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ARTICLE

Effect of biorationals and Predatory Mites incontrolling the Two-Spotted Spider Mite (Tetranychus urticae L) on Eggplant field in the Greenhouse

Altantsesteg Zul [1], Siiriimaa Amarsaikhan [2], Oyungerel Janlav [3] and Undarmaa Davaasambuu [*1]

[1]Laboratory of applied biological control of plant pests, School of Agroecology, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia
[2]Laboratory of microbiology, Plant Protection Institute, Ulaanbaatar Mongolia,
[3]Department of Crop science, Forestry and Landscape Architecture, School of Agroecology, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia.

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Abstract: The Two-Spotted Spider Mite (TSSM) is a prevalent mite pest found worldwide and is extensively distributed in Mongolian greenhouses. In this study, we conducted a field experiment using predatory mites and botanical pesticides on eggplants infested by TSSM. We observed promising results in terms of reducing pest populations and achieving overall improved outcomes. The experiment took place in a 120 sq.m. greenhouse located at the "Agropark" training-research center of the Mongolian University of Life Sciences.

The aim of this experiment was to test the effectiveness of the following treatments in controlling Two-Spotted Spider Mite (TSSM): Treatment A (predatory phytoseiid mites at a ratio of 1:5), Treatment B (predatory phytoseiid mites + Neem), Treatment C (Neem alone), Treatment D (Neem + BEB), and Treatment E (untreated control). The experiment utilized a total of 20 plots, each with a size of 6 square meters, and 24 plants were grown in each plot, resulting in a total of 480 plants. Each plot was replicated four times.

To assess TSSM control, mortality rates of TSSM were calculated for each treatment as follows:

- Treatment A (Predatory phytoseiid mites): 44.4% to 94.7%
- Treatment B (Predatory phytoseiid mites - 1:5 + Neem - 30ml/4.5L of water): 76.3% to 96.3%
- Treatment C (Neem - 30ml/4.5L of water): 61.8% to 90.8%
- Treatment D (Neem - 30ml/4.5L of water + BEB - 20ml/10L of water): 68.6% to 88.2%.

The combination of all these treatments have shown apromising results in controlling TSSM in the greenhouse, significantly reducing the TSSM population, according to our research findings. However, it is important to release the predatory mites after a 5-day interval following the application of botanicals (Neem).

We recommend a release of predatory mites (Phytoseiilus persimilis and Amblyseius swirskii) either alone or in combination with botanicals for controlling TSSM in the greenhouse, because it is effective and a safe plant protection measurement to use in the greenhouse.

Keywords: *biological control; predatory mites; Phytoseiulus persimilis, Amblysieus swirskii, botanical pesticide and plant growth regulator*

***Corresponding author, email:undarmaa@muls.edu.mn** **https://orcid.org/0000-0001-6592-2805**

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INTRODUCTION

The two-spotted spider mite (TSSM),*Tetranychus urticae*, is a harmful arthropod that is widespread in many countries around the world. TSSM is a pest that infests various plants and causes damage in non-agricultural areas, such as forested regions and natural habitats, as well as cultivated fields [3]. According to the latest data, TSSM (*T.urticae*) infests approximately 4,000 different plants worldwide (Migeon and Dorkeld, 2021) [5]. It feeds on a wide range of plant species, including but not limited to trees, shrubs, ornamental plants, vegetables, and various crops. It can adversely affect crops such as beans, tomatoes, cucumbers, melons, strawberries, raspberries, currants, gooseberries, apples, pears, grapes, and many other fruit-bearing and ornamental plants [12; 13].

The strategy to control TSSM involves relying on synthetic acaricides and insecticides, which are widely used worldwide (Van Leeuwen et al., 2015) [9]. In the greenhouse vegetable field, biological methods of pest control, particularly in dealing with TSSM, have gained significant traction in practice across many countries [10; 11]. However, in Mongolia, reliance on chemical synthetic pesticides (acaricides) has remained prevalent, posing risks to human health and the environment. The use of chemical pesticides in crop fields can have detrimental effects, both in terms of health and the environment.

Biological methods, when applied appropriately, offer a safer and more environmentally friendly alternative. In conditions where spider mite infestations are prevalent, the use of biological methods has been found to be the most effective and sustainable approach. Therefore, this study explores the use of biological methods and the potential benefits they offer in terms of both effectiveness and environmental impact.

