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# THE EFFECTIVENESS OF THE ENTOMOPATHOGENIC FUNGI *Beauveria bassiana* ON THE ADULT FEMALE OF *Tetranychus urticae*

By

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## ABSTRACT

The aim of this study is to evaluate the toxic effect of entomopathogenic fungi *Beauveria bassiana* on adult females of the two-spotted spider mite *Tetranychus urticae* under laboratory conditions. Mortality by the commercial formulation of *B. bassiana* (Biofly) decreased with decreasing concentrations of spore inoculum, the $LC_{50}$ was $4.12 \times 10^6$ spores/ml.

The effect of spraying with the biocide Biofly on the mite population, and mortality on green bean plants was increased with increasing spore concentrations. The most effective treatment was the plot sprayed 4 times at the concentration of 2 ml/l (78.55% reduction in the mite population).

When biocide Naturalis L and WP (*Beauveria bassiana*) were sprayed on the moving stages of the mite infesting strawberry, Naturalis L sprayed 3 times at the concentration of 3 ml/l showed reduction percentage of 95.39 in the moving stages of the mite population and the other formulation, Naturalis WP showed 95.98% reduction in the mite population after 15 days from the first application.

## INTRODUCTION

Tetranychid mites are common pests in agricultural systems, causing, in many cases, greater economic losses than any other arthropod pests. Two-spotted spider mite, *Tetranychus urticae*, is considered one of the major pests attacking different agricultural crops such as field crops, vegetables, fruits and ornamental plants. The infestation by mite caused a great damage to these infested plants followed by infestation by various pathogens such as virus, bacteria and fungi (Hoy *et al.*, 1980 and Penman and Chapman, 1980). The latter organism caused serious problems in the field of agriculture. Great attention is offered to survey and control of both pests to protect the crops and then minimize the loss in agricultural economy. The severity of plant injury caused by spider mites depends on numerous factors influencing feeding and reproduction of the mites Of particular importance are the nature and conditions of the host plants and the influence of physical factors on both host and mite (Abou-Awad and El-Banhawy, 1985).

A wide range of chemicals have been marketed for controlling the two-spotted spider mite. The wide use of the chemical compounds resulted in many problems such as population outbreaks and chemical resistance endangering human health and wealth Therefore, world is going to reduce chemicals use and trying to introduce the entomopathogens such as virus, bacteria and fungi in I.P M programmes (Huffaker *et al.*, 1970; McMurtry *et al.*, 1970 and Van De Vrie *et al.*, 1972).

The pesticides referred to as microbial pest control agent or microbial pesticides are preparations based on disease inducing organisms (entomopathogens) which normally infect or poison an insect or mite and ultimately cause its death. The integrated control of these pests includes parasitic fungi which are capable of penetrating the insect cuticle directly after piercing the cuticle then the fungus multiplies inside the insect producing lethal metabolites (Kumor, 1984). Many microbial pesticides have been formulated with the use of the following fungi (for instance) *B. bassiana, Verticilium lacanii. Entemophaga asiatic* and *E. grylli*. Formulations of *Verticilium lecanii* are used to control aphids, scales, thrips and red spider mites in green house crops (Pena *et al.*, 1996). Furthermore, *Hirsuttello thompsonii*, a parasitic fungus from which a spore-formulation is produced that kills citrus-rust mites and also infects spider mite. Parasitic fungi, generally are host-specific and are not hazardous to non-target organisms. They are cheep to produce and may be formulated and applied similar to chemical pesticides.

Klubertanz *et al.*(1991) showed that a pathogenic fungus (*Neozygites* sp.) caused dramatic reduction in population of *T. urticae* in artificially infested soybean plots. Yousri (1994) recorded that the mortality of the adult female mite, *Tetranychus urticae*, exposed to a series of spore dilutions of *Beauveria bassiana*, from $1.26x10^9$ to $1.26x10^6$, decreased with decreasing amounts of inoculum. At concentration of $1.26x10^6$, the mortality of the tested mites was significantly greater than that of uninoculated control, the $LC_{50}$ after 8 days of exposure was $2.22x10^7$ spores/ml. Abdel-Samad (1998) studied the efficiency of Biofly (*Beauveria bassiana*) with $3x10^7$ conidia, at 100 cc/100 l of water on different egg stages and adult females of *T. urticae*. The results showed that the effect of this compound against egg stages was higher than the adult females of *T. urticae*. Moreover, three days old eggs were most effectual of the others. The biological aspects of *T. urticae*, when treated one day old eggs with Biofly, indicated that the average number of deposited eggs per female decreased to 95.9%. Moreover, it caused 90% mortality during immature stages. Under field conditions, the average reduction in population density of *T. urticae* was 71.06% after the fourth application with *B. bassiana*. Biofly can be used in the integrated control programmes to control *T. urticae*. Derballa (1999) showed that the microibial pesticide Biofly was highly toxic to the adult females at zero hour after treatment of the discs, $LC_{50}$ = 83948.73 conidia/ml, followed by the treatment at 6 hours after dipping (LC50 = 133647 conidia/ml). Also, results showed that Biofly was highly toxic to eggs of 24 hours age with $LC_{50}$ value of 31514.95 conidia/ml, followed by eggs of 12 hours with $LC_{50}$ value of 42773.94 conidia/ml but there was no significant differences between them.

