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# Evaluation of the effects of *Beauveria bassiana* on the predatory mite *Phytoseiulus persimilis*

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Plant Science

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## Abstract

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) and the predatory mite *Phytoseiulus persimilis* are effective biological control agents of *Tetranychus urticae* (Acari: Tetranychidae), an economically important pest of greenhouse vegetables, horticultural and ornamental crops worldwide. To evaluate whether *B. bassiana* may affect *P. persimilis* negatively, *P. persimilis* mortality, fecundity and *T. urticae* preying capacity exposed to the lowest and highest recommended dose of a commercial formulation of the *B. bassiana* strain GHA at three different temperatures (15, 20 and 25 °C) and 95% RH were evaluated in a detached leaf disk assay and potted plant assay. Adult *P. persimilis* was infected by *B. bassiana* in leaf disk assay and potted plant assay where 100% mortality was reached in 12 days at 20 °C and 25 °C in both assays. However, only 75% mortality was recorded in 12 days of the experiment at 15 °C. There was significant difference between 100% and 75% mortality at 0.05 significance level. Furthermore, the number of eggs laid by *P. persimilis* and *T. urticae* eaten by *P. persimilis* in four days was also affected at 0.05 significance level. The current study shows that *B. bassiana* has significant effect on the survival, egg laying and feeding capacity of *P. persimilis.*

**Keywords:**
*Beauveria bassiana*, *Phytoseiulus persimilis*, *Tetranychus urticae*, natural enemy, biological control, interactions, non-target effect.

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## 1. Introduction

Strawberry, *Fragaria* × *ananassa* (Duch.), has great economic value and approximately 8.86 million tons of strawberries were produced worldwide in 2020. Commercial strawberries are grown in almost all parts of Norway and around 7,030 tons of strawberries were produced in 2020 (FAO, 2020). Strawberries are cultivated mostly in open fields or high plastic tunnels; however, strawberry production is shifting from open field to tunnel production because cultivation of strawberries in plastic tunnel gives higher yield than cultivation in open fields (Kadir et al., 2006). Though there are numerous advantages of cultivation of strawberries in tunnels, it also provides warm and dry condition favorable for pests like the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae).

*Tetranychus urticae*, is a polyphagous and herbivorous pest that can damage several crops and cause substantial yield losses (Helle & Sabelis, 1985; Navajas, 1998). It has been well-studied and documented as the pest of different greenhouse vegetables, horticultural and ornamental crops all over the world. *Tetranychus urticae* attack more than 1100 plant species belonging to more than 250 plant families (Migeon, 2007). Vegetables and fruits are the major host plants attacked by *T. utricae* but it also infests maize, cotton, and soybean (Migeon, 2007). It is a major pest of strawberry which may create huge problems to farmers (Choi et al., 2022). Feeding by *T. urticae* can cause loss of chlorophyll, defoliation, leaf bronzing, and even plant death can occur in severe infestation (Smith, 1996). It can rapidly increase its population because of the short generation time, within and between farms (So, 1991). *Tetranychus urticae* can complete the life cycle in just 10 days if the ambient temperature is near 27 °C (Hoy, 2011). Factors like temperature (Riahi et al., 2013), humidity (Duso et al., 2004), and natural enemies such as predators and pathogens(Fonseca et al., 2020) affect the population size of *T. urticae*.

Biological control, chemical control, or a combination of biological and chemical control are the commonly used control methods worldwide. Since the first serious and widespread outbreak of *T. urticae* reported worldwide in 1950s, different generations of diverse synthetic acaricides have been produced and used to control this pest. Extensive use of the acaricides has resulted in the acaricide resistance in plant-feeding mites which is a seriously increasing phenomenon, especially in spider mites which have high inherent potential for fast evolution of resistance (Knowles, 1997; Van Leeuwen et al., 2010). Several *T. urticae* populations has developed resistance to one or more acaricides across the world(Van Leeuwen et al., 2010). Increasing problem with acaricide resistance and the legal EU framework (Directive 2009/128/EC) that was implemented by Norway in 2015 that promotes integrated Pest management (IPM) has triggered a growing interest in alternative control strategies with a lower impact on environment and health. Because of the widespread use of pesticides and the consequent complications of pesticide resistance, there is a growing demand for sustainable, ecologically sustainable pest control approaches.

Cost-effective management methods must be applied on commercial farms to avoid economic losses caused by elevated populations of *T. urticae* in strawberries. The conservation of natural enemies and the introduction of predatory mites have been the focus of research and development for biological control methods for *T. urticae* (Zhang, 2003). Biological control agents like entomopathogenic fungi and natural enemies can be important parts of *T. urticae* management (Wekesa et al., 2015). Since entomopathogenic fungi have the potential to control arthropod pests, the entomopathogenic fungus species like *Lagenidium, Entomophaga, Neozygites, Hirsutella, Metarhizium, Beauveria, Entomophytora, Erynia, Aschersonia, Lecanicillium, Nomuraea,* and *Isaria* have gained attention (Altinok et al., 2019). *Beauveria bassiana* have been well studied as a microbial control agent of crop pests all over the world because of their pathogenicity to a variety of insects and mites(Islam et al., 2017; St Leger et al., 1992; Ullah & Lim, 2015).

*Beauveria bassaina* is formulated and used as a curative microbial control agent, which is considered a safer alternative to traditional chemical-based pesticides with less non-target effects on health and environment (Jacobson et al., 2001; Maina et al., 2018). *Beauveria bassiana* has numerous advantages, including the ability to infect up to 80% of single pest populations, high genetic variation among isolates, great diversity, potential mortality of all stages of the targeted pest (Groden & Lockwood, 1991). A commercially available formulation BotaniGard based *B. bassiana* strain GHA is recommended for the control whiteflies and thrips in greenhouse crops and strawberry in high plastic tunnels in Norway. Since *T. urticae* is among one of the major pests of strawberry in Norway, farmers use *P. persimilis* as the preventive control measure of *T. urticae*. When applying *B. bassiana* in strawberry tunnels against whiteflies, aphids, and spider mites, one should also consider the possible negative impacts of its use on natural predatory mites like *P. persimilis.*

*Tetranychus urticae* is regulated by predatory arthropods in natural environments, indicating that conservation biological control may suppress this pest in managed agroecosystems (Gardiner et al., 2003; James et al., 2001). Phytoseiid predatory mites has been successfuly used as a biological control method of *T. urticae* within protected environments but also in open fields in many parts of the world (Zhang, 2003). On commercial strawberry farms with high populations of *T. urticae*, inundated release of predatory mites are generally recommended. The predatory mite *Phytoseiulus persimilis* (Acarina: Phytoseiidae) has been shown to be a beneficial arthropod that is effective in controlling *T. urticae*, and it is widely used in IPM and biological control programs (Santi & Maccagnani, 2000) and is currently being commercially produced across the world.

