# Selective Toxicity of the Entomopathogenic Fungus *Beauveria bassiana* (Balsamo) to the Two Spotted Spider Mite *Tetranychus urticae* Koch and the Predator *Stethorus gilvifrons* (Muls.)

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## ABSTRACT

The selective toxicity efficacy of entomopathogenic *Beauveria bassiana* (Balsamo) fungus was evaluated against the two spotted spider mite *Tetranychus urticae* Koch and its predator *Stethorus gilvifrons* (Muls) under laboratory condition. The tested *B. bassiana* strain had been brought from Plant Protection Institute, Agricultural Research Center, Jokioinen, Finland. The activation of *B. bassiana* was carried out using *Tenebrio molitor* (L.) larvae as a bait insect. Conidia of growing fungus were extracted and counting of spores was computed. Five concentrations of the conidial suspension were prepared ($3.92 \times 10^4$, $3.92 \times 10^5$, $3.92 \times 10^6$, $3.92 \times 10^7$ and $3.92 \times 10^8$ spores/ml). Results revealed that the mortality rates for both adult females of the mite *T. urticae* and the predator *S. gilvifrons* infected by *B. bassiana* were positively correlated with stranded stage concentration and time dependent. There was a latent eight-day period between infestation and mortality achievement in both tested organisms. On the eighth day, $LC_{50}$ values of *B. bassiana* were $2.702 \times 10^5$ and $2.208 \times 10^{12}$ spores/ml for adult females of *T. urticae* and 2nd instar larval stage of *S. gilvifrons*, respectively. Such selective toxicity was also noted at $LC_{90}$ level where the values were $6.1987 \times 10^{10}$ and $2.839 \times 10^{17}$ for adult females of *T. urticae* and 2nd instar larvae of *S. gilvifrons* respectively. These results showed that the entomopathogenic fungus *B. bassiana* has a potent selective toxicity against adult females of *T. urticae* compared with second instar larvae of *S. gilvifrons.*

**Key Words:** *Tetranychus urticae*, *Stethorus gilvifrons*, *Beauveria bassiana*, Biological control.

## INTRODUCTION

The fungus *Beauveria bassiana* (Balsamo) was firstly recognized as a disease-causing organism by Bassi (1835) and has been used as a biological control agent for about 100 years (De Bach 1948). It is non-specific entomogenous fungus with a broad geographical distribution (Tanada 1953; Leatherdale 1970; Kenneth *et al.* 1971). In Britain, Dresner (1949) recorded the fungus infecting and causing mortality of *Tetranychus urticae* in the field. He also found that 0.5% spore preparation of *B. bassiana* was effective against *T. urticae* on beans. The experiments were carried out in July in very favorable weather for infection and development of the fungus, at 91.3% R.H. and 23.3°C in the morning.

In Egypt, Yousri, 1994 and 1999 studied the pathogenicity of *B. bassiana* against *T. urticae* at various temperatures. On the other hand, several studies indicated that all species of *Stethorus* weise are varacious obligate spider mite predators (Helle & Sabelis, 1985; Sarhan *et al.* 1989a&b and Rott & Ponsonby, 2000) with high capabilities to control spider mites both in open fields and greenhouses (Congdon *et al.*, 1993). On the other hand, the toxicity of some bio-insecticides such as Spinosad and Vertimec and the predation response of some preditors on *T. urticae* were investigated (El-Basha *et al.*, 2005; Raudonis, 2006 and El-Kady *et al.* 2007).

The present study evaluated the effect of *B. bassiana* on adult stage of the two-spotted spider mite *T. urticae* and its predator *S. gilvifrons* in order to assess the selective toxicity of the fungus to be implemented in IPM of *T. urticae.*

## MATERIALS AND METHODS

The techniques used for laboratory assays were similar to those used by Tanada *et al.* (1953), Dunn & MacLeod (1963); Yevlakhova & Shvetsova (1965); Roberts & Yendol (1971); Vanninen & Hokkanen (1988) and Yousri (1994).

