



Enhancing Biological Control Efficiency: Predatory Potential of *Phytoseiulus Persimilis* Against *Tetranychus Urticae* in Greenhouse Conditions



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Abstract: This study examines the predatory efficiency and biological characteristics of *Phytoseiulus persimilis* in managing *Tetranychus urticae* (two-spotted spider mite) populations under laboratory and greenhouse conditions. Laboratory assessments were conducted to evaluate feeding preferences and reproductive performance by providing *Ph. persimilis* with different prey types, including *T. urticae* eggs, *Sitotroga cerealella* eggs, and decapsulated *Artemia salina* cysts. Findings indicated a marked preference for *T. urticae* eggs, with *Ph. persimilis* consuming an average of 23.5 eggs per day, significantly surpassing other prey types in consumption rate. Greenhouse trials in cucumber cultivation systems evaluated the predator's efficacy in reducing *T. urticae* populations at a predator-to-prey ratio of 1:10. Within 10 days, *Ph. persimilis* achieved a reduction of over 70% in *T. urticae* populations, underscoring its effectiveness as a biological control agent in greenhouse settings. Statistical analyses, conducted using dispersion analysis via Microsoft Excel and SigmaStat 3.1 software, validated these findings. Controls comprised untreated greenhouse sections and laboratory containers devoid of predators to ensure accurate comparative assessments. The results support *Ph. persimilis* as a highly effective biological control agent, demonstrating significant predation rates and reproductive success, which underscores its potential to reduce chemical pesticide reliance and promote sustainable, eco-friendly pest management in integrated pest management (IPM) frameworks.

Keywords: *Phytoseiulus persimilis*; *Tetranychus urticae*; Biological control; Integrated pest management; Predatory mites

1. Introduction

People are witnessing a rapid development of protected agriculture. The range of cultivated plants is expanding, and traditional vegetable crops (cucumber, tomato, and sweet pepper) are joined by eggplants, green crops, strawberries, melons, flowers and ornamental plants (Baiseitova et al., 2023; Naliukhin et al., 2024).

The world knows over 1,200 vegetable plant species; only 80 species are actively used in agriculture. Multivorous phytophages reduce the weight and quality of the harvests. To ensure a stable supply of vegetables, it is crucial to cultivate them year-round on protected land (Amirova et al., 2024).

Plant protection from pests and diseases plays an important role in increasing crop yield in protected soil. However, the protected environment of greenhouses with controlled temperature and humidity also fosters conditions favorable to pest infestations (Alimbekova et al., 2021). The uninterrupted use of greenhouses for the same crops creates favorable conditions for the spread and development of many pests (whiteflies, spider mites, aphids, and thrips).

Among the most problematic pests in greenhouse agriculture is *T. urticae*, commonly known as the two-spotted

spider mite. This species, highly adaptable and polyphagous, affects over 200 plant species and is capable of causing significant yield losses, with damage often reaching 40-60% in heavily infested crops. In the industrial production of vegetables in protected soil, herbivorous mites can cause great damage to the product (Kashutina et al., 2019; Korolkevich, 2009). Spider mites cause irreversible damage to cucumber and tomato crops a month after the attack if no control measures are taken. The main method of pest control is treatment with chemical plant protection agents. However, continuous chemical application has led to the development of resistance in pest populations, reducing pesticide efficacy and contributing to environmental contamination. Furthermore, chemical treatments can pose health risks to both farm workers and consumers, driving a need for more sustainable, eco-friendly pest control methods (Agasyeva et al., 2021).

The most well-developed method of biological plant protection against spider mites in greenhouses is using the predatory mite *Ph. persimilis*. This is the only way to overcome the resistance of indoor pests and obtain ecologically clean products consumed fresh. To reduce the chemical load on plants and ensure environmental safety, methods using pesticides and beneficial arthropods have been developed (Adilkhankyzy et al., 2019; Maksimova, 2013; Temreshev et al., 2023; Tixier, 2018). It is risky to rely on chemical plant protection products alone to control spider mites, as they have a great genetic potential for resistance to acaricides (Helle & Vrie, 1974).

2. Literature Review

The two-spotted spider mite belongs to the family *Tetranychidae*, order *Trombidiformes*, genus *Tetranychus*, species *Urticae* (Migeon et al., 2019).