Clearly, if chemical, microbial pesticides and biological methods are successfully integrated, then the impact of pesticides used to control key pests and diseases must be minimized while the beneficial arthropods must be introduced. So, the present study was carried out to:

1. Evaluate the efficiency of the commercial products of the entomopathogenic fungus, *B. bassiana*, namely Biofly against the two-spotted spider mite, *T. urticae*.

2. Biochemical control of the two-spotted spider mite moving stages infesting green beans by using the commercial product of entomopathogenic fungus, *Beauveria bassiana*, namely Biofly.

3. Biochemical control of the two-spotted spider mite moving stages infesting strawberry by using two formulations of the entomopathogenic fungus, *Beauveria bassiana*, namely Naturalis-L and Naturalis WP, which were sprayed 3 times with three concentrations (1, 2 and 3 ml/l for Naturalis L) and (1, 2 and 3 g/l for Naturalis WP).

## MATERIALS AND METHODS

### Commercial biocides used:

The fungus *B. bassiana*, as a commercial products were:
Naturalis-L (entomopathogenic fungus *B. bassiana* $2.3 \times 10^{7}$ conidia /ml).
Naturalis WP (entompathogenic fungus *B. bassiana* $1.94 \times 10^{13}$ conidia/kg).

Biofly (entomopathogenic fungus *B. bassiana* $3 \times 10^{7}$ conidia/ml).
They were supplied by El-Nasser for Fertilizers and Pesticides, Egypt.

### Techniques used:

The techniques used for laboratory assays on the effect of the entomopathogenic fungus *B. bassiana* on the adult female mites were used by Vanninen and Hokkanen (1988) and Yousri (1994).

**Biological control of the two-spotted spider mite *T. urticae* infesting strawberry:**

The fungus *B. urticae* as a commercial product namely Naturalis-L in two forms, liquid with a concentration of $2.3 \times 10^7$ conidia/milliliter and W.P with a concentration of $1.94 \times 10^{13}$ spores/kg were used as biocide to control the two-spotted spider mite *Tetranychus urticae*, by using 3 concentrations: 1, 2 and 3 ml/litre for EC formula and 1, 2 and 3 g/litre for WP formula. The experiment was designed as random complete block design, the replicate area was 7x12 m (i.e, 84 $m^2$) and 4 replicates for each treatment to evaluate the efficiency of the certain concentrations applied three times at different rates against spider mites The samples were taken pretreatment, after 1 ,3 and 5 days from each application, the sample of the $5^{th}$ day was considered as pretreatment for the second spray and also for the $3^{rd}$ spray. A sample of 10 leaves was picked up from each replicate, thus the sample of each treatment contained 40 leaves. The leaves were examined using a binuclear microscope, the alive moving stages were counted on each leaf The average number in treated and untreated leaves was calculated and the percentages reduction in infestation were calculated by the Henderson and Tilton (1955) equation.

**Biological control of the two-spotted spider mite *T. urticae* infesting green bean:**

This trial was carried out in the experiment which was sprayed 4 times with the commercial biocide "Biofly" (*Beauveria bassiana*), its concentration was $3 \times 10^7$ spores/ml. The tested area was divided into 4 plots every plot was divided into 3 sub-plots, the rates of application were 0.5, 1 and 2 ml/litre. The $1^{st}$ plot was sprayed once, the $2^{nd}$ plot was sprayed twice, the $3^{rd}$ plot was sprayed 3 times and the $4^{th}$ plot was sprayed 4 times. The interval between every spray and the other was 5 days. The samples had been taken regularly after 1, 3 and 5 days from each application and the 5 day was considered as pretreatment for the next application. A sample of 10 leaves was picked up from each replicate, thus the sample of each treatment contained 40 leaves. The leaves were examined using a binuclear microscope, the alive moving stages were counted on each leaf The average number in treated and untreated leaves was calculated and the percentages reduction in infestation were calculated by the previous Henderson and Tilton (1955) equation.

**Statistical analysis:**

Data obtained in the field experiment were subjected to computerized statistical analysis. Duncan's multiple range test was used to determine the significance of the differences between the mean values of the treatments (Duncan, 1995).

