fluorescens* and *B. basianna* | 23.80 | 12.86 | 6.00 | 9.43 | 71.51b |
| **T5** | Foliar application of *B. basianna* | 26.35 | 20.44 | 8.73 | 14.58 | 63.12c |
| **T6** | Ortus | 30.00 | 12.23 | 4.09 | 8.16 | 80.44a |
| **T7** | Untreated check | 28.42 | 36.40 | 42.64 | 39.52 | - |

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

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 activities in field strain of *T. urticae* were summarized in Table (2). There is no significant effect on the protease by all treatments. While the chitinase enzyme shows that its activity 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 is (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. The chemical pesticide has had an effective effect on the lack of activity of the enzyme acetylcholinesterase (AchE). Where the cause of this pesticide inhibition in this enzyme compared to the other the treatments where some of them increased and decreased from the control with simple significant differences. The chemical pesticide ortus had an effective and strong effect on the activity of this enzyme where the activity of this enzyme decreased to the extent of inhibition, reaching 35.43 (µgAchBr/min/g.b.wt) while the activity of this enzyme increased in the other the treatments, where the activity reached nearly control or increase after the first spray. In view of the activity of this enzyme after the second spray we find that it decreased with high rate from the other the treatments that appeared with a fluctuating level between the increase and decrease compared to the control. Bacterial chitinases have been reported to be effective in controlling the insects and mites by hydrolyzing chitinous exoskeleton, Kramer and Muthukrishnan, (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

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

| Tr. No. | Treatments | Enzyme activity | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Protease** (O.D. units x 103/g.b.wt.) | | **Chitinase** (µgNAGA/min/g.b.wt) | | **Acetyl-cholinestrase** (µgAchBr/min/g.b.wt) | |
| | | **1st spray** | **2rd spray** | **1st spray** | **2rd spray** | **1st spray** | **2rd spray** |
| **T1** | Foliar application of *P. fluorescens* | 36.25a | 31.08a | 95.18a | 81.03a | 124.15a | 129.31a |
| **T**2 | Soil and Foliar application of *P. fluorescens* | 33.68a | 37.26a | 105.43a | 73.56a | 107.30b | 97.43b |
| **T**3 | Foliar application of *P. fluorescens* and *B. basianna* | 29.54b | 33.31a | 80.73b | 60.2b | 120.13a | 112.35a |
| **T**4 | Foliar application of *B. basianna* | 34.49a | 30.07a | 73.98c | 55.36b | 117.21b | 126.67a |
| **T**5 | Ortus | 30.23b | 28.32b | 30.39e | 35.42c | 35.43d | 19.44c |
| **T**6 | Untreated check | 35.61a | 32.56a | 48.26d | 40.67c | 115b | 102.43b |

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 *P. fluorescens* and some compounds used in the control of mite resulted in significant differences in the amount of crop produced during the growing season. This shows the importance of these treatments in the control against major mite and harmful to the cucumber crop where the chemical pesticide had an effective effect in increasing the crop where the area treated with chemical pesticide (T6) has given 9.5 tons/fed. of cucumber followed by the treatment (T3) then (T4) where both treatments recorded 7.98 and 7 tons/fed., respectively, while the other of the treatments recorded non-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 is 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) 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* | 5.8b | 24.31 | 80.49 |
| **T2** | Soil application of *P. fluorescens* | 5.12b | 14.25 | 75.34 |
| **T3** | Soil and Foliar application of *P. fluorescens* | 7.98a | 44.98 | 95.03 |
| **T4** | Foliar application of *P. fluorescens* and *B. basianna* | 7.00a | 37.28 | 89.54 |
| **T5** | Foliar application of *B. basianna* | 4.98b | 11.85 | 78.19 |
| **T6** | Ortus | 9.5a | 53.78 | 105.11 |
| **T7** | Untreated check | 4.39b | - | 76.43 |

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

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