**Pathogenic:**

*B. bassiana*, a laboratory strain was brought from Plant Protection Institute, Agricultural Research Center, Jokioinen, Finland host organism of the strain benefit.

**Activation of the strain**:

The strain of *B. bassiana* was stored in deep freezer and reinoculated regularly every three months using potato dextrose agar (PDA) medium in Plant Protection Lab., Ismailia Agricultural Research Station. The activation was done using *Tenebrio molitor* L. larvae as a bait insect. Ten larvae were placed in a petri-dish and infected by *B. bassiana* by spraying with the suspension of the spores using manual atomizer (Sigma glass spray unit No. S 3135). The petri-dishes were covered with wetted filter papers to increase the relative humidity then placed at 25°C and about 100%RH. After about seven days, the

larvae died and the hyphae of the fungus appeared on the upper surfaces of the larvae. The dead larvae were rinsed for three minutes in 0.1% sodium hypochloride, then thoroughly washed with tap water by placing the larvae in a sieve, then dried by rolling gently on tissue paper. Larvae were then placed in a petri-dish containing a selective medium which allowed *B. bassiana* to grow only without contaminating strains of saprophytic fungi. The petri-dishes were kept at 25°C to notice the fungus growth. This activation process was repeated several times to increase the pathogenicity of the tested strain.

**Harvesting of spores from agar to prepare the original treatment suspension:**

The spores of growing fungus were harvested by adding a mixture of 100 ml of sterile water + 50 µl of tween 20 to each petri-dish to reduce the surface tension of the spores suspension. The spores were harvested from the surface of agar by scraping with spatula into the mixture of water + tween 20 then the crude suspension was collected in sterilized bottle (250 ml in size). The glass pearls were added to the bottle and shacked well using a laboratory horizontal shaker to separate the medium, mycelium and spores from each other. The big lumps of mycelium and growth media, were removed from the suspension by using an ordinary tea sieve or muslin cloth.

**Preparation of spore suspension:**

Determination of spores concentration was made by using the Burker chamber (hemocyte counter) as follow:

The spores were harvested from the mass culture media of the fungus into sterile water containing 0.05% tween 20 (surfactant). Dilution was done to facilitate the spores suspension counting. One ml of initial spores suspension was added to 9 ml of sterile water containing 0.05% tween 20 and then shacked well. Using 1/100 dilution rate was enough, taking the concentration of the initial suspension into consideration.

A small drop of the final dilution was put into the dish of a Burker counter by using a pasteur pipette. The dish should be full, but the water should not run out of it. A cover slip was carefully placed over the dish, so that the water drop spread over the sets of squares (the counting cells). After at least 2 minutes the spores sediment to the bottom of the counting cell, then the number of spores was counted under amicroscope, in each of the nine squares surrounded by three lines, altogether nine squares in the upper counting cell and nine in the lower counting cell. (NB: the lines of the counter and the number of the counting squares can be differently arranged according to the type of the chamber: some time for example the number of squarer being twelve instead of nine, but the counting principle remains the same). The counting was repeated for four times (4 drops) to obtain a good estimate of the spore concentration.

The spore concentration was calculated by using the following formula:-

$$\text{No. of spores in suspension} = m \times 2.5 \times 10^5 \times d$$

Where: m = mean number of spores per one square,
d = dilution rate.

The volume of the square was usually, at least in Burker chamber, 1/4000 ml, *i.e* $2.5X10^5$ is used in the formula.