## RESULTS AND DISCUSSION

**A. Laboratory evaluation of *Beauveria bassiana* (Balsamo) VUHL pathogenecity against the adult female stage of the two-spotted spider mite *Tetranychus urticae* Koch.:**

The mortality rate of the adult female of the mite *T. urticae* by commercial formulation of *B. bassiana* decreased with decreasing concentration of spore inoculum (Tables 1, 2 and 3). Levels of statistical significance between mortalities at each spore dilution after 8 days of exposure are given in Table (2). The $LC_{50}$ after 8 days of exposure was calculated to be $4.12\times10^{6}$ spores/ml (Table 3). After 10 days of exposure at $3\times10^{7}$ and $3\times10^{8}$ spores/ml, all the tested mites had died; these mortalities were significantly greater than control mortality ($P<0.001$). All treated mites at the rest spore concentration, i.e., $3\times10^{5}$ spores/ml, were killed after 12 days. Control mortality at this time was significantly lower than treatment mortality ($P<0.001$). The mortality rates of *T. urticae* infected by *B. bassiana* are, in part, a function of spore inoculum concentration. More than $10^{6}$ spore/ml of *B. bassiana* gave a rapid kill of *T. urticae* in 8 days, whereas at lower spore concentrations all treated mites were killed after 12 days (Table 1). Levels of mortality between treatments and control in the pathogenicity tests were compared using $X^2$ contingency tables (Table 2). The probit line exp[illegible]es the mortality of the mite *T. urticae* related to log of the concentration of *B. bassiana* spores. The slope of this line from the computerized probit analysis program (Table 3) is b = 1.025.

**Table (1): Mortality (%) of the adult females of *Tetranychus urticae* exposed to a series of spore concentrations of *Beauveria bassiana* (commercial formulation).**

| Spore concentration | Days after incubation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 5 | 6 | 8 | 10 | 12 |
| $3 \times 10^8$ | 69.76 | 75.60 | 86.80 | 97.22 | 97.22 | 97.22 | 100 | - |
| $3 \times 10^7$ | 18.36 | 24.48 | 48.78 | 78.00 | 82.90 | 82.90 | 100 | 100 |
| $3 \times 10^6$ | 7.14 | 7.14 | 7.14 | 17.50 | 22.22 | 46.15 | 47.22 | 100 |
| Control | 0.00 | 0.00 | 0.00 | 2.00 | 2.00 | 5.00 | 7.50 | 11.25 |

**Table (2): Statistical significance between the mite mortalities at each spore concentration after 8 days of exposure to the biocide Biofly (*Beauveria bassiana*).**

| Spore concentration | P values | | | |
|---|---|---|---|---|
| | $3 \times 10^8$ | $3 \times 10^7$ | $3 \times 10^6$ | Control |
| $3 \times 10^8$ | - | | | |
| $3 \times 10^7$ | NS | - | | |
| $3 \times 10^6$ | $P < 0.001$ | NS | - | |
| Control | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | - |

**Table (3): Probit analysis data *Beauveria bassiana*. (commercial product Biofly)**

| | |
|---|---|
| Slope (b) | 1.025581 |
| Intercept (a) | 1.7846110 |
| Variance of slope | 0.0180717 |
| Chi-square | 0.0348358 |
| DF | 1 |
| Log ED50 | 6.6153820 |
| Variance of log ED50 | 1.261E-02 |
| ED50 | 4.124E+06 |

**B. Biological control of the two-spotted spider mite *Tetranychus urticae* on green bean, using the commercial product of *Beauveria bassiana* (Biofly):**

The effect of spray with the biocide Biofly, containing the entomopatrhogenic fungus *Beauveria bassiana* ($3x10^7$ spores/ml) on the mite population infesting green bean was presented in Tables (4 and 5). In this trial, the Biofly was sprayed at three concentrations (0.5, 1 and 2 ml/litre water). Four plots were sprayed by the tested concentrations, the 1st plot was sprayed once, the 2nd plot was sprayed twice, the 3rd plot was sprayed 3 times and the 4th plot was sprayed 4 times, the interval between sprays was 5 days. The data showed that the percentage of mortality increased with increasing the concentration used and number of sprays. In the 1st plot, which was sprayed once with the rates 1 and 2 ml/l, there was no significant difference between the effects of the two rates after 20 days from application on mite population. On the other hand, the concentration of 2 ml/l was the most effective rate.