Research objective

The objective of this study is to test a treatment for mitigating TSSM infestations, with a particular focus on meeting organic cultivation requirements. Within the scope of this study, our goal was to investigate and assess the effectiveness of biological control agents by applying predatory mites and botanical pesticides on eggplants inhabited by the TSSM population in the greenhouse.

MATERIAL AND METHODS

Location of experimental field

A field study was conducted in the eggplant greenhouse with a size of 120 m^2 at the "Agropark" training-research center of the Mongolian University of Life Sciences during the plant growth seasons in 2017-2019. This center is situated in the Zaisan Valley in the Khan-Uul District of Ulaanbaatar, the capital of Mongolia.

The territory of Mongolia lies within the middle latitudes of Central Asia, in the Northern Hemisphere. According to the world's soil and climate classification, Mongolia exhibits a cool climate in terms of heat resources and falls under the category of a dry and arid region with regards to humidity. It is situated in a region characterized by a continental climate, covering an area of 1,569.9 thousand km^2, with elevations reaching 1,380 meters above sea level. The study was conducted in the Zaisan Valley, which is a part of Bogdkhan Mountain, a subrange of the Khentii Mountains [4]. In terms of its geographical location, the Zaisan Valley is located within the humid and temperate sub-region of Mongolia's central agricultural region [4].

Annual rainfall ranges from 250 to 270 mm. The cumulative heat units during the plant growth period range from 1500 to 2200^0 C degree-days, and the plant growth period lasts for 90 to 100 days. The soil exhibits a light mechanical composition with a high water absorption capacity,

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predominantly consisting of dark brown, brown, and light brown soils. The cultivated area is situated at an elevation of 800 to 1100 meters above sea level [4,12].

Research materials

In these experiments, we utilized predatory mites, *Phytoseiulus persimilis* and *Amblyseius swirskii*, which were purchased from the Koppert company in the Netherlands and have been reared in the laboratory for research purposes since 2015. Additionally, we imported the botanical pesticide NEEM from the USA and obtained a natural plant growth regulator, BEB, from China, which reduces plant stress and promotes growth.

Field Experimental layout and design

Between 2017 and 2019, following the onset of TSSM infestation in the greenhouse eggplant field, research was conducted to evaluate various treatments. We compared four different treatments against TSSM, including an untreated control, each with four replications.

This experiment for controlling TSSM was tested in 20 plots, each with a size of 6 sq.m., and in each plot, 24 plants were grown. In total, 480 plants were used for the experiment, with each plot being replicated four times. The experimental layouts were arranged in a Completely Randomized design (CR), with each plot positioned 60 cm apart and separated by a mesh curtain. Subsequently, we randomly allocated the four treatments to the plots in each row, so that there is one replicate per row. By doing so, no treatment as to be penalized or favored.

In this experiment as a variant, the following treatments were applied: 'A' variant (Predatory phytoseiid mites with a 1:5 ratio (used 2 species of predatory mites such as Phytoseiilus persimilis and Amblyseius swirskii), 'B' variant (Predatory phytoseiid mites - 1:5 ratio + Neem - 30 ml/4.5 liters of water), 'C' variant (Neem - 30 ml/4.5 liters of water), and 'D' variant (Neem - 30 ml/4.5 liters of water + BEB - 20 ml/10 liters of water), E (untreated control).

For 'B' variant (Predatory phytoseiid mites - 1:5 ratio + Neem - 30 ml/4.5 liters of water), Neem treatment was applied first at a rate of 30 ml/4.5 liters of water, followed by the application of predatory phytoseiid mites at a 1:5 ratio five days after Neem spraying.

Data collection and analysis

The results of these biological control treatments were determined by assessing the percentage of TSSM mortality. The assessment involved counting the number of surviving mites before the treatment and at intervals of 24 hours, 5 days, 10 days, 15 days, and 20 days after the treatment application on marked plants, using a digital magnifier with 200x-1000x magnification.

Data analysis was conducted using Excel Data Analysis and ANOVA (DATA tab). The mortality rate was calculated using Belyaev's method, which involved the following formulas:

$$\text{Mortality rate} = \frac{A-B}{A} \text{ x } 100;$$

A - number of individuals before treatment application
B - number of individuals after treatment application

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Figure 1. A-Two spotted spider mite (TSSM), predatory phytoseid mites (in red colour; B-Phytoseiilus persimilis, in pale colour; D-Amblyseius swirskii), C-damaged leaf with pest and predatory mite population. Photos taken with* DIMIS pro digital microscope 400x-1000x *by D.Undarmaa and Z.Altantsetseg