**Check of fungal spores viability**

The viability of spore suspension was checked before preparing the final suspension with known concentration. The percentage viability was used in calculating the amount of the original suspension needed to prepare the final suspension. A drop of 50 µl from suspension (subjected for spore viability) was added to autoclaved test tubes containing one ml of PDA and mixed well by the help of a vortex-mixer. The tube was corked and incubated at 25 °C (the optimum temperature for *B. bassiana* growth) for about 20 h. After the incubation period, the counting of spores was made for germinated spores by taking three drops of agar from the tube into a glass-slide and cover them with glasses. A total of 100 spores from each drop, randomly chosen vision fields, then the germinated and non-germinated spores were counted separately. The percentage of germination (= viability) spores was calculated. The proportion of viable spores should be over 85%.

**Spraying of fungal suspension on the adult female mites *T. urticae*:**

This test was carried out by placing 25 adult females on the lower surface of sweet potato discs (one inch in diameter), all discs were placed in petri-dishes on moist cotton wool. Each petri-dish contained four discs as replicates. From the stock suspension which was prepared of known concentration of spores, five concentrations of the conidial suspension by dilution with sterile water were prepared ($3.92 \times 10^4$, $3.92 \times 10^5$, $3.92 \times 10^6$, $3.92 \times 10^7$, $3.92X10^8$). A constant amount 4ml/petri-dish (4 replicates) was sprayed by a glass manual atomizer (Sigma glass spray unit No. S3135) for each concentration. The control check discs were treated with 4 ml of sterile water mixed with a wetting agent (tween 20). The petri-dishes were incubated for

12 days under constant temperature 25±2 °C and relative humidity approximately 100%. The surviving mites were counted daily and the mortality percentage was calculated. Cumulative percent mortalities were corrected and analyzed as described before (Abbott, 1925 and Finney 1971).

**Spraying fungal suspension on the second instar of larval stage of the predator *S. gilvifrons*:**

Fifty moving individuals of the two-spotted spider mite and 10 individuals of second instar larval stage of the tested predator were transferred by the aid of a fine brush onto the sweet potato leaf discs. From the stock suspension, which was prepared of a known concentration of spores, five concentrations of the conidial suspension by dilution with sterile water were prepared. A constant amount of four ml/petri-dish (4 replicates) was sprayed by a glass manual atomizer (Sigma glass spray unit No. S3135) for each concentration. The control check discs were treated with four ml of sterile water mixed with a wetting agent (tween 20). The petri-dishes were incubated for eight days under constant temperature 25±2 °C and relative humidity approximately 100%. The survivor larvae were counted daily and the percentage mortality was calculated.

Koch's Postulates test was carried out to ensure the positive involvement of the fungus. The cadaver-tested mites were simply placed on selective agar medium which had been poured on petri-dishes to form a layer of approximately three mm thickness. The composition of this selective medium MacConkey agar and modification as follows:- Distilled water (1 Liter); agar (10 g); glucose (5 g); yeast extract (2 g); chloramphenicol (50 mg); cycloheximide (200 mg) and 1 g for each Sodium nitrate; ($NaNo_3$); magnesium phosphate ($MgPO_4.7H_2O$); potassium phosphate ($KH_2PO_4$); sodium propionate; Oxen gall. (Alves *et al*., 2002).

The selective medium efficiently prevents the growth of other bacteria and, saprotophic fungi, but allows *B. bassiana* to grow freely.

## RESULTS AND DISCUSSION

**Effect of *B. bassiana* on the adult female stage of the two spotted spider mite *T. urticae*:**

The mortality rate of the adult females of the mite *T. urticae* infected by *B. bassiana* increased with increasing concentration of spore inoculum (Tables 1 & 2). Statistical significance levels between moralities at each spore dilution after eight days from exposure are given in Table 2. The $LC_{50}$ after eight days of exposure was calculated to be 2.702 $x10^5$ spores/ml. After 10 days of exposure at 3.92 $x10^6$, 3.92 $x10^7$ and 3.92 $x10^8$ spores/ml, all the tested mites died. These mortalities were significantly greater than control mortality. All treated mites at the remaining spore concentrations, *i.e.*, 3.92 $x10^4$ and 3.92 $x10^5$ spores/$mL^{-1}$, were killed after 12 days (Table 2) and control mortality at this time was significantly lower than treatment mortality. The mortality rates of *T. urticae* infested by *B. bassiana* were in part a function of spore inoculum concentration. More than $10^4$ spores/ml of *B. bassiana* resulted in significant mortality of *T. urticae* after 10 days, whereas at lower spore concentrations all treated mites were killed after 12 days. Level of mortality between treatments and control in the pathogenicity tests was compared using $X^2$ contingency tables (Table 2). The slope was calculated from the computerized probity analysis program is $b = 0.239$.