At the end of the 1st application (5 days after spraying), data showed that there was no significant difference between all concentrations and control. This result indicated that the fungus did not start its activity on the mite population. Table (5) showed that the treatment with 0.5 ml/l had the lowest reduction percentage in all plots, being 13.59, 7.74, 12.4 and 14.9% in the 1st, 2nd, 3rd and 4th plots, respectively. The treatment with 1 ml/l showed medium reduction percentage, being 22.33, 16.73, 15.4 and 14.4% in the 1st, 2nd, 3rd and 4th plots, respectively. Whereas, at the treatment with 2 ml/l the reduction in infestation was 21.98, 27.18, 27.9 and 23.29% for the plots from 1 to 4, respectively.

After 10 days (5 days from the second application), data showed that the mite population decreased with increasing the spore concentration. Table (5) showed that the percentages reduction in the treatment with 0.5 ml/l were 22.58, 14.24, 28.9 and 19.52% for the 1st, 2nd, 3rd and 4th plots, respectively. While the treatment with 1 ml/l showed 24.17, 36.49, 32.44 and 28.35% reduction in mite infestation in the same plots. The treatment with 2 ml/l showed 29.86, 32.49, 34.4 and 31.97% reduction in the mite population in the 4 plots. These data indicated that there were no significant differences between the treatments with 1 and 2 ml/l.

After 15 days from the 1st application (i.e., 5th day after the 3rd application), there was no significant difference between the plot treated once and the control, but the other treatments (i.e., plots which were sprayed twice and three times) showed significant differences as compared with the check control. Data also showed that using 0.5 ml/l reduced the population density by 28.86, 18.6, 26.15 and 24.17%, compared with the treatment with 1 ml/l which caused 25, 39.77, 28.36 and 36.13% reduction in the mite population in the 1st, 2nd, 3rd and 4th plots, respectively. The corresponding values in treatment with 2 ml/l were 37.23, 36.6, 32.36 and 48.49% reduction in the mite population in the four plots.

After 20 days from the 1st application (5 days from the 4th application), data showed that the plot which was sprayed 4 times with the rate of 2 ml/l showed the highest reduction percentage in infestation (78.55%) compared with all rates of application. The treatment of 0.5 ml/l reduced mite population by 38.9, 36.34, 40.2 and 57.02% in the plots sprayed once, twice, three and four times, respectively. While the treatment with 1 ml/l showed 56.14, 49.09, 54.56 and 66.14% reduction and the treatment with 2 ml/l showed 57.8, 58.34, 59.21 and 78.55% reduction in the mite population in the plots which were sprayed 1, 2, 3 and 4 times, respectively.

Many investigators showed that fungous pathogen (Biofly) was effective for control mites. Kanagaratnam *et al.*(1981) found that *V. lecanii* was effective fungous pathogen of a gall causing mite *Cecidophytopsis ribis.* Also, there were low levels of infectivity with *Hiswtella thomposnii* and *Metarhizium anisopliae* but no invectivity with *B. bassiana* (Barkowski *et al.*, 1988). The present results are in agreement with Karadzhov (1973), Humber *et al.*(1981) and Brandenburg and Kennedy (1982).

**Table (4): Number of *Tetranychus urticae* moving stages/leaf infesting green bean as affected by a foliar spray with the biocide Biofly under field conditions.**