Yield losses in protected soil due to *T. urticae* damage reach 40-60%. *T. urticae* can develop on over 200 plant species from 30 different botanical families (Bondarenko et al., 1993). As a result of the damage, light spots are formed on leaves in the places where the pest feeds, which gradually merge and acquire a marbled color. The mite also infests stems and flowers, covering them with cobwebs. The plants damaged by the mite suffer from the disruption of transpiration and photosynthesis, leading to plant depression and a significant reduction in yield. The development of one generation of the pest is completed within 7-26 days, depending on environmental conditions. *T. urticae* develops in a wide range of temperatures and humidity, but the optimal conditions for its development are 29-31°C and 35-59% humidity.

One of the most effective biological methods of controlling *T. urticae* is the predatory mite *Ph. persimilis*. It is highly biologically effective in suppressing pest populations. *Ph. persimilis* belongs to the family *Phytoseiidae* and superorder *Parasitiformes* and predominantly preys on plant-eating *T. urticae* (van Wijk et al., 2006).

Ph. persimilis adult stage differs from *T. urticae* by its much greater mobility. Compared to other species of the *Phytoseiidae* family, this predatory mite has a much higher rate of development and is extremely voracious (Popov & Kondriakov, 2008). *Ph. persimilis* can significantly reduce spider mite populations in crops such as cucumbers, roses, and other ornamental plants under controlled greenhouse conditions. Research highlights the mite's rapid life cycle and high reproductive success, which allow it to quickly establish and control pest populations. However, the success of *Ph. persimilis* in pest suppression is closely tied to environmental conditions, as its development is optimal within specific temperature and humidity ranges (Zhang, 2003).

Ph. persimilis is successfully used on different vegetable and ornamental crops. On chrysanthemum in England, with 10 specimens per plant, the predator destroyed the pest in 3-4 weeks. In China, on the potted crops *Salvia splendens*, *Ageratum conyzoides*, *Zantedeschia aethiopica*, and *Pelargonium lateripes*, the mite was effective at rates of 1 to 50 specimens/plant depending on the density of the prey and the size of the plant. In Sicily, Italy, the predator came from the wild into gerbera greenhouses and successfully controlled *T. urticae*. In rose cultivation, *Ph. persimilis* successfully killed the phytophage in a few weeks as long as a predator-prey ratio of 1:10 was maintained (Gacheri et al., 2015). The commonly recommended application rates for this predator are as follows: 2 specimens/m² once every 21 days for prevention; 6 specimens/m² every 7 days for small infestations in small foci until the necessary effectiveness is achieved; and 20-50 specimens/m² every 7 days for large infestations (Dogan et al., 2019). Successful long-term control of *T. urticae* on greenhouse roses is subject to repeated releases of *Ph. persimilis* (Dubovskiy et al., 2012; Gough, 1991).

Ph. persimilis release rates depend largely on the density of *T. urticae* infestation, plant species, and hygrothermal conditions. On average, the annual rate of *Ph. persimilis* colonization in greenhouses on cucumber plants is 0.5-1 million specimens/ha. When the first pest foci appear, soybean or other crop leaves with the predator accumulated on them should be spread across the mite foci at the rate of 10-60 predator specimens/infested plant. In neglected pest outbreaks, the predator-victim ratio should be 1:20-1:50, depending on the crop (Agasyeva et al., 2021; Dubovskiy et al., 2012).

While *Ph. persimilis* has shown promise as a biological control agent, gaps remain in understanding the exact predator-prey dynamics under different environmental conditions. Previous studies have focused on single environmental setups or specific crop types, limiting the generalizability of findings across diverse greenhouse conditions.

The study aims to explore the biological characteristics, feeding behavior, and predatory efficiency of *Ph.*

persimilis under controlled conditions.

Researchers have been searching for affordable and technologically feasible methods to breed *Ph. persimilis* in the laboratory. The newly developed types of feed are more cost-effective to produce in laboratory conditions compared to the traditional use of feed mites bred on bran. Studies have been conducted on artificial diets consisting of honey, sucrose, pollen, bee pollen, tryptone, egg yolk, the hemolymph of the black soldier fly (*Hermetia illucens* Linnaeus), mill moth eggs (*Ephesia kuehniella* Zeller), and the eggs of *Artemia crustaceans* (Debolt, 1982; Nguyen, 2015). Nevertheless, foraging mites remain the main type of feed for predatory mites. In Kazakhstan, the species used for these purposes include the mold mite (*Tyrophagus putrescentiae* Schrank), the dried-fruit mite (*Carpoglyphus lactis* Linnaeus), and the flour mite (*Acarus farris* Oudemans), raised on wheat bran and then transferred to predatory mites (Dobrokhotov, 2008).