The efficacy of entomopathogenic fungi are influenced not only by the host-pathogen interactions, but also by abiotic factors such as humidity, temperature, precipitation, and ultraviolet radiation (Fernandes et al., 2015; Jaronski, 2010). These abiotic factors also has a significant effect on population growth of insects and mites (Bommarco, 2001; Logan et al., 2006). Certain abiotic factors like temperature and relative humidity(RH) are known to affect the performance and reproductive capacity of of *P. persimilis* (Zhang, 2003). We need to study the effects of different range of temperatures to the different aspect of *P. persimilis* like egg laying capacity, change in feeding capacity and days of survival as the temperature range fluctuates in strawberry growing areas in Norway. We used three different temperatures to study the effects of temperature and *B. bassiana*. The combined effects of using both predatory mites and *B. bassiana* should be studied before using them in the same field (Agboton et al., 2013; Roy & Pell, 2000). Control of *T. urticae* by predatory mite can vary depending on a range of factors such as predator-prey ratio, host plant and climate (Escudero & Ferragut, 2005). Hence, we need to study the effect of temperature on the different aspects of life cycle of *P. persimilis* to use it in different temperature zones.

Studies suggest that multiple natural enemies can be used to enhance the biological control of the pests if simultaneous use of the multiple biological control agents does not lead to antagonistic intraguild interactions (Alma et al., 2007). Combining multiple biocontrol agents may allow for a better biological pest management approach, but at the same time the interaction between them might result into the disruption of each other's function. Research done by Roy and Pell in 2000 suggested that using multiple natural enemies for biological control may act synergistically, additively, or antagonistically. Most studies focus on the host mortality due to the use of pathogenic fungus but recently focus has been on the sublethal effects too. Effect on the fecundity of different insects and mites due to pathogenic fungus has been studied in recent years (Chandler et al., 2005; Roy et al., 2006).

A broader understanding of the different variables that reduce the negative impact microbial control agent might have on other biocontrol agents (e.g., predatory mites) can help to improve their combined use against insect- and mite pests of different crops. *Phytoseiulus persimilis* and *B. bassiana* has been successfully used in different parts of world to control *T. urticae* in green house crops (Oliveira et al., 2007) and controlling aphids, leafhoppers, and whiteflies (Pu et al., 2005). *T. urticae* could also be controlled by releasing *P. persimilis but* we need to find an entomopathogenic fungus which could also be used as curative agent in combination with this predatory mite without harming it. Some laboratory studies have found that *B. bassiana* may affect *P. persimilis* negatively (Ullah & Lim, 2017; Wu et al., 2015). A study by Chandler in 2005 showed, however, that combined application of *P. persimilis* and *B. bassiana* in a glasshouse experiment reduced the *T. urticae* by 98%. Though there have been some studies on the lethal effects of *B. bassiana* on *P. persimilis*, sub lethal effects like change in fecundity and praying capacity due to this mycopesticide are yet to be studied at different temperature levels.

The aim of the current study was to assess the potential negative side effect of the commercially available *B. bassiana* strain GHA on the predatory mite *P. persimilis* at three different temperatures. Highest and lowest doses of *B. bassiana* recommended by the manufacturers were used as the treatments along with control for the comparison. Leaf disk assay and potted plant assay were carried out in the climatic chambers to evaluate the possible effects of *B. bassiana* on number of days until death, feeding capacity, and egg laying capacity of *P. persimilis.* The information obtained in this study will help to know the possible outcome of combining both biocontrol agents at different temperatures and doses of *B. bassiana* to control *T. urticae* control.

## 2. Materials and methods

### 2.1 Rearing of Strawberry plants:

Strawberry, (*Fragaria* x *ananassa)* used for the experiment were grown in a climatic chamber at 22 ± 5 °C and 70% RH situated at NIBIO Ås using potting soil(P-Jord) in plastic pots of dimension (8 x 8 x 9 cm). The climatic chambers were calibrated for photoperiod of 16 h (6:00 AM to 22: PM) using artificial light (3 x 120 cm fluorescent tubes, Philips, TL-D 90 Graphica 36W 965). Two weeks old plants grown from the runners were used in all experiments.

### 2.2 Rearing of *Tetranychus urticae*:

*Tetranychus urticae* were reared on two-week-old strawberry (*Fragaria* X *ananassa*) plants, in insect cage (size: 70x50x50 cm) at 23 ± 2 °C and 60% RH. The mites were collected from Aspidistra in Son, Norway, in 2000 and have been reared on cucumber and strawberry in climatic chamber using photoperiod of 16 h since then. The artificial light is being produced by three 120 cm fluorescent tubes (Philips, TL-D 90 Graphica 36W 965) with a light intensity of 75-95 µmol/m$^2$ /s measured at average height of plants, with an Apogee model MQ-200 sensor.

### 2.3 Rearing of *Phytoseiulus persimilis*:

*Phytoseiulus persimilis* was obtained from the biological product Phytoseilus-system (Biobest Group, Belgium). They were stored in refrigerator at overnight 9 °C in darkness and used next day for the experiments. Around 20 gravid females were transferred from the Phytoseilus-system to a *T. urticae* infested strawberry leaf in 4 petri dishes of diameter 6 cm. Female *mites* were left to lay eggs for eight hours and were removed after laying the eggs. The eggs laid were removed and put in the climatic chambers at 25 °C at 90% RH on detached strawberry leaves until they moulted into the adults. The newly hatched offspring were fed with eggs of the *T. urticae*. All experiments were conducted using newly emerged female predators of the same age.

### 2.4 Fungal pathogen and preparation of conidial suspension

The entomopathogenic fungus (*Beauveria bassiana*) tested was obtained as the commercial product BotaniGard WP (GHA strain: 220 g/kg in the form 4,4 x 10$^{10}$ CFU/g  Mycotech Europe Ltd, England). The experiments were conducted using the minimum and maximum recommended dose of the product: 75 gram of product in 60 (5.5 x 10$^{10}$ CFU/liter) and 120 (2.75 x 10$^{10}$ CFU/liter) liters of water. To ensure the conidial viability, before the experiments, the recommended lowest dose of product was diluted in three series of 10X, 100X and 1000x and plated on potato dextrose agar (PDA) and water agar for 24 h at 25 ± 2 °C for incubation in seven petri plates for each media. The 1000x dilution of *B. bassiana* on PDA was used to determine the germination percentage of the product. Four sections of each plate were counted for the number of germinated conidia per 100 conidia. When the length of the germ tube exceeded the diameter of the conidium, it was considered to have germinated(Luz & Fargues, 1997).The germination percentage of the conidia on PDA was determined to be greater than 90% and the product was used for the experiment.