| Number of sprays | Rates of application | Average number / leaf at indicated days after applications | | | | | | | | | | | | Means |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 6 | 8 | 10 | 11 | 13 | 15 | 16 | 18 | 20 | |
| Untreated | 0.0 | 125 e* | 116 a | 81.3a | 75.8 b | 73.8 a | 91.3 c | 90 b | 90 b | 68.3b | 77.8 b | 79.3 d | 87.3 d | 87.94 g |
| One Spray | 0.5 ml/l | 120 bc | 89 a | 70.5 a | 65.8 ab | 62.8 a | 60.3 b | 56.8 a | 56.5 a | 48.8 ab | 50.3 a | 47.0 c | 44.8 c | 64.35 f |
| | 1 ml/l | 118 abc | 91 a | 63.5 a | 64.3 ab | 52.5 a | 50 ab | 55.3 a | 50 a | 43.3 ab | 42.8 a | 39.3 bc | 38.5 bc | 58.27 ef |
| | 2 ml/l | 104 abc | 84.8 a | 59 a | 52.5 ab | 67 a | 33.3 a | 45.3 a | 48.3 a | 47.5 ab | 41.8 a | 41.5 bc | 34.3 abc | 54.08 cd |
| two sprays | 0.5 ml/l | 97 abc | 81.3 a | 58 a | 48.8 ab | 57 a | 56 ab | 44.8 a | 38.5 a | 40.8 a | 37.3 a | 32 abc | 41 bc | 52.44 cde |
| | 1 ml/l | 98 abc | 72 a | 63 a | 46 a | 66.3 a | 53.3 ab | 47.5 a | 40.5 a | 38 a | 36.3 a | 33.3 abc | 33 abc | 51.33 cde |
| | 2 ml/l | 95 abc | 71 a | 56 a | 47.5 ab | 53 a | 50 ab | 46 a | 42 a | 36 a | 34.3 a | 34.3 abc | 30.3 abc | 49.6 bcd |
| Three Sprays | 0.5 ml/l | 89 abc | 74 a | 54 a | 49 ab | 48.5 a | 48 ab | 39 a | 35.8 a | 38.3 a | 37.3 a | 33.8 abc | 29 abc | 47.96 abcd |
| | 1 ml/l | 81 abc | 68.5 a | 52 a | 45 a | 47.5 a | 45.5 ab | 40.3 a | 35 a | 37 a | 35.8 a | 28.8 abc | 30 abc | 45.52 abc |
| | 2 ml/l | 80.5 abc | 71.3 a | 52 a | 46.5 ab | 49.8 a | 45.5 ab | 41.5 a | 35.8 a | 36 a | 33.8 a | 30 ab | 27.8 abc | 45.85 abcd |
| Four Sprays | 0.5 ml/l | 80 abc | 72.3 a | 51.5 a | 57.3 ab | 47 a | 47.8 ab | 39.3 a | 36 a | 34.5 a | 31.5 a | 29.3 abc | 28 abc | 45.94 abcd |
| | 1 ml/l | 72 ab | 70 a | 50.5 a | 45.3 a | 47 a | 44.3 ab | 38.3 a | 32.3 a | 30.3 a | 29.5 a | 22.5 ab | 20.5 ab | 41.88 ab |
| | 2 ml/l | 70 a | 68.8 a | 48 a | 45.5 a | 47.5 a | 41.3 ab | 36 a | 32 a | 33.3 a | 28.5 a | 17.8 a | 12.8 a | 40.1 a |

* By Duncan's multiple range test, means followed by the same letter are not significantly different at 5% level.

Table (6): Mean number of *Tetranychus urticae* moving stages/leaf infesting strawberry as affected by a foliar spray with the biocide Naturalis** under field conditions.

| Treatments | Rate a.i/l | Average number / leaf at indicated days after applications | | | | | | | | | Means |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 6 | 8 | 10 | 11 | 13 | 15 | |
| Naturalis-L | 1 ml/l | 4.05 e | 0.70 a | 1.15 ab | 1.00 b | 2.95 e | 1.55 bc | 0.70 bcd | 0.45 a | 0.90 cd | 1.494 b |
| Naturalis-L | 2 ml/l | 1.00 bc | 1.20 b | 1.30 abc | 1.15 b | 1.00 b | 1.25 b | 0.55 abc | 0.60 ab | 0.70 bc | 0.972 a |
| Naturalis-L | 3 ml/l | 0.55 a | 0.65 a | 0.95 a | 0.40 a | 0.15 bc | 0.85 a | 0.45 ab | 0.35 a | 0.10 a | 0.606 a |
| Naturalis WP | 1 g/l | 1.05 c | 3.70 c | 1.90 d | 1.30 b | 1.40 c | 1.70 c | 1.00 de | 0.80 b | 1.90 e | 1.606 b |
| Naturalis WP | 2 g/l | 2.65 d | 5.60 d | 2.85 e | 1.25 b | 1.90 d | 1.95 c | 1.40 e | 0.55 ab | 1.45 e | 2.178 c |
| Naturalis WP | 3 g/l | 0.95 bc | 0.75 a | 1.40 bc | 0.40 a | 0.70 a | 0.85 a | 0.35 ab | 0.30 a | 0.40 ab | 0.678 a |
| Untreated | 0.0 | 8.05 f | 6.90 e | 6.20 f | 6.50 c | 7.65 f | 7.35 d | 8.40 f | 7.60 c | 8.70 f | 7.383 d |

Jegina and Cinowskis (1970) indicated that they obtained 97% mortality in *T. urticae* 24 hours after treatment with the suspension of *Conidiobolus obscurs*. Spadafora and Lindquis (1972) found that spray application of 0.03% benomyl as a systemic fungicide to leaf discs of lima bean in laboratory tests, reduced the percentage hatch of eggs of *T. urticae*. Garcia-Mari *et al.* (1983) evaluated the toxicity of dicofol to eggs of *T. urticae*. The toxicity to eggs and larvae was similar causing 100% mortality at 200 ppm. Nakayama *et al.*(1986) and Mizukoshi (1988) examined the effect of acaricide sprays for control of eggs, nymphs and adults of *T. urticae*. The results showed that bromopropylate plus tetradifon were effective up to 7 days after spraying to eggs.