The primary purpose of this study is to evaluate the potential of this predatory mite as a biological control agent against *T. urticae* in laboratory and greenhouse environments to reduce reliance on chemical pesticides and promote sustainable pest management practices in protected agriculture.

3. Methods

The research was conducted in the Laboratory of Beneficial Insects and Mass Bioagent Production, Department of Biological Plant Protection, Kazakh Research Institute of Plant Protection and at the Nurakhunov peasant farm, Almaty region, Enbekshikazakh district, Kazakhstan. The subjects under study were *T. urticae* (Acari: Tetranychidae) and *Ph. persimilis* (Acari: Phytoseiidae).

The breeding technique adapted to laboratory conditions was utilized to produce the predatory mite *Ph. persimilis* (Agasyeva et al., 2021; Debolt, 1982; Dobrokhotov, 2008; Dubovskiy et al., 2012; Nguyen, 2015). The principal scheme of acariphage production technology included the cultivation of beans, which is a food substrate of its prey *T. urticae*, the accumulation of the phytophage on the grown plants, and then the production of the bioagent itself.

To ensure a reliable supply of both *Ph. persimilis* and *T. urticae* for the study, breeding techniques were adapted to laboratory conditions. *T. urticae* colonies were reared on common bean plants in a separate, controlled room at 25-30°C and 50-60% relative humidity with a 16-hour light period to simulate natural day-night cycles. Bean plants were regularly replaced to prevent overpopulation and ensure continuous growth of healthy mite populations. For *Ph. persimilis* breeding, the temperature was maintained at 26-28°C and humidity at 70-85%, which is optimal for its development.

The number of *Ph. persimilis* specimens was counted on day 10 after release by directly counting mites on plants using MBS-10 binoculars. The voracity of the predatory mite was determined according to the methodology of technological regulations for *Ph. persimilis* production. For this purpose, bean leaves with the predator and different types of eggs were placed in prepped plastic containers. The container was kept at 25°C for two days, after which the number of eggs eaten by *Ph. persimilis* was counted (Dobrokhotov, 2008).

The experiment to determine fecundity was conducted with *Ph. persimilis*. The predatory mites were kept with different feeds at 25°C and 85% relative humidity for 5 days. The experiment was carried out in four variants, i.e., *T. urticae* (control); *S. cerealella*; *A. salina*; and artificial nutrient medium *D1*, with each repeated three times. The results show that the female predator laid an average of 13 eggs in 5 days when fed with *T. urticae*. In the second and third variants, the number of laid eggs averaged 8 and 6, respectively. In the version with an artificial nutrient medium, the female predator laid only 3 eggs. The laid eggs of predators were observed in laboratory conditions for 7 days to determine the viability of predator nymphs at an air temperature of 25°C and a relative humidity of 75%. In all variants, nymph viability was determined after 3-4 days.

Laboratory experiments were conducted to determine the voracity of predatory phytoseiid mites (*Ph. persimilis* and *Amblyseius swirskii*) with different food options: *C. lactis* (control), *S. cerealella*, and *A. salina*. The voracity of first-generation phytoseiids (G1) was evaluated by counting intact *T. urticae* eggs at the end of the experiment. The results of the experiment show that G1 *Ph. persimilis* and *A. swirskii* bred on the tested diets did not lose their ability to kill phytophages. Only the voracity of G1 when fed decapsulated *A. salina* eggs was significantly lower (1.2 units) than in the control variant (3.5 units).

Experiments were also conducted to determine the selectivity of phytoseiids for different types of food. The results suggest that *Ph. persimilis* and *A. swirskii* prefer *S. cerealella* eggs out of the provided food. All tests were repeated three times.

Voracity (*Vor*), understood as the number of *T. urticae* eaten by a female predator in 1 day, was calculated using the formula:

$$Vor = \frac{(M - n)}{2n} \quad (1)$$

where, *M* is the initial number of *T. urticae* specimens placed in the cage; *n* is the remaining number of *T. urticae*

specimens; and n is the initial number of female specimens placed in the cage.