## 2.5 Experimental design

### 2.5.1 Leaf Disk Experiment

To determine the possible effects of *B. bassiana* on number of days until death, feeding capacity, and egg laying capacity of *P. persimilis*, leaf disk experiment was conducted. Experimental units consisted of 3 cm in diameter strawberry leaf disks excised from strawberry plants and placed with abaxial side up in the plastic cup of 30 ml volume filled with 15ml of 1.5% water agar (figure 1). Lids having 40 holes to circulate the air were used to seal the cups which prevented the escape of the mites. For control, leaf discs were dipped in the sterile water with 0.05% Tween 80(EMD Millipore Corporation, Germany). For *B. bassiana*, the leaf discs were dipped in the conidial solution containing 5.5 x $10^{10}$ CFU/liter and 2.75 x $10^{10}$ CFU/liter of water as two different treatments with 0.05% Tween 80 for 30 seconds before fixing them on the plastic cups. After the leaf discs were air dried for 30 minutes, 5 adult *T. urticae* and 1 female *P. persimilis* were transferred into each leaf disc. The plastic cups were covered with the lids. 15 replications were used for each treatment and the cups were placed in the climatic chambers in the evening at same time. The climatic chambers (MLR-352H-PE, PHC corporation, Japan) produced artificial light with light intensity of 50 µmol $m^{-2}$ $s^{-1}$ using Panasonic fluorescent tubes. The experiments were conducted at three different temperatures: 15 ± 0.5 °C, 20 ± 0.5 °C and 25 ± 0.5 °C and 90 % RH. This experiment was conducted twice at the same setup.

Death of *P. persimilis* each day was observed for once per day for 12 days while number of adult *T. urticae* eaten and number of eggs laid by *P. persimilis* were monitored once per day for 4 days. Eggs laid by both *P. persimilis* and *T. urticae* were removed from the leaf discs each day. Dead predatory mites were transferred to Petri dishes containing moist filter paper and incubated in complete darkness at at 25 ± 2°C in climatic chambers to encourage sporulation. After a 10-day incubation period, the plates were checked for the presence of white mycelium containing conidiophores and conidia, which are typical characteristic of *B. bassiana* (Batta, 2007*)*. Death by mycosis was confirmed by sporulation in the dead cadavers. The fungal growth on cadavers was confirmed by microscopic observation at magnification X630.

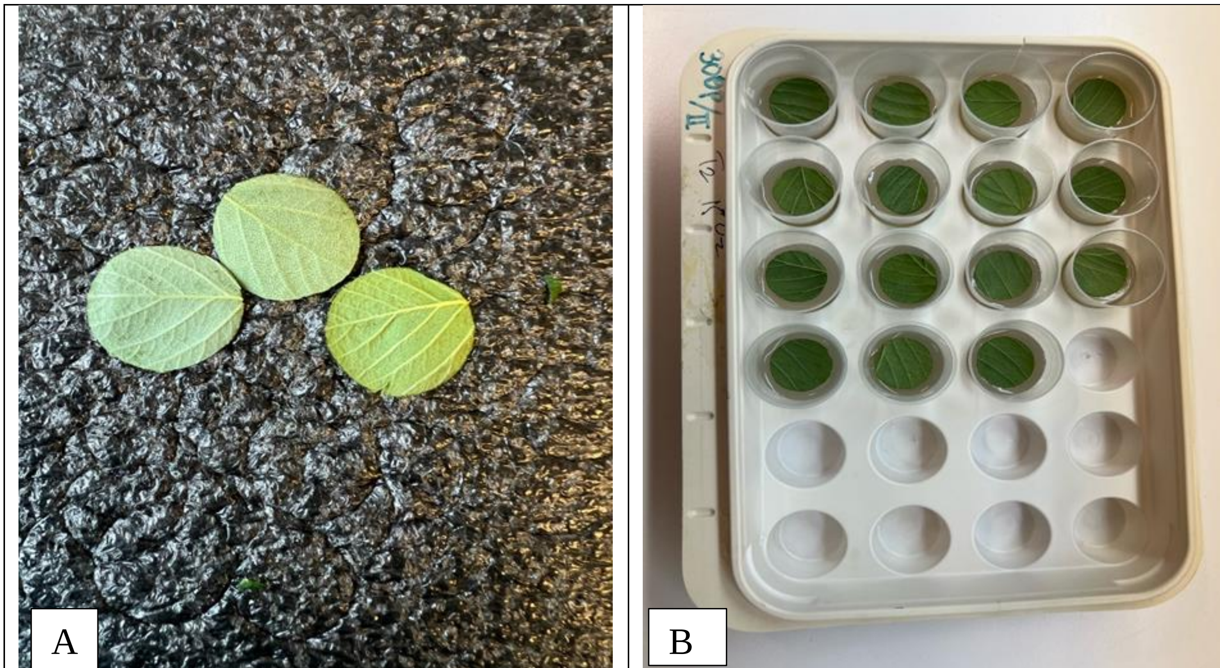


*Figure 1. Leaf disks excised from the two-week-old strawberry plants(A). leaf disks were placed in the plastic cups filled with 1.5% agar and  5 adults Tetranychus urticae and one female Phytoseiulus persimilis were transferred and cups were covered with ventilated lids to prevent the escape of mites(B).*

### 2.5.2 Potted Plant experiment

To evaluate effects of *B. bassiana* on number of days until death, feeding capacity, and egg laying capacity of *P. persimilis*, a potted plant experiment was conducted where each potted plant was trimmed to have only one leaf as shown in figure 2. Two-week-old strawberry plants prepared from the runners were used in this experiment. This experiment examined the effects of lowest recommended dose of *B. bassiana*. Since in leaf disk assay all the *P. persimilis* died in 12 days at both doses at 20 ± 0.5 °C and 25 ± 0.5 °C, we only chose lowest recommended dose to find the similarities between the leaf disk assay and potted plant assay. One female predatory mite and 10 two-spotted spider mites were released on each plant. The experiments were conducted at three different temperatures: 15 ± 0.5 °C, 20 ± 0.5 °C and 25 ± 0.5 °C and 90 % RH. Plants in the control were sprayed with sterile water with 0.05% Tween 80. Solution containing 2.75 x $10^{10}$ CFU/liter of water of *B. bassiana* was sprayed with the handheld sprayer until the solution started to drip from the leaves. Plants were left to air dry, and mites were transferred on the plants as mentioned above. Measurement of the same parameters as in the leaf disk experiment was done and similar process for the confirmation of fungal infection was used. Hand lens X90 magnification was used to observe the dead and alive predatory mites, eggs laid by them and *T. urticae* eaten by them each day.