**C. Biological control of the two-spotted spider mite *Tetranychus urticae* on strawberry:**

The effect of spray with the biocide Naturalis (*Beauveria bassiana*) in two formulations Naturalis-L and Naturalis WP, on the mite population infesting strawberry was presented in Tables (6 and 7). The fungus *B. bassiana*, as a commercial product Naturalis-L in liquid form at a concentration of $2.3 \times 10^7$ spores per milliliter and WP at a concentration of $1.94 \times 10^{13}$ spores/kg, was used as biocide to control the two-spotted spider mite *Tetranychus urticae*. Data obtained revealed that all treatments significantly reduced the mean numbers of the mite population compared with the untreated check control. Both formulations; Naturalis-L and Naturalis WP, were sprayed three times with 3 concentrations (1, 2 and 3 ml or g/l). The data at the end of the trial (after 3 sprays) showed that the mean percentage of reduction in infestation increased with increasing the concentration. The concentrations 2 ml/l, 3 ml/l and 3 g/l were the most effective treatments and there were no significant differences between each other and they followed in descending order by treatments of 1 ml/l, 1 g/l and 2 g/l. This means that the rate of 2 ml/l was economically enough to be used as efficient rate.

After 5 days from the first application (end of the 1st spray), the

Naturalis-L formulation, at concentrations of 1, 2 and 3 ml/l, caused 75.69, 89.6 and 91.69% reduction in the population of the mite, respectively. While using of WP formulation at the same concentrations caused 75.28, 76.4 and 90.66% reduction in the mite population, respectively.

After 10 days from the 1st application (end of the 2nd spray), data showed that treated with Naturalis-L formulation reduced the population of the mite by 88.2, 80.29 and 93.73% by using 1, 2 and 3 ml/l, respectively. Whereas, WP formulation caused 75.27, 81.34 and 95.2% reduction at the same concentrations in gram, respectively.

After 15 days from the 1st application (5 days after the 3rd spray), the data obtained showed that the treatment by Naturalis-L reduced the infestation by 90.67, 94.2 and 99.37% at the concentrations of 1, 2 and 3 ml/l, respectively. While using WP formulation reduced the population by 84.06, 85.16 and 98.09% at the concentrations of 1, 2 and 3 g/l, respectively.

Finally, after 3 sprays, the treatment with the liquid formulation of Naturalis showed 86.2, 88.57 and 95.39% reduction in mite population by using the concentrations of 1, 2 and 3 ml/l, respectively. Whereas, WP formulation showed 76.19, 84.38 and 95.98% reduction with using the concentrations of 1, 2 and 3 g/l, respectively.

### D. Comparative susceptibility between the biocide Naturalis and some conventional acaricides against *T. urticae* on strawberry:

The effect of spraying with Naturalis (*Beauveria bassiana*) in two formulations, Naturalis-L (at 1, 2 and 3 ml/l) and Naturalis WP (at 1, 2 and 3 g/l), and some conventional acaricides (one spray) dicofol, dicofol-M and propargite, against *T. urticae* is shown in Table (8). The data obtained showed that, after 1 day from application, the conventional acaricides were more effective than Naturalis L and WP.

**Table (7): Reduction in *Tetranychus urticae* moving stage infesting strawberry** as affected by a foliar spray with the biocide Naturalis under field conditions.**

| Treatments | Rate a.i/l | Average number / leaf at indicated days after applications | | | | | | | | | Means |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 6 | 8 | 10 | 11 | 13 | 15 | |
| Naturalis-L | 1 ml/l | 85.60 | 97.80 | 75.69 | 76.20 | 87.43 | 88.20 | 92.40 | 90.85 | 90.67 | 86.20 |
| Naturalis-L | 2 ml/l | 71.87 | 94.30 | 89.60 | 9.01 | 84.85 | 80.29 | 95.34 | 96.68 | 94.20 | 88.57 |
| Naturalis-L | 3 ml/l | 96.29 | 94.89 | 91.69 | 96.13 | 91.85 | 93.73 | 97.09 | 97.50 | 99.37 | 95.39 |
| Naturalis WP | 1 g/l | 62.35 | 56.75 | 75.28 | 78.65 | 76.24 | 75.27 | 84.06 | 93.07 | 84.06 | 76.19 |
| Naturalis WP | 2 g/l | 89.84 | 72.38 | 76.04 | 81.27 | 85.24 | 81.34 | 90.39 | 98.16 | 85.16 | 84.38 |
| Naturalis WP | 3 g/l | 95.11 | 95.50 | 90.66 | 97.04 | 95.67 | 95.20 | 98.27 | 98.36 | 98.09 | 95.98 |
| Untreated | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