**Effect of *B. bassiana* against *S. gilvifron*s:**

The mortality rate of the second instar of larval stage of the predator *S. gilvifrons* infested with the laboratory strain of *B. bassiana* was decreased with decreasing concentration of spore inoculum (Tables 3 & 4). Statistical significance levels between mortalities at each spore dilution after eight days of exposure are given in table 3. The $LC_{50}$ after eight days exposure period resulted in 2.208 $x10^{12}$ spores/ml, whereas $LC_{90}$ after eight days from exposure recorded 2.839 $X10^{17}$ spores/ml (Table 4).

After 10 days of exposure at 3.92 $x10^7$ mortality recorded 50%, whereas 3.92 $x10^8$ spores/ml, resulted in 70%. The rest of concentrations resulted in 20% mortality after 8 days of exposure. That makes the laboratory strain safe to the predator comparing with its effect on the mite at the same concentrations.

The mortality rates of *S. gilvifrons* infested by *B. bassiana* are in part a function of spore inoculums concentration. More than $10^7$ spores/ml of *B. bassiana* gave an effective kill of *S. gilvifrons* after 10 days, whereas at lower spore concentrations all treated insects had less effect on the treated insects. Level of mortality between treatments and control in the pathogenicity tests was compared using $X^2$ contingency tables (Table 4). The probity line expresses the mortality of the predator *S. gilvifrons* related to log of the concentration of *B. bassiana* spores. The slope of this line from the computerized probity analysis program (Table 4) is $b = 0.251$.

Results of selectivity ratio of *B. bassiana* to adult female of *T. urticae* and the predator *S. gilvifrons* are shown in table 5. It revealed that the mortality

Table (1): Mortality % of the adult female of *T. urticae* exposed to a series of spore concentrations of *B. bassiana* (lab. strain).

| Concentration (Spores No./ ml) | Days after treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 |
| 3.92 $x10^8$ | 0 | 4 | 12 | 22 | 64 | 82 | 92 | 100 |
| 3.92 $x10^7$ | 0 | 2 | 6 | 8 | 46 | 60 | 68 | 100 |
| 3.92 $x10^6$ | 0 | 0 | 2 | 12 | 38 | 52 | 66 | 100 |
| 3.92 $x10^5$ | 0 | 0 | 2 | 4 | 28 | 42 | 58 | 86 |
| 3.92 $x10^4$ | 0 | 0 | 0 | 6 | 14 | 38 | 48 | 64 |
| Control | 0 | 0 | 0 | 0 | 4 | 6 | 10 | 10 |

Table (2): Probit analysis data for *B. bassiana*-treated mites.

| | |
|---|---|
| slope (b) | = 0.239 |
| intercept (a) | = 3.701 |
| Chi-square | = 10.26 (Tabulated $X^2$= 7.8) |
| DF | = 3 |
| Heterogenecteity factor | = 3.421 |
| $LC_{50}$ | = (2.7019) $x10^5$ spores/ml |
| $LC_{90}$ | = (6.1987) $x10^{10}$ spores/ml |
| r (Correlation coefficient) | = 0.915 (Tabulated r = 0.878) |
| P (probability of $X^2$) | = 0.0165 |

Table (3): Mortality percentage of the second instar of larval stage of *S. gilvifrons* exposed to a series of spore concentrations of *B. bassiana*.