*Figure 2 Experimental set up for the potted plant experiment. Each plant was a replicate for the treatments in the experiment. The trays were transferred into the climatic chambers after applying the required treatments*

### 2.5.3 Observations

To study the effect of *B. bassiana* on in the *P. persimilis* egg laying capacity and *T. urticae* praying capacity, number of eggs laid by *P. persimilis* and number of adult *T. urticae* eaten were recorded daily for four days in both leaf disk assay and potted plant assay. Similarly, dead *P. persimilis* in each treatment groups were recorded daily for 12 days to find the effect on days to death of the adult|*P. persimilis*.

## 2.6 Statistics

Two experiment each for both the detached leaf assay and potted plant experiment were done to study the effects of two different dose of *B. bassiana* and three different temperatures on number of days until death of adult *P. persimilis*, number of *T. urticae* eaten and effect in egg laying capacity of *P. persimilis*. The three different treatments: control and two doses of *B. bassiana*, and temperature were fixed factors in the experiment, while number of days to death of *P. persimilis*, number of eggs laid by *P. persimilis* for four days and no. of *T. urticae* eaten for four days were the response variables registered in both leaf disk assay and potted plant experiment. Experiment number for each setup was considered as random factor. Each set of data from detached leaf assay and potted plant assay were analysed separately. Data was analysed using the mixed effect model. When significant effect was observed, comparisons were made at 95% significance level using Tukey test.  The analysis was performed using Minitab 21.1.1.

# 3. Result

## 3.1 Leaf Disk Assay

### 3.1.1 Number of days until death:

To confirm death caused by *B. bassiana*, dead *P. persimilis* were observed under microscope(630x) to confirm for fungal growth and sporulation after the adults started to die. All the dead *P. persimilis* were observed until day 12 of the experiment. The death of adult *P. persimilis* started from day three and all the adults were dead by day 10 at 20 °C and 25 °C in both doses of the *B. bassiana*. The adults of *P. persimilis* started to die after day 5 of the start experiment at 15 °C. As shown in Table 1, the average number of days before all the replicates died is lowest for 5.5 x $10^{10}$ CFU/liter *B. bassiana* at 25 °C. Results show that there was significant effect of temperature (P=0.04) and treatment (P=0.02)  (df=2 for both variables) and an interaction between temperature and treatment (df=4) on the mortality of the *P. persimilis.* There was 100% mortality in 12 days at 20 °C and 25 °C for both doses of the *B. bassiana* while at 15 °C the mortality was lower than for 20 °C and 25 °C for both doses (73% for 5.5 x $10^{10}$ CFU/liter and 80% for 2.75 x $10^{10}$ CFU/liter ). Survival of *P. persimilis* tested at day 12 days of starting the experiment was as significantly ($P<0.05$) lower at 25 °C compared to control  for both *B. bassiana* doses.

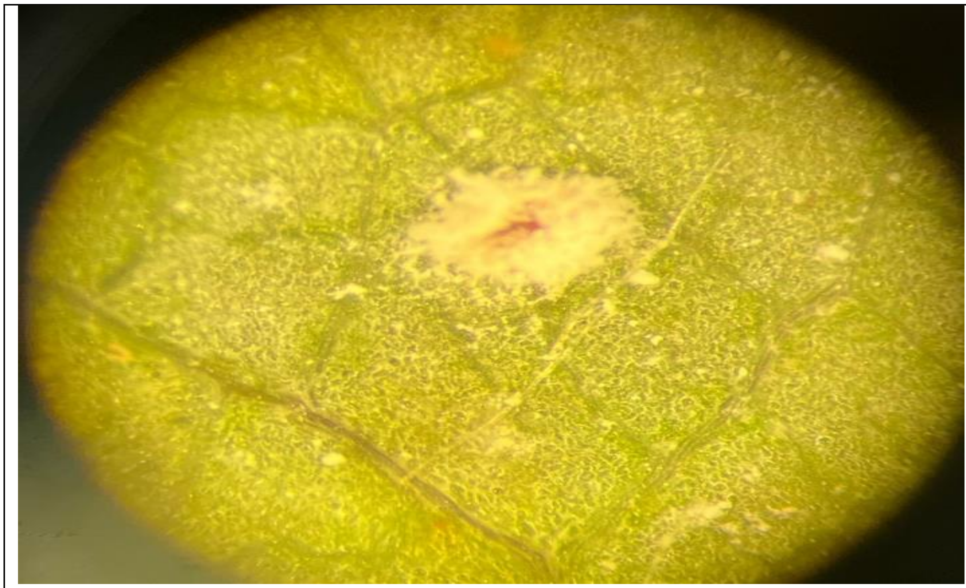

*Figure 3: White mycelial growth of Beauveria bassiana seen on dead Phytoseiulus persimilis four days after incubating dead Phytoseiulus persimils.*

*Table 1. Effect of Beauveria bassiana concentration and temperature on days before death of Phytoseiulus persimilis*. Mean represents the average number of days required to kill all the adult *Phytoseiulus persimilis* due to infection of *Beauveria bassiana.*

| **Treatment** | **Temperature (°C)** | **N** | **Mean** | **Grouping** | | |
|---|---|---|---|---|---|---|
| Control | 15 | 30 | 13.0000 | A | | |
| Control | 25 | 29 | 12.9899 | A | | |
| Control | 20 | 30 | 12.6667 | A | | |
| 5.5 x $10^{10}$ CFU/liter | 15 | 29 | 9.0790 | | B | |
| 2.75 x $10^{10}$ CFU/liter | 15 | 30 | 8.2333 | | B | |
| 2.75 x $10^{10}$ CFU/liter | 20 | 29 | 5.8721 | | | C |
| 5.5 x $10^{10}$ CFU/liter | 20 | 30 | 5.8333 | | | C |
| 2.75 x $10^{10}$ CFU/liter | 25 | 30 | 5.3000 | | | C |
| 5.5 x $10^{10}$ CFU/liter | 25 | 30 | 4.8667 | | | C |

*The groups which do not have same letter are the means which are significantly different after Tukey HSD test at 95% confidence level.*

### 3.1.2 Effect on Egg laying:

Eggs laid for four days were not significantly affected by the different fungal treatments (df=2, $p>0.05$), however, temperature had a significant effect (df=2, $p<0.05$). As shown in Fig. 4 the average number of eggs laid at 15 °C were 4.55 (control), 4.25 (2.75 x $10^{10}$ CFU/liter ) and 4.05 (5.5 x $10^{10}$ CFU/liter), at 20 °C 6.9 (control), 6.85 (2.75 x $10^{10}$ CFU/liter ) and 7.0 (5.5 x $10^{10}$ CFU/liter ) eggs were laid and at 25 °C 10.45 (control), 10.7 (2.75 x $10^{10}$ CFU/liter ) and 10.7 (5.5 x $10^{10}$ CFU/liter).


