



**ORIGINAL ARTICLE**

## **Biological Control of Two-Spotted Spider Mite Using *Phytoseiulus persimilis* (Athias-Henriot) and *Neoseiulus californicus* (McGregor)**


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DOI: <http://doi.org/10.4038/sljae.v4i2.98>

### **Abstract**

Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch is a major pest wherever strawberry plants are grown in Sri Lanka. This pest was not controlled by the Department of Agriculture recommended synthetic insecticide Abamectin in many instances. Use of biological agents for the control of TSSM is globally a common practice and thus *Phytoseiulus persimilis* (Athias-Henriot) and *Neoseiulus californicus* (McGregor) were imported from Koppert biological systems, Netherlands. An experiment was conducted to investigate the possibility of using and *N. californicus* (McGregor) and *P. persimilis* (Athias-Henriot) in combination and *N. californicus* (McGregor) alone to control TSSM under laboratory conditions. The results indicated that the use of *N. californicus* alone was not effective in controlling the TSSM, though the TSSM population was maintained at low levels. However, the combination of biological agents was very effective by controlling the TSSM population with an 85% reduction. There was no population growth of biological agents after introduction and their population was reduced over the time. Further, there was a significant reduction in TSSM population after fifth week when the biocontrol agents were applied in combination. It was concluded that the application of *P. persimilis* and *N. californicus* was effective in controlling *T. urticae* rather than applying *N. californicus* alone. The biological control can be proposed as an alternative to insecticide, Abamectin.

**Keywords** - Abamectin, Biological control, *Tetranychus urticae*, Strawberry

## 1. Introduction

Strawberry (*Fragaria* spp.) that belongs to genus *Fragaria* and family *Rosaceae* is native to Europe, Asia, and some other parts of North America where indigenous people use them in many dishes. (Preet et al. 2021). In Sri Lanka, the wild strawberries (also known as Indian or mock strawberries) were named under the genus *Duchesnea* by, Focke in 1888 in the Revised Handbook to the Flora of Ceylon (Wadhwa et al. 2000). The cultivated plant produces a succulent, red, conical fruit from tiny white flowers, and sends out runners to propagate.

The growth of strawberry plants is mainly affected by a complex and dynamic set of interacting environmental factors, such as temperature, day length, light intensity (Sønsteby et al. 2016), and soil temperature (Jun et al. 2008). Strawberry growth and fruit yield, therefore, could be predicted by environmental data (Døving and Måge 2001). In Sri Lanka, strawberry is cultivated in greenhouses. There are a number of insect and mite pests that can have detrimental effects on strawberry production. Among them leaf pests can be divided into two main groups, sucking pests and chewing pests. Sucking pests include twospotted spider mites (TSSM), cyclamen mites, thrips, aphids, whiteflies, leafhoppers and spittlebugs. Injuries caused by these pests lead to a reduction of the plants' ability to photosynthesize hence, reduces the quality and quantity of fruits produced. In perennial production, these injuries can affect the yield the following season too (Childers 2003).

The TSSM, *T. urticae* is a major pest reported in places where strawberry plants are grown. Other major pests are reported in certain regions or under specific production systems (Flint 2012). TSSM are oval shaped and tiny. Its life cycle which takes 19 days to complete progresses through five stages: egg, six-legged larvae, protonymph, deutonymph and adult (Tasnin & Khalequzzaman 2016). The adults are about 0.5 mm in length, which is about the size of a period in 12-point font. They are light greenish-yellow in colour with two large dark spots on the dorsum of abdomen. However, brown, red, orange, and darker green forms also occur depending on the host plants. The eggs are spherical and clear to tan in colour. The eggs and all stages of mite are usually found on the underside of the leaves (Mitchell 2003). Females are larger and rounder than males. Optimum condition necessary for development is known to be high temperatures (up to 38°C) and low humidity (RH 60%) (Krantz 1978; White and Liburd 2005).

Pesticide resistance in general is an issue when controlling many agricultural pests with synthetic chemicals. The TSSM is a notorious example as it has been proven capable of developing resistance to more than 92 active ingredients in pesticides (Neuman 2019). In addition, miticide/acaricide applications might also affect beneficial arthropods population among TSSM's natural enemies, and therefore, "foster" spider mite outbreaks. The shorter life cycle and higher reproductive potential of TSSM also help them to develop resistance against pesticides.

Biological control of pests of strawberry is common worldwide and there are many control agents such as predators, pathogens, parasites and parasitoids, and even other plants. The only requirement is that they work to eliminate a pest or augment a predator population. Among them, a wide range of natural enemies, such as *Phytoseiulus*-System and *Californicus*-System, for controlling spider mite has been recorded (Vidrih et al 2021). Koppert Biological systems, Netherlands offer two species of predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* which can be used for effective control of TSSM. Releasing these predatory mite species can give the cultivators a season-long control of TSSM. Releasing predatory mites may also encourage and promote the population build-up of other beneficial insects that feed on TSSM in home gardens.

Time of release of predatory mites is very important for the successful control of TSSM. Before introducing predatory mites, one must inspect the strawberry plants for TSSM or their eggs and there must be TSSM for the predatory mites to feed once they are released. If food sources are not available, the predatory mites will either die