| Concentration (Spores No./ ml) | Days after treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 3.92 $x10^8$ | 0 | 5 | 5 | 10 | 15 | 25 | 30 | 45 | 70 |
| 3.92 $x10^7$ | 0 | 5 | 10 | 10 | 20 | 25 | 25 | 40 | 50 |
| 3.92 $x10^6$ | 0 | 0 | 5 | 5 | 10 | 20 | 20 | 30 | 40 |
| 3.92 $x10^5$ | 0 | 0 | 0 | 10 | 10 | 20 | 20 | 25 | 45 |
| 3.92 $x10^4$ | 0 | 0 | 0 | 0 | 5 | 15 | 15 | 20 | 40 |
| Control | 0 | 0 | 0 | 0 | 5 | 10 | 15 | 25 | 30 |

Table (4): Probity analysis data for *B. bassiana*-treated predator.

| | |
|---|---|
| slope (b) | = 0.251 |
| intercept (a) | = 1.9 |
| Chi-square | = 0.198 (Tabulated = 6) |
| DF | = 3 |
| Heterogenecteity factor | = 9.9 |
| $LC_{50}$ | = (2.208) $x10^{12}$ spores/ml |
| $LC_{90}$ | = (2.839) $x10^{17}$ spores/ml |
| r (Correlation coefficient) | = 0.949 (Tabulated r = 0.95) |
| P (probability of $X^2$) | = 0.69 |

Table (5): Selectivity ratio (SR) of *B. bassiana* against adult female mite *T. urticae* and the predator *S. gilvifrons*.

| | *T. urticae* | *S. gilvifrons* | (SR) |
|---|---|---|---|
| LC50 | 2.7019 $x10^5$ | 2.208 $x10^{12}$ | ≈ 7 fold |
| LC90 | 6.1987 $x10^{10}$ | 2.839 $x10^{17}$ | ≈ 7 fold |

S.R.=LC50 or 90 of No. target / LC50 or 90 of target.

increasing rate for both adult females of the mite *T. urticae* and the predator *S. gilvifrons* infested by *B. bassiana* was correlated with increasing concentration and time of infestation. There were latent periods between infestation and mortality achievement in both tested organisms noted after about 8 days. The $LC_{50}$ value of *B. bassiana* was 2.702 $x10^5$ spores/ml after eight days from infestation, while the $LC_{50}$ value of 2nd larval instar of *S. gilvifrons* was 2.208 $x10^{12}$ spores/ml after 8 days of infestation. Such selective toxicity was also noted at $LC_{90}$ level as the values were 6.1987 $x10^{10}$ and 2.839 $x10^{17}$ for adult females of *T. urticae* and 2nd instar larval stage of *S. gilvifrons*. These results show that the entomopathogenic fungi *B. bassiana* enhanced selective toxicity power between the adult females of *T. urticae* and 2nd instar larva of *S. gilvifrons* which recorded about 7 folds after 8 days from infestation.

**Disease Symptoms**:

There was experimental evidence, particularly which presented by Schaerffenberg (1957), showed that *B. bassiana*, like many other entomogenous fungi, kills its insect host by the action of hyphae that germinate from spores, penetrate the exocuticle, and subsequently invade and destroy the internal tissues.

Shigeo (1978) reported that a toxin named bassianolide has been isolated from the *B. bassiana* bodies which accounted for the lethality and toxicity accompanying the atonic symptom detected in the dead silkworm, *Bombyx mori*, pupae infected with *B. bassiana*. This finding is completely in agreement with that of Yousri (1994). The adult females of *T. urticae* were probably infected and killed by the same way. At the time of death, infected mites become dark in colour, unlike their usual appearance. About 48 h. after death and incubation at 24°C and approximately 100% RH, using the selective medium their colour changed to gray-white and the body surface partially filled with fungal mycelium, after further 48 h., aerial mycelium appeared and gradually covered the whole body surface. Sporulation on the surface of the cadaver mites occurred on 9th day after death.

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