*Figure 4. Effect of Beauveria bassiana on average number of eggs laid by each female Phytoseiulus persimilis in four days at three different temperatures (15 °C, 20 °C, 25 °C ). Treatments: 1=control, 2=2.75 x $10^{10}$ Beauveria bassiana CFUs/liter, 3=5.5 x $10^{10}$ Beauveria bassiana CFUs/liter. Means that do not share a letter are significantly different.*

### 3.1.3 Effect on Feeding:

The number of *T. urticae* eaten by *P. persimilis* was significantly affected by temperature and the different doses of *B. bassiana* (df=2, p<0.05). Number of *T. urticae* eaten after four days were subjected to analysis.There was significant effect of interaction between the treatments and temperature (df=4, p<0.05). The differences among the different groups of interaction of treatment and temperature are presented in the Table 2. The number of *T. urticae* eaten was highest in the control treatment at 25 °C (Fig 5) and at 15 °C the feeding rate decreased sharply.

Table 2. *The table shows the grouping information using the Tukey method and mean represents the average number of Tetranychus urticae eaten by the adult female Phytoseiulus persimilis in first four days of its life span. It shows that there is significant difference in different combination of temperature and doses of the Beauveria bassiana.*

| **Treatment** | **Temperature** | **N** | **Mean** | **Grouping** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Control | 25 | 30 | 6.43333 | A | | | | | |
| 2.75 x $10^{10}$ CFU/liter | 25 | 30 | 4.73333 | | B | | | | |
| 5.5 x $10^{10}$ CFU/liter | 25 | 30 | 4.00000 | | B | C | | | |
| Control | 20 | 30 | 3.40000 | | | C | D | | |
| 2.75 x $10^{10}$ CFU/liter | 20 | 30 | 2.50000 | | | | D | E | |
| 5.5 x $10^{10}$ CFU/liter | 20 | 30 | 2.03333 | | | | | E | F |
| Control | 15 | 30 | 1.93333 | | | | | E | F |
| 2.75 x $10^{10}$ CFU/liter | 15 | 30 | 1.86667 | | | | | E | F |
| 5.5 x $10^{10}$ CFU/liter | 15 | 30 | 1.10000 | | | | | | F |

The groups which do not share common letters are significantly different at 95% confidence level.

There was significant effect of treatment and temperature in different groups. The mean in the table 2 shows that the highest number of *T. urticae* eaten in four days by each female *P. persimilis* was highest in control treatment.

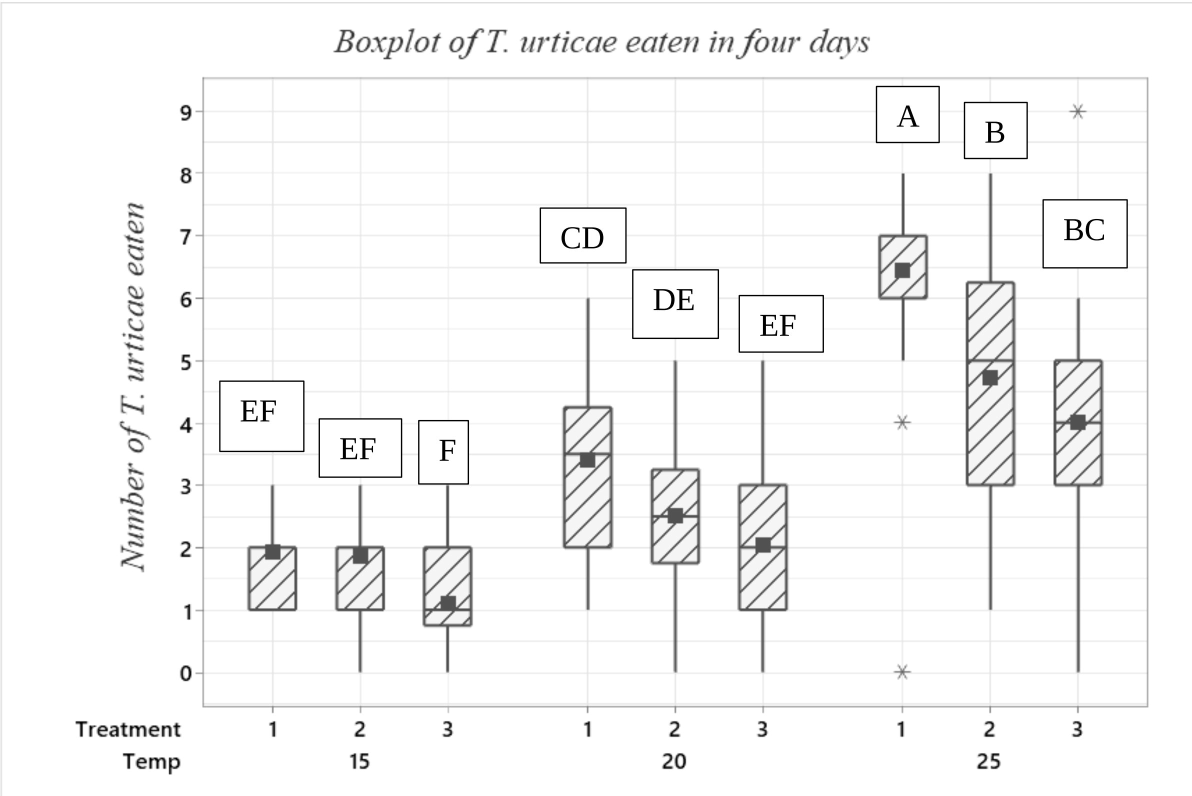


*Figure 5 Average number of Tetranychus urticae eaten by one female Phytoseiulus persimilis at different temperature and treatments in detached leaf assay. X-axis shows the three different treatments and Y-axis represents the average number of Tetranychus urticae eaten by Phytoseiulus persimilis in four days. 1 represents control, 2 represents lower recommended dose of Beauveria bassiana while 3 represents highest dose of B. bassiana used as a treatment in the experiments. Means that do not share a letter are significantly different.*

## 3.2 Pot Experiment:

### 3.2.1 Number of days until death:

The mycosis caused death in potted plant experiment began from day 5 onwards to day 9 at 20 °C and 25 °C while the death started after 8 days of the experimental set up at 15 °C. As in the detached leaf assay, significant effect of temperature, treatment (df=1 for temperature and df=1 for treatment, $p<0.05$) and interaction between temperature and treatment (df=2, $p<0.05$) on number of days to death of the *P. persimilis* were studied. There was 100% mortality in 12 days at 20 °C and 25 °C due to infection of *B. bassiana*. As observed in detached leaf disk assay, mortality at 15 °C was comparatively lower (75%).