The method of determining phytoseiid viability essentially consists in counting adults of phytoseiids. The counting was performed on day 7 after the beginning of the trials.

Viability, denoted as O , which is the share (%) of developed phytoseiid specimens from the eggs laid by females over the first 2 days of testing, was calculated according to the following formula:

$$O = \frac{(k-n)}{N} \times 100 \quad (2)$$

where, k is the number of phytoseiid adult specimens; n is the initial number of female specimens placed in the cage; and N is the number of eggs laid by female specimens in 2 days (Nguyen, 2015).

The biological effectiveness of *Ph. persimilis* application was determined according to the death rate of or the decrease in the *T. urticae* population on the protected crop. For this purpose, before the release of the predator, the pest population on the plants infested with *T. urticae* was counted. Sometime after the release of *Ph. persimilis* (3, 5, and 7 days), the number of *T. urticae* was counted again. The obtained data allowed us to determine the biological effectiveness of the bioagent (B), calculated using the following formula:

$$B = \frac{(a-c)}{a} * 100 \quad (3)$$

where, a is the *T. urticae* population on plants before *Ph. persimilis* release (specimens); and c is the *T. urticae* population on plants 7 days after the release of the acariphage (specimens) (Antonenko et al., 2024; EcoProverka.ru, 2019).

Statistical processing of the data was carried out by the method of dispersion analysis using Microsoft Office Excel spreadsheets and the SigmaStat 3.1 application software package.

Biological effectiveness of *Ph. persimilis* was calculated as the percentage reduction in *T. urticae* populations on treated plants compared to control sections.

4. Results

Laboratory populations of *T. urticae* and *Ph. persimilis* were bred in the Laboratory of Beneficial Insects and Mass Production of Bioagents, Department of Biological Plant Protection (Figure 1).



Figure 1. Laboratory population of *Ph. persimilis*

The study of the biological indicators of *Ph. persimilis* in laboratory conditions shows that decreased temperature and relative humidity significantly affect the development of the acariphage (Figure 2).