**Table (8): Number of *Tetranychus urticae* moving stage infesting strawberry as affected by a foliar spray with the biocide Naturalis and some conventional acaricides under field conditions.**

| Treatments | Rate a.i/l | Average number / leaf at indicated days after applications | | | | | | | | | Means |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 6 | 8 | 10 | 11 | 13 | 15 | |
| Naturalis-L | 1 ml/l | 4.05 f | 0.70 a | 1.15 bc | 1.00 b | 2.95 f | 1.55 cd | 0.70 bcd | 0.45 b | 0.90 cd | 1.494 c |
| Naturalis-L | 2 ml/l | 1.00 cd | 1.20 b | 1.30bcd | 1.15 b | 1.00 c | 1.25 c | 0.55 abc | 0.60 bc | 0.70 bc | 0.972 b |
| Naturalis-L | 3 ml/l | 0.55 b | 0.65 a | 0.95 ab | 0.40 a | 0.15cd | 0.85 b | 0.45 ab | 0.35 b | 0.10 a | 0.606 ab |
| NaturalisWP | 1 g/l | 1.05 d | 3.70 c | 1.90 e | 1.30 b | 1.40 d | 1.70 d | 1.00 de | 0.80 c | 1.90 e | 1.606 c |
| NaturalisWP | 2 g/l | 2.65 e | 5.60 d | 2.85 f | 1.25 b | 1.90 e | 1.95 d | 1.40 e | 0.55 bc | 1.45 e | 2.178 d |
| NaturalisWP | 3 g/l | 0.95 cd | 0.75 a | 1.40 cd | 0.40 a | 0.70 b | 0.85 b | 0.35 ab | 0.30 ab | 0.40 ab | 0.678 ab |
| Dicofol | 250ml/100l | 0.65 bc | 0.50 a | 0.75 a | 0.45 a | 0.50 b | 0.70 b | 0.95 cd | 0.75 c | 1.55 e | 0.756 b |
| Dicofol M | 250ml/100l | 0.30 ab | 1.45 b | 1.60 de | 1.05 b | 0.55 b | 0.80 b | 0.75 bcd | 0.55 bc | 1.20 de | 0.917** b |
| Propargite | 60ml/100l | 0.15 a | 0.45 a | 0.70 a | 0.25 a | 0.05 a | 0.10 a | 0.15 a | 0.05 a | 0.10 a | 0.222 a |
| Untreated | 0.0 | 8.05 g | 6.90 e | 6.20 g | 6.50 c | 7.65 g | 7.35 e | 8.40 f | 7.60 d | 8.70 f | 7.383 e |

After 3 days from application, the conventional acaricides were still the most effective and the treatment with Naturalis (liquid at the concentration of 3 ml/l and WP at the concentration of 3 g/l) were more effective than the other biocide concentrations.

After 5 days, propargite and dicofol were the most effective treatments, followed by Naturalis-L (3 ml/l), whereas the other treatments were less effective. There were significant differences between all treatments and the untreated check control.

At the $6^{th}$ day (1 day after the $2^{nd}$ spray), there were no significant differences between propargite, dicofol, Naturalis-L (3 ml/l) and Naturalis WP (3 g/l).

After 8 days, propargite was still the most potent treatment followed by Naturalis-L 3 ml/l, dicofol, dicofol M, Naturalis WP 3 g/ml, Naturalis-L 2 ml/l, descendingly.

After 10 days (5 days from the $2^{nd}$ application with the two formulations of the biocide Naturalis, data showed that propargite was the most effective treatment followed by dicofol, dicofol M and Naturalis WP 3 g/l or Naturalis-L 3 ml/l.

After 11 days (1 day from the $3^{rd}$ application with the two formulations of the biocide Naturalis), data showed that propargite, Naturalis WP 3 g/l and Naturalis-L 3 ml/l were the most potent treatments followed by Naturalis-L 2 ml/l, Naturalis-L 1 ml/l, dicofol M, dicofol.

After 13 days (3 days from the $3^{rd}$ application with the two formulations of the biocide Naturalis), data showed that propargite and Naturalis WP 3 g/L were the most potent treatments followed by Naturalis-L 3 ml/l, Naturalis-L 1 ml/l, Naturalis-L 2 g/l, or dicofol M, Naturalis-L 2 ml/l, dicofol and Naturalis WP 1 g/l.

After 15 days (5 days from application with the two formulations of the biocide Naturalis), data showed that propargite and Naturalis-L 3 ml/l or Naturalis WP 3 g/l were the most potent treatments followed by Naturalis-L 2 ml/l, Naturalis-L 1 ml/l, dicofol M, Naturalis-L 2 g/l, dicofol and Naturalis WP 1 g/l.