Table 3. *Effect of Beauveria bassiana and temperature on days before death of adult Phytoseiulus persimilis. Mean represents the average number of days required to kill all the adult Phytoseiulus persimilis due to infection of Beauveria bassiana.*

| Treatment | Temperature | N | Mean | Grouping | | | |
|---|---|---|---|---|---|---|---|
| Control | 15 | 8 | 13.000 | A | | | |
| Control | 20 | 8 | 13.000 | A | | | |
| Control | 25 | 8 | 13.000 | A | | | |
| 2.75 x $10^{10}$ CFU/liter | 15 | 8 | 10.750 | | B | | |
| 2.75 x $10^{10}$ CFU/liter | 20 | 8 | 7.125 | | | C | |
| 2.75 x $10^{10}$ CFU/liter | 25 | 8 | 5.500 | | | | D |

*The groups which do not share the same letters are significantly different at 95% confidence level.*

In table 3, group A contains all three temperatures and control and groups B, C, D contain 15 °C, 20 °C and 25 °C combined with lowest dose of *B. bassiana* as treatment. There was no death of the adult *P. persimilis* in the control treatment. There was significant difference between the different temperature and dose of the *B. bassiana*. The average number of days to death was 5.5 at 25 °C but the average number of days to death at 15 °C was 10.75. This shows that temperature has significant effect on the days before death of the adult *P. persimilis.*

### 3.2.2 Effect on Egg laying:

The egg laying capacity was significantly affected by the different treatments (df=1, $p<0.05$), and temperatures (df=2, $p<0.05$). Mean of eggs laid in control were 4.88, 7.00, and 8.38 at 15 °C, 20 °C and 25 °C respectively. Similarly, on average 3.88, 5.88 and 7.38 eggs were laid by each female in four days at 15 °C, 20 °C and 25 °C respectively (fig. 6). However, there was not significant effect of interaction between treatment and temperature in eggs laid in four days (df=2, $p=0.973$). There was decrease in number of eggs laid in four days because of *B. bassiana* and temperature.

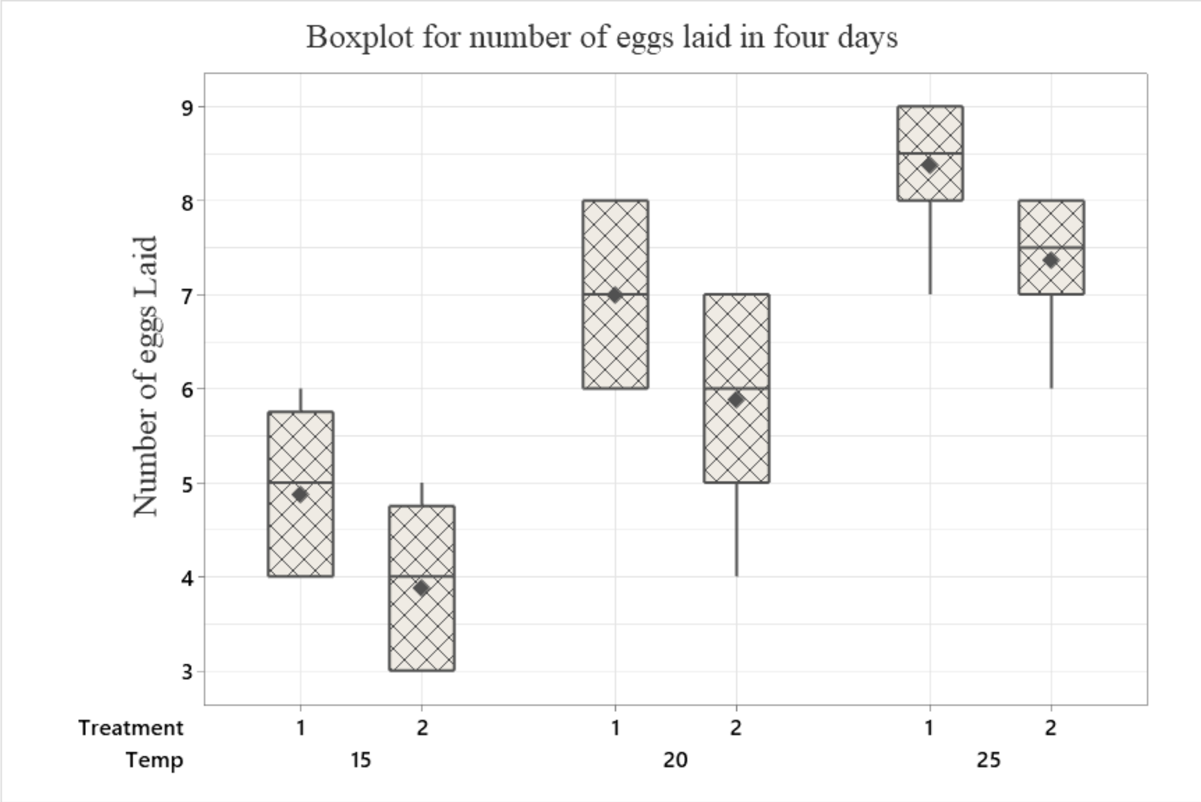


Figure 6 *Average number of eggs laid by the female Phytoseiulus persimilis at different temperature levels in potted plant assay. X-axis shows the different treatments and Y-axis represents the average number of eggs oviposited by Phytoseiulus persimilis in four days. 1 represents control, 2 represents lower recommended dose of Beauveria bassiana.*

### 3.2.3 Effect on Feeding:

The number of *T. urticae* eaten by per female for four days was significantly affected by temperature (df=2, p<0.05), and the treatment (df=1, p<0.05). On average 4.5 and 4.13 *T. urticae* were eaten in four days by each female *P. persimilis* at 15 °C in control and lowest dose of the *B. bassiana* treated plants. At 20 °C and 25 °C, 5.38 and 8.5 *T. urticae* were eaten in control plant respectively (fig. 7). Similarly, 4.88 and 6.63 *T. urticae* were eaten at 20 °C and 25 °C were eaten in the plant treated with lowest dose of *B. bassiana* in four days by each *P. persimilis*. There was no significant effect of interaction between the treatments and temperature (df=2, P=0.089).