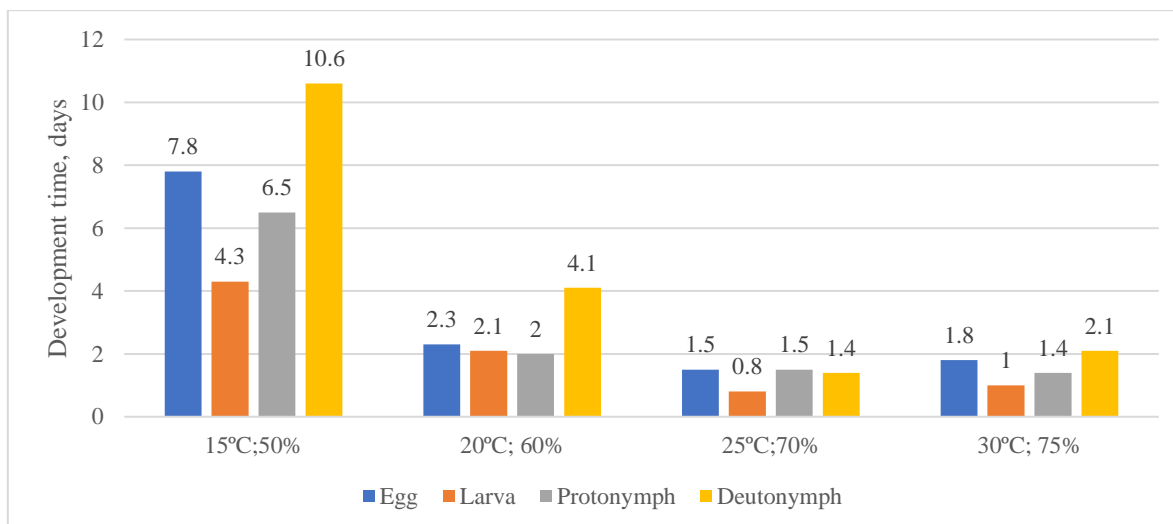


Figure 2. The influence of temperature on *Ph. persimilis* development

At more favorable temperatures of 25 and 30°C and the relative humidity of 70 and 75%, the development period of the acariphage from egg to adult is 5.2-6.3 days, respectively, which is 2 times faster than that of *T. urticae*. The female on average lays up to 80 eggs during her lifetime and destroys about 30 eggs and more than 20 *T. urticae* adults. Comparatively, previous studies indicate that *Ph. persimilis* performs optimally within this range, with temperatures above 30°C negatively affecting its survival and predation (Urbaneja-Bernat & Jaques, 2022). For *T. urticae*, which can tolerate a broader range of conditions, lower humidity levels tend to decrease mite reproduction and survival, a finding corroborated by Messelink & Leman (2020).

To determine the voracity and selectivity of *Ph. persimilis* in laboratory conditions, the acariphages were provided with *T. urticae* eggs, *S. cerealella* eggs, and decapsulated *A. salina* eggs as feed (Figure 3).

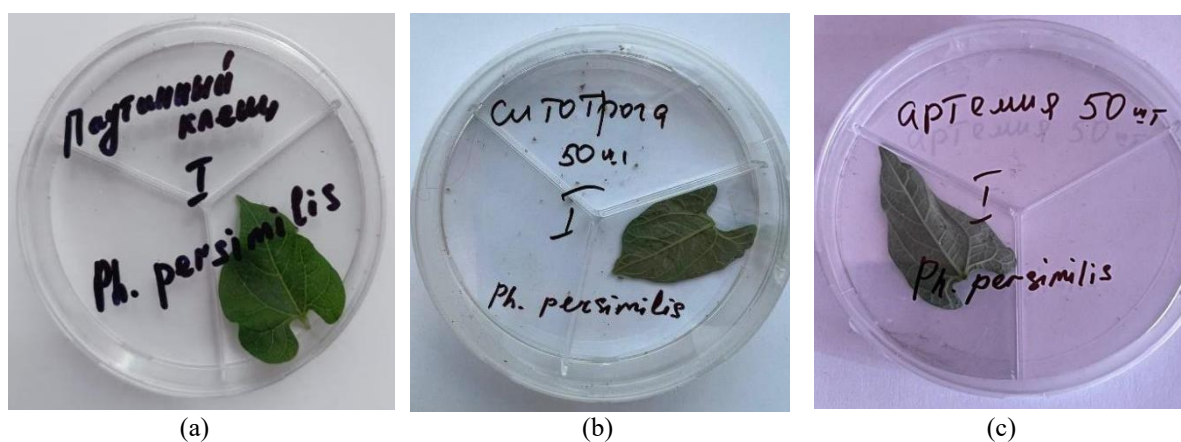


Figure 3. (a) *T. urticae*; (b) *S. cerealella*; and (c) *A. salina*

The acariphage adults and nymphs were fed daily with the proposed feed and monitored. The studies demonstrated that the voracity indices of *Ph. persimilis* adults and nymphs do not differ significantly when fed the proposed feed (Figure 4).

Ph. persimilis adults and nymphs attacked all presented types of feed but favored the eggs of *T. urticae*. An adult of the predatory mite consumed an average of 23.5 *T. urticae* eggs/day, 12.2 *S. cerealella* eggs, and 7.2 decapsulated *A. salina* eggs/day. Predator nymphs also preferred to feed on *T. urticae* eggs over other feed offered.

The experiment determining the biological activity of *Ph. persimilis* in laboratory conditions against *T. urticae* yielded the results below. The average number of *T. urticae* eggs gradually increased, and the number of larvae, nymphs, and adults remained relatively constant. On day 6, the number of *T. urticae* eggs and specimens decreased markedly. The peak of *T. urticae* nymph growth was observed on day 7 (146 specimens). The maximum number of adults on day 8 was 108 specimens with about 1,000 eggs. On day 12, the number of *T. urticae* eggs, nymphs, and adults decreased by 88.3, 97, and 77.8%, respectively. While the phytophagous population showed a decrease in density, the acariphagous population was growing (Figure 5).