RESULTS AND DISCUSSION

In the experimental eggplant field or controlling TSSM, the following treatments were applied, and results represented by the mortality rate of each treatment by year, were as follows:

In 2017, the mortality rate were observed as 'A' (Predatory phytoseiid mites): 44.4% ± 3.39 - 83.8% ± 0.83, 'B' (Predatory phytoseiid mites - 1:5 + Neem - 30ml/4.5L of water): 83.7% ± 1.69 - 93.5% ± 0.86, 'C' (Neem - 30ml/4.5L of water): 79.7% ± 1.71 - 83.5% ± 1.36, 'D' (Neem - 30ml/4.5L of water + BEB - 20ml/10L of water): 71.8% ± 2.58 - 85.7% ± 1.25.

In 2018, the results in terms of mortality rate were as follows: 'A' (Predatory phytoseiid mites): 63.3% ± 2.12 - 92.2% ± 0.67, 'B' (Predatory phytoseiid mites - 1:5 + Neem - 30ml/4.5L of water): 77.0% ± 1.31 - 93.9% ± 1.21, 'C' (Neem - 30ml/4.5L of water): 70.0% ± 1.68 - 95.2% ± 0.43, 'D' (Neem - 30ml/4.5L of water + BEB - 20ml/10L of water): 71.6% ± 2.29 - 90.8% ± 0.67.

In 2019, the results in terms of mortality rate were as follows: 'A' (Predatory phytoseiid mites): 57.5% ± 3.45 - 94.7% ± 0.79, 'B' (Predatory phytoseiid mites - 1:5 + Neem - 30ml/4.5L of water): 76.3% ± 3.49 - 96.3% ± 0.86, 'C' (Neem - 30ml/4.5L of water): 61.8% ± 4.27 - 90.8% ± 1.36, 'D' (Neem - 30ml/4.5L of water + BEB - 20ml/10L of water): 76.2% ± 2.91 - 88.2% ±

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1.74.

These treatments showed effective results for controlling TSSM in respective of study years. The highest mortality was recorded after 5 days of application of 'B' variant (Predatory phytoseiid mites - 1:5 + Neem - 30ml/4.5L of water) and impact of this combined treatment was sustainable after 20 days.

Table 1. Mortality rate of Two Spotted Spider Mites after treatments

Variants	Mortality rate of two spotted spider mites %,(mean±standard error), 2017				
	24 hours after	5 days after	10 days after	15 days after	20 days after
A	44.4±3.39	82.3±2.13	82.8±2.16	83.8±0.83	81.8±1.42
B	83.7±1.69	92.1±1.22	92.6±1.17	93.5±0.86	93.1±0.73
C	79.7±1.71	80.6±1.09	83.5±1.36	68.1±2.41n	80.6±1.18
D	71.8±2.58	81.4±0.72	85.7±1.25	83.0±1.20	80.6±1.90

Variants	Mortality rate of two spotted spider mites %, (mean±standard error), 2018				
	24 hours after	5 days after	10 days after	15 days after	20 days after
A	50.6±7.11	80.4±2.93	87.1±2.56	87.5±2.13	89.0±1.35
B	71.4±3.04	92.3±1.41	87.8±1.91	89.5±1.62	93.0±0.96
C	76.4±3.16	84.4±1.72	80.7±2.55	85.0±2.03	87.4±1.05
D	68.6±2.58	84.1±0.86	74.6±0.86	80.2±1.66	84.5±1.69

Variants	Mortality rate of two spotted spider mites%, (mean ±standard error), 2019				
	24 hours after	5 days after	10 days after	15 days after	20 days after
A	57.5±3.45	81.6±2.75	87.9±2.31	93.2±0.61	94.7±0.79
B	76.3±3.49	94.9±0.92	95.1±0.99	95.4±0.81	96.3±0.86
C	61.8±4.27	87.0±1.61	90.8±1.36	82.1±2.41	89.1±1.18
D	76.2±2.91	86.2±1.12	88.2±1.74	87.7±1.33	86.7±1.89

Based on the ANOVA results, there were no significant differences in the average mortality of TSSM over three years, either between observation times (df=4, F=52.11, P<.001) or among treatments (df=3, F=0.52, <.671), which are shown below in Figure 2.