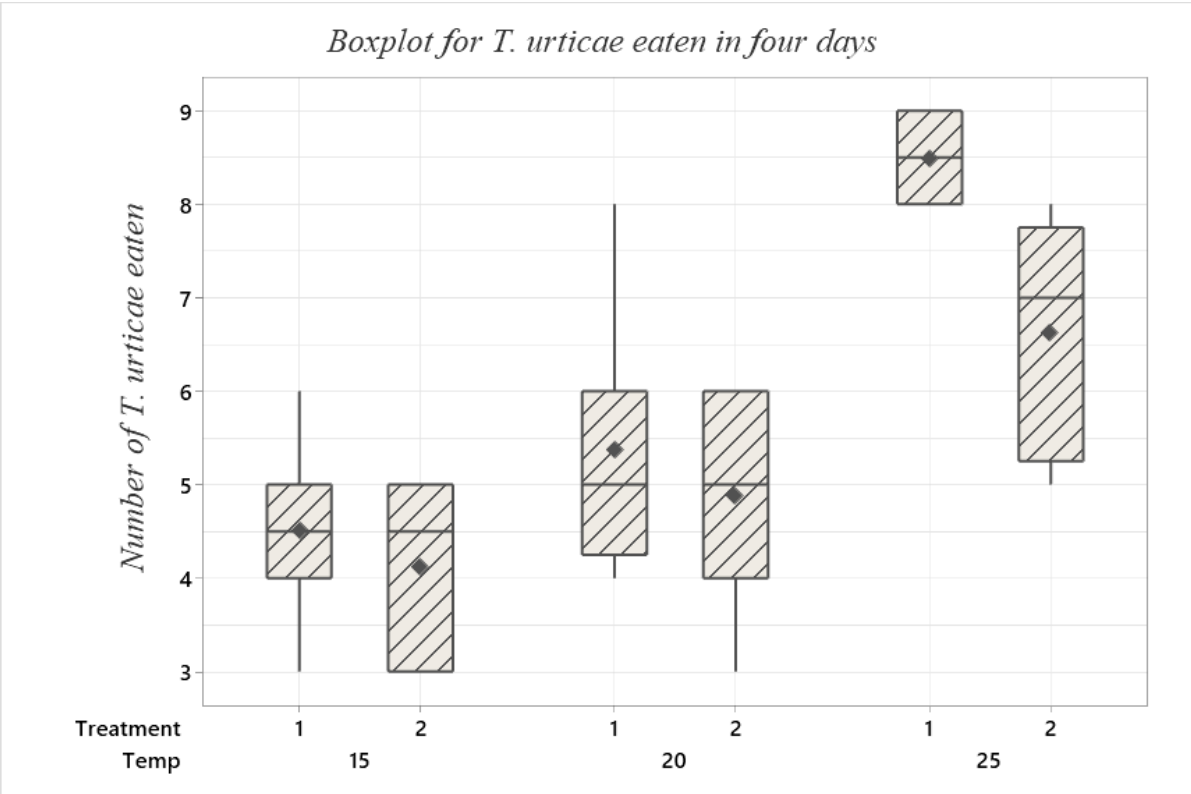


*Figure 7 Average number of Tetranychus urticae* eaten *by the Phytoseiulus persimilis at different temperature and treatments in potted plant assay. X-axis shows the two different treatments along with three temperatures and Y-axis represents the average number of Tetranychus urticae eaten by Phytoseiulus persimilis in four days. 1 represents control, 2 represents lowest recommended dose of Beauveria bassiana.*

## 4. Discussion

Entomopathogenic fungi are employed to control a variety of pests; nevertheless, they may have a detrimental impact on other? natural insect enemies, either directly or indirectly, by reducing the number of prey (Roy, 2000). In both application and theory, it has proven challenging to get the benefit of combined use of the entomopathogens as it might affect the beneficial insects too. An article by Vandermeer et al in 2015 suggested that entomopathogenic agents might have a strong negative effect on both natural enemies and pests of the crops which might lead to failure of IPM.

In our study, *Beauveria bassiana* strain GHA was found to infect *P. persimilis* when exposed to the fungus in leaf disk experiment and potted plant conditions. The method employed here in both experiments mimicked the field condition where *B. bassiana* has been recently sprayed in the crops. Both the doses (5.5 x $10^{10}$ CFU/liter and 2.75 x $10^{10}$ CFU/liter) of the *B. bassiana* used in the leaf disk assay caused significant amount of infection to the *P. persimilis.* Similarly, the lowest dose (2.75 x $10^{10}$ CFU/liter), the only dose used in potted plant assay, also showed significant amount of infection and caused death of the predatory mite. This study also showed that there is significant effect of temperature and different doses of *B. bassiana* in the feeding capacity of the *P. persimilis.* The feeding rate of the *P. persimilis* in the *B. bassiana* treated group was lower than in control.

A laboratory study by Pozzebon and Duso in 2010 to assess the effects of *B. bassiana* on *P. persimilis* where they exposed the predatory mite to *B. bassiana* in three different ways: by infecting the *T. urticae*, topical application and through fresh residues on leaves of beans. They reported the significant effect of *B. bassiana* on survival (mortality ranged from 35 to 60%) and fecundity of *P. persimilis*. The detrimental effect of the *B. bassiana* was independent of methods of exposure used in the experiments. They also found that detrimental effects on fecundity and egg hatching increased when the predatory mite was fed with infected *T. urticae.* In our study we found that the survival of *P. persimilis* was affected by the *B. bassiana*. The mean days until death in leaf disk assay was 5.8 and 4.8 at 20 °C and 25 °C respectively for the highest dose of the *B. bassiana*.

Laboratory evaluation of the effects of *B. bassiana* on the survival, longevity, fecundity, and egg hatch rate of the predatory mite *P. persimilis* done by Ullah et al in 2017 showed significant negative effects on fecundity and life table parameters (net reproductive rate, intrinsic rate of natural increase, mean generation time, finite rate of increase, and doubling time) when $1 \times 10^8$ conidia/ml *B. bassiana* was applied directly on the adults. This study also reported 44% reduction in the oviposition period, 26% in adult longevity, and 63% in fecundity due to application of *B. bassiana* on newly emerged adults of *P. persimilis.* They found that amount of eggs/female/day of *P. persimilis* were significantly reduced by *B. bassiana* spray which supports the results of current study. Our results show that there is significant effect of *B. bassiana* on number of eggs laid during the first four days of the lifespan of *P. persimilis*. The number of eggs laid was higher in control in both laboratory bioassay and potted plant experiment in comparison to the *B. bassiana* treated group.