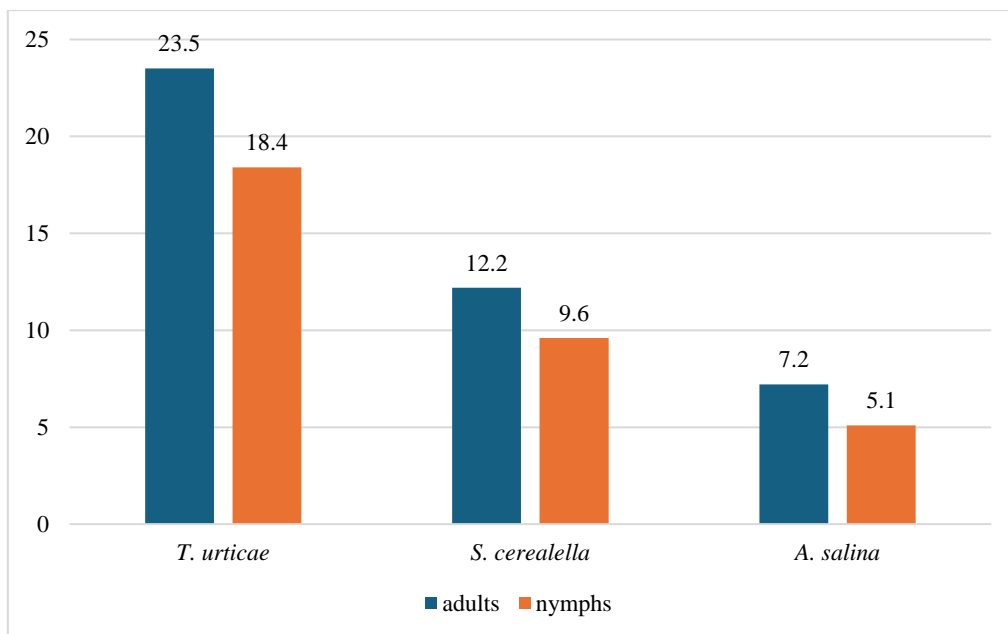


Figure 4. Assessment of the voracity of *Ph. persimilis* adults and nymphs

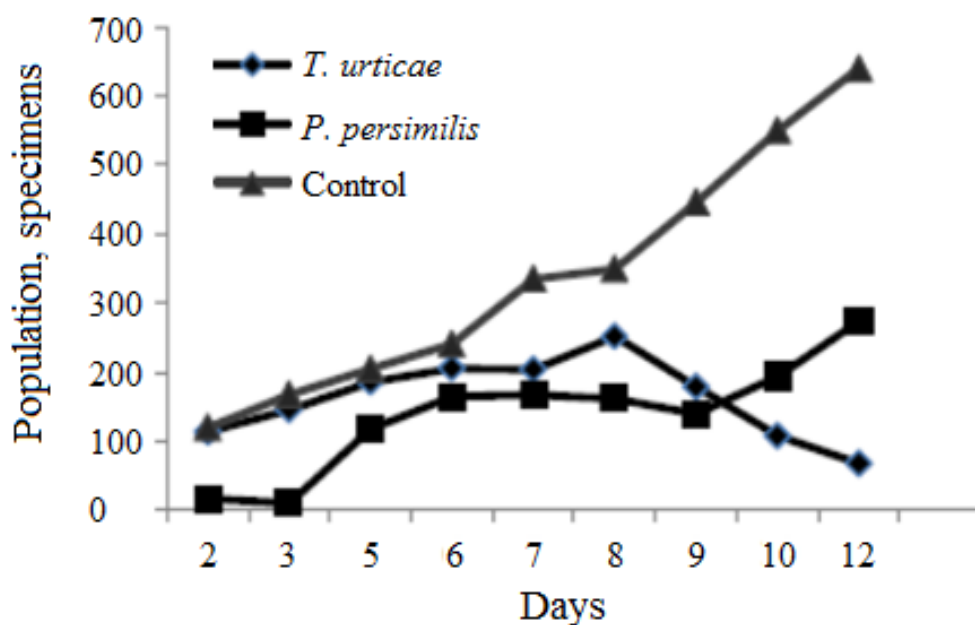


Figure 5. Biological activity of *Ph. persimilis* against *T. urticae*

Thus, the yield of *Ph. persimilis* on day 12 amounted to 180 nymphs and 94 adults. In the control variant, the number of phytophages reached more than 1,000 specimens. According to the data obtained, the 20 specimens released (total predator population) at the beginning of the experiment produced a total of 180 specimens (all age stages) by the end of the experiment. They actively suppressed the growth of *T. urticae* in the experiment in a short period. The predator also laid 152 eggs. The predator actively preys on, restrains, and actively suppresses the growth of *T. urticae*. The maximum number of eggs laid by *Ph. persimilis* females was observed on the 10th day after its release and then went down as the amount of feed began to decrease.