Finally, by the end of this trial, data showed that propargite, Naturalis-L 3 ml/l and Naturalis WP 3 g/l were the most potent treatments followed by dicofol, dicofol M, Naturalis-L 2 ml/l, Naturalids-L 1 ml/l, Naturalis-L 1 g/ml, descendingly.

The above results are similar to those obtained by Dresner (1949) and El-Adawy *et al.*(1995).

The toxic effect of *Beauveria bassiana* on the adult stage of *Tetranychus urticae* is referred to the spores present on mite cuticle germinate to produce a young hypha termed as a germ tube. Such thin hypha can either penetrate both the epicuticle and procuticle of mite or only epicuticle, then it expands into normal size hyphae, these hyphae produce numerous dispersal spores and cover the surface of the cadaver. The conidial fungi of *Beauveria bassianas* synthesize and secrete a variety of hydraulic enzymes against chitin and lipids of mite cuticle (Smith *et al.*, 1981; Leger *et al.*, 1986) which could aid the penetration of the inner epicuticle (Plymerized lipoprotein). An insecticidal metabolite one of the bassianolid (cyclodepsipeptide) was isolated from *Beauveria bassiana* (Suzuki *et al.*, 1977). Also, Beauvericin is another toxin isolated and indentified as having insecticidal activity (Hamill *et al.*, 1969), such toxicant could be responsible for mite mortality.

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## الملخص العربى

## التأثيرات الفعالة للفطر الممرض للحشرات بيوفيريا باسيانا على الإناث الكاملة للعنكبوت تترانيكس أورتيكا

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تهدف هذه الدراسة إلى تقييم فاعلية التأثير السام للفطر الممرض للحشرات بيوفيريا باسيانا على الإناث الكاملة للأكاروس ذو البقعتين تترانيكس أورتيكا تحت الظروف المعملية والذى أثبت كفاءة عالية

فـي مكافحـة بعـض الآفـات والأكاروسـات. وقـد أوضحـت نتـائج الدراسـة مـايلي:

١) تـم إسـتخدام تركـيزات مختلفـة مـن المركـب الحيـوى بيوفـلاى وإختبـار مدى فاعليـة علـى طـور الأنثـى الكاملـة للعنكبـوت ذو البقعتيـن وأثبتـت التجربـة أن نسـبة المـوت تـزداد بزيـادة التركـيز المسـتخدم مـن الجراثيـم وكـان التركـيز النصفـى القـاتل ٤,١٢ × ١٠^٦ جرثومـة بينمـا تركـيز وهـو ٣ × ١٠^٨ أعطـى نسبة موت بلغـت ٩٧,٢٢% بعـد ٧ أيـام مـن المعاملـة.

٢) تـم رش المركـب الحيـوى بيوفـلاى علـى نبـات الفاصوليـا المصـاب بالأكاروس فـى الحقـل وأثبتـت المركـب بيوفـلاى تـأثيرا متوسـطا مقارنـة ببـاقى المركبـات حيـث أعطـت ٤ رشـات مـن المركـب بتركـيز ٢ مـل / لـتر مـاء خفضا فـى التعـداد وصـل إلـى ٧٨,٥٥%.

٣) تـم رش المركـب الحيـوى نـترالس ثـلاث رشـات متتاليـة فـى صورتيـن إحداهـا سـائلة والأخـرى كمسـحوق قـابل للبلـل علـى نباتـات الفراولـة المصابـة بالأكـاروس وذلـك بإسـتخدام ثلاثـة تركـيزات مختلفـة مـن كـلا المسـتحضرين فى الحقـل. وقـد أثبتـت المركبـات الحيويـة جـدارة حيـث أعطـت ثـلاث رشـات متتاليـة مـن المركـب الحيـوى نـترالس -ل بتركـيز ٣ مـل / لـتر مـاء نسـبة خفـض وصلـت ٩٥,٣٩% مـن تعـداد الأكـاروس وأيضـا أعطـت نسـبة خفـض ٩٥,٩٨% فى حالة المسـحوق القـابل للبلـل مـن نفـس المركـب.

ومن النتائج السـابقة التـى تظـهر أن المركبـات الحيويـة تبشـر بنتـائج كبـيرة فـى المسـتقبل القريـب فـى القضـاء علـى العنكبـوت ذو البقعتيـن الـذى بـات يـهدد جميـع محاصيلنـا بعـد أن ازدادت مقاومتـه للمركبـات التقليديـة المسـتخدمة ممـا يشـير أنـه فـى المسـتقبل سـتحل هـذه المركبـات الحيويـة محـل المركبـات الكيماوية ذات الأثر الضـار علـى البيئـة والإنسـان