Laboratory experiment test conducted by Wu et al. in 2018 to assess the change in predation of *P. persmilis* using 1 X $10^7$ conidia/ml of *B. bassiana* in comparison to untreated bean leaves. The result showed that *P. persimilis* mites inevitably encounter fungal conidia while walking and handling the eggs of *T. urticae* on bean leaves. Furthermore, this study reported significant effect in the predation behaviour of the *P. persimilis* due to exposure to *B. bassiana* after 12 hours of spraying *B. bassiana*. The adults spent more time on grooming the leaf treated with the *B. bassiana* in comparison to the untreated leaves. This might be one of the reasons of lower feeding in our experiment. Since *p. persimilis* significantly spends time on groomimg the *B. bassiana* conidia, it might have significantly reduced the number of mites eaten in both experiments.

We found that temperature has significant role in predation of *T. urticae* by *P. persimilis*. The number of *T. urticae* eaten by *P. persimilis* in four days was lowest at 15 °C. Similarly, there was significant effect of different fungal doses on the *T. urticae* eaten by *P. persimilis*. Similar, research done by Bugeme et al. in 2008 reported the significance of temperature on the virulence of three different strains of *B. bassiana and Metarhizium anisopliae (Metschnik.)* to cassava green mite, *Tetranychus evansi*. All the fungal isolates tested were pathogenic to the adult females of *T. evansi*, and there were significant differences in mortality between fungal isolates. They also found that all the isolates tested germinated and grew at all temperatures, but germination and radial growth varied with isolate and temperature. The virulence of different strains of *B. bassiana* varied with the temperature on *T. evansi. Beauveria bassiana* isolate ICIPE279 caused 72.5% mortality at 20 °C. Since, our experiment was done in three different temperatures, the growth of *B. bassiana* could have been affected and hence, the effects on mortality of *P. persimilis*, predation and number of eggs laid could have been affected.

When tested under laboratory condition, several studies have shown that *P. persimilis* are susceptible to *B. bassiana* topical application and exposure to dry residues. Our study also confirms that there are effects on *P. persimilis* caused by *B. bassiana* at different temperature and doses. Slight to moderate susceptibility to the *B. bassiana* was reported by research done by Pozzoben and Duso in 2010 where they observed the effects on survival caused by *B. bassiana* on for three days. Similarly, a study conducted by Numa et al. in 2011, where they observed the effects on the longevity of *P. persimilis* for 12 days, reported the moderate virulence.

Laboratory investigation of interaction of *B. bassiana* and *P. persimilis* by Seiedy et al. in 2012 to control the *T. urticae* reported significant effect on fecundity and longevity of *P. persimilis* due to combined use with *B. bassiana*. 1 X $10^6$ (conidia/ml) was sprayed on leaf disks excised from cucumber to investigate effects on developmental stages and life table parameters of *P. persimilis* and they considered four-time intervals (0, 24, 48 and 72 hours post-inoculation of spider mites) for studying the effect on fecundity and longevity. According to their findings, releasing *P. persimilis* following *B. bassiana* treatment could have a negative impact on the development of *P. persimilis*. In our experiments the number of eggs laid were significantly affected in both assay where the number of eggs laid were reduced.

The laboratory experiment conducted by Vergel et al. in 2012 reported 43% mortality caused by *B. bassiana* on *P. persimilis* when a concentration of 1.25 x $10^7$ conidia/ml was used. They also found that *P. persimilis* was more susceptible to *B. bassiana* than the predatory mite *N. californicus*. Further they concluded that *P. persimilis* used to control *T. urticae* in roses was susceptible to *B. bassiana*. Our research shows that *B. bassiana* GHA could have an adverse effect on the development of the *P. persimilis* population if it is released after the application of *B. bassiana*. Baverstoclk et. al (2009) also suggest that  entomopathogenic fungi used as microbial control agents  may infect and hence cause negative effects to predatory mites. In our study it was observed that female predatory mites laid eggs until they were killed by *B. bassiana*. Similar results were also reported by Vergel et. al. in 2011.

## 5. Conclusion

Based on the results from the leaf disk and the potted plant assay we can confirm that *P. persimilis* is susceptible to both the lowest and the highest recommended field dose of *B. bassiana* strain GHA approved against whiteflies of strawberries in greenhouse by Norwegian authorities. *Beauveria bassiana* caused 100% mortality of *P. persimilis* within 12 days at 20 °C and 25 °C at both concentrations used in the experiments. Similarly, the number of eggs laid and number of *T. urticae* eaten by *P. persimilis* also decreased significantly in four days when treated with *B. bassiana* GHA. Though *Beauveria bassiana* GHA has been recommended to control whitefly and it should be remembered that the strawberry is also attacked by *T. urticae*. Farmers use *P. persimilis* as a preventive measure of *T. urticae* and our study emphasizes that the possible side effects *B. bassiana* has on *P. persimilis* should be taken into consideration before using it in the fields. Further work should be done, however, to understand the compatibility of *B. bassiana* and *P. persimilis* in field condition as the climatic factors might be different in the greenhouses and fields.

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## Appendix

1. **Pairwise Comparison of eggs laid for first four days in leaf disk experiment at different temperature**

**Grouping Information Using the Tukey Method and 95% Confidence**

| Temperature | N | Mean | Grouping | | |
|---|---|---|---|---|---|
| 25 | 60 | 10.6167 | A | | |
| 20 | 60 | 6.9167 | | B | |
| 15 | 60 | 4.2833 | | | C |

*Means that do not share a letter are significantly different.*

2. **Comparison of *T. urticae* eaten in four days in potted plant experiment**

**Grouping Information Using the Tukey Method and 95% Confidence**

| Treatment | Temperature | N | Mean | Grouping | | |
|---|---|---|---|---|---|---|
| Control | 25 | 8 | 8.500 | A | | |
| 2.75 x $10^{10}$ CFU/ltr | 25 | 8 | 6.625 | | B | |
| Control | 20 | 8 | 5.375 | | B | C |
| 2.75 x $10^{10}$ CFU/ltr | 20 | 8 | 4.875 | | | C |
| Control | 15 | 8 | 4.500 | | | C |
| 2.75 x $10^{10}$ CFU/ltr | 15 | 8 | 4.125 | | | C |

*Means that do not share a letter are significantly different.*

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