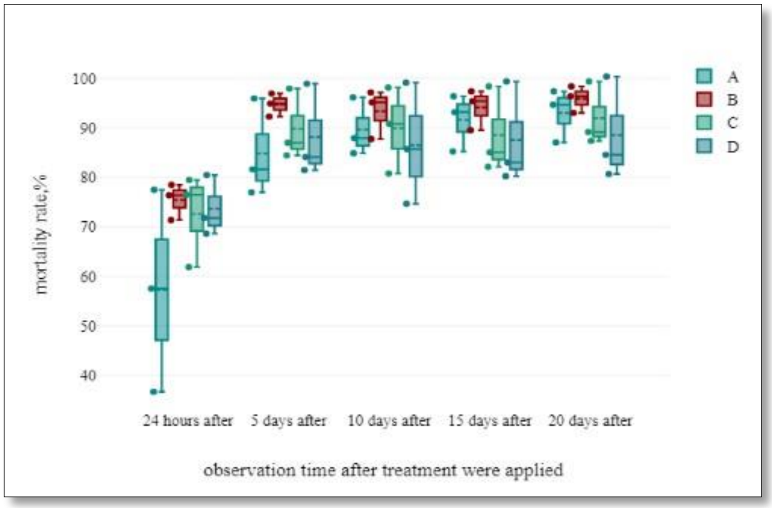

Figure 2. Effect of treatments on TSSM control in the eggplant field over a 3-year average

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In the treatment plots, the growing season over three years also involved recording the density of TSSM. The results are illustrated in Figure 3. As depicted in the chart, the mean ***plots (E).*** the mean number of individuals increased constantly (R^2=*0.8548*) in the control (E-untreated) plots. In contrast, the number of individuals in the treatment plots decreased, indicating that the treatments in this experiment effectively inhibited the population growth of TSSM during th rowing season, ultimately protecting the yield of eggplant.

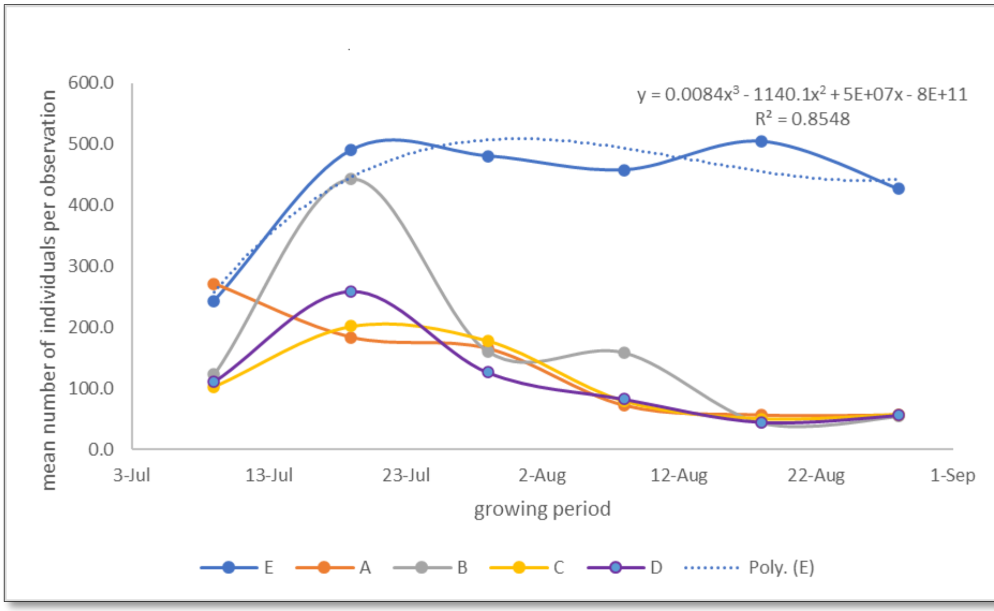

Figure 3. Population dynamics of TSSM in treatment plots compared to untreated control

In Mongolia, eggplants are exclusively cultivated in greenhouse conditions, and TSSM (Tetranychus urticae), commonly found in greenhouses, also infests many different plants, including eggplants [3,6,11]. Initially, scientist Ch. Chogsomjav discovered TSSM (Tetranychus urticae) in 1962 and 1963 in Juun Kharaa and Amgalan within a protected crop field. It was documented as a newly introduced harmful arthropod (invasive species) in Mongolia [11]. Researchers, including Selenge et al., have observed that TSSM predominantly spreads in greenhouse cucumber cultivation areas, leading to a reduction in yield per unit area by 25-52.6% [3,6]. In findings in Mongolia, two types of spider mites are identified, and their presence have been recorded in greenhouses in such cities as Ulaanbaatar, Selenge, Erdenet, and other urban areas, where the potential for crop damage exists [10].

Many research studies have been conducted to investigate various aspects of predatory mites' life, especially when dealing with TSSM, which have yielded promising results[1;3;9].

For example, studies that have utilized predatory mites in controlling TSSM have shown promising results. Barber and others have indicated that Phytoseiulus persimilis and Neoseiulus californicus are

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more effective in controlling TSSM than other predatory mites, with the former being faster in consumption (Barber et al)[1].

The predatory phytoseid mites (Phytoseiulus persimilis and Amblyseius swirskii) used in our study have shown effective predation on TSSM, with an average mortality rate ranging from 82.3% ± 2.13 to 83.8% ± 0.83% during the experimental period. However, the predation performances of each predatory mite species on TSSM have not been assessed separately, and the results are based on the combined action of both predator mite species.

Based on the studies conducted by Elena M. Rhodes, among the combination treatments, the P. persimilis/N.californicus treatment significantly reduced TSSM numbers compared to the control. However, it was not as effective as N.californicus alone during the 2003–2004 field season[7].

In this study, we used the botanical pesticide NEEM in combination with predatory mites. Initially, NEEM was applied separately, and then, starting from 5 days later, it was used in combination with the predatory mites. This approach aimed to mitigate the negative effects of NEEM on these predators and determine the overall effectiveness of this treatment combination.

Among the treatment variations we tested, the combination treatment 'B' (Predatory phytoseiid mites - 1:5 + Neem - 30ml/4.5L of water) variant produced the highest results, effectively reducing the populations of two-spotted spider mites, with a mortality rate ranging from 83.7% ± 1.69 to 93.5% ± 0.86. This demonstrates that the botanical pesticide NEEM can be used in conjunction with predatory mites, with application intervals over a certain number of days, and achieve a significant reduction in the population of two-spotted spider mites, making it an effective strategy.

According to a study conducted by researchers Saloni et al., various biorational treatments were tested for the control of TSSM. These treatments included Propargite, Spiromesifen, the botanical pesticide Ozoneem, entomopathogenic fungi such as Beauvaria bassiana and Lecanicillium lecanii, as well as the predatory mite Neoseiulus longispinosus. The results indicated a significant reduction in mite populations, with Propargite and Spiromesifen achieving more than 80% reduction. In contrast, the homemade neem fruit aqueous extract (HMO) demonstrated the least effectiveness, with a mortality rate of only 35–41%. Among the botanical treatments, Ozoneem exhibited higher mite mortality rates (47–78%) compared to the homemade neem fruit extract (41–62%)[5].

Among the entomopathogenic fungi tested, Beauvaria bassiana demonstrated higher effectiveness, resulting in a mortality rate ranging from 43% to 58%, compared to Lecanicillium lecanii. The predatory mite, Neoseiulus longispinosus, successfully reduced the pest mite population by 40% to 60% at various ratios. All of the treatments proved to be effective in reducing the pest mite population, suggesting that these biorational methods can be integrated into comprehensive mite management programs in protected environments, as recommended by the researchers[5].

In these experiments, we tested different treatments as follows: 'A' (Phytoseiid predatory mites - Phytoseiulus persimilis and Amblyseius swirskii), 'B' (Phytoseiid predatory mites + Neem - 30ml/4.5L water), 'C' (Neem - 30ml/4.5L water), and 'D' (Neem - 30ml/4.5L water + BEB growth regulator - 20ml/10L water), comparing them to 'E' (untreated control) plots. For the 'B' variant, the predatory mites were released after a 5-day interval following the application of the botanical (Neem).

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CONCLUSIONS

- For controlling the TSSM population on the eggplant field, the treatments tested within this experiment have effectively reduced the TSSM population. The mortality rates of the following treatments were calculated: 'A' (Predatory phytoseiid mites): 44.4% to 94.7%, 'B' (Predatory phytoseiid mites - 1:5 + Neem - 30ml/4.5L of water): 76.3% to 96.3%, 'C' (Neem - 30ml/4.5L of water): 61.8% to 90.8%, 'D' (Neem - 30ml/4.5L of water + BEB - 20ml/10L of water): 68.6% to 88.2%.
- As seen in the above-mentioned results, these treatments are recommendable for controlling TSSM in greenhouse vegetables, and the use of predatory mites either alone or in combination with botanicals is important for controlling TSSM in the greenhouses. It is crucial to release the predatory mites after a 5-day interval following the application of botanicals (Neem).

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