In addition, the biological effectiveness of *Ph. persimilis* against *T. urticae* on cucumber plants in protected soil was studied. As a result of regular phytosanitary monitoring in the greenhouse, the first foci of *T. urticae* were detected on plants in early January. At that time, the pest population was low, and the infestation of plants did not exceed 1 point on average on a 5-point scale. A significant increase in the population of the phytophage was observed one week later, with the infestation of plant leaves reaching an average of 3 points. *Ph. persimilis* was released on cucumber plants at a predator-prey ratio of 1:10 (Table 1).

Table 1. The biological effectiveness of *Ph. persimilis*

Variant	<i>Ph. persimilis</i> Count/1 Running Meter			Biological Effectiveness (%)
	Pre-Treatment	5 Days Later	10 Days Later	
Control	22	43	68	-
<i>Ph. persimilis</i>	20	15	6	70

On day 5 after release, the predatory mite dispersed, actively fed, and reproduced in the presence of a sufficient amount of prey. During subsequent observations, predatory mite specimens were observed practically on all plant leaves infested by the pest. The biological effectiveness of the predator on day 10 reached 70%. The cucumber plants were sufficiently protected from *T. urticae* with a single release of the acariphage, which subsequently survived on the plants and effectively restrained the pest population. The high biological effectiveness of *Ph. persimilis* observed in this study supports its viability as an alternative to chemical pesticides, aligning with existing research that highlights the predator's efficiency in greenhouse settings. For instance, study by Yari et al. (2023) also reported significant reductions in *T. urticae* populations on various crops with a single release of *Ph. persimilis*. This level of effectiveness demonstrates the predator's ability to reduce *T. urticae* populations to economically insignificant levels, minimizing plant damage and promoting healthier crop yields without the need for repeated chemical treatments.

The implications of these findings suggest that while *Ph. persimilis* is highly effective in reducing *T. urticae* populations, its success is partly dependent on consistent environmental conditions, which may not always be feasible in all agricultural settings.

5. Conclusions

The study confirms that *Ph. persimilis* is a highly effective biological control agent against *T. urticae*, with a marked preference for the eggs and postembryonic stages of the pest. *Ph. persimilis* exhibited stable feeding behavior across life stages, with adults tending to target larger *T. urticae* specimens. Adult *Ph. persimilis* tended to feed on larger *T. urticae* specimens. *T. urticae* eggs were the preferred prey stage for the predatory mite. The adults and nymphs of the predator also showed a preference for prey specimens in the postembryonic stages over the feeding period.

In the study of the predator's biological activity against the phytophage, the predator was observed to be active in the presence of abundant food and able to suppress its population by up to 97%. Due to the accumulation of the acariphage during its active predatory activity, there is no need to use acaricides.

Pre-release surveys showed that the pest population in the identified foci was within 2 points, i.e., the infestation of leaves by mites in the foci amounted to about 30%. *Ph. persimilis* was released into the identified foci in a predator-prey ratio of 1:10, i.e., one predatory mite/15 pest specimens. A second release was performed 10 days later. At that time, the number of *T. urticae* averaged 170 specimens/plant on 10 observed plants. In subsequent surveys following *Ph. persimilis* release, the number of mites on the leaves decreased to an average of 50 specimens/plant.

The calculations indicated that the biological effectiveness of *Ph. persimilis* application on cucumbers against *T. urticae* reached 70%. Additional releases of *Ph. persimilis* suppressed the development of the mite to an economically insignificant size. Due to the activity of *Ph. persimilis*, no further increase in the number of mites was observed until the end of the crop rotation.

These findings suggest that *Ph. persimilis* can be a viable alternative to chemical treatments in greenhouse environments. Future research should focus on evaluating the predator's adaptability to different environmental conditions, exploring optimal release strategies for various crop types, and assessing long-term control efficacy in diverse greenhouse and field conditions.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Conflicts of Interest

The authors declare no conflict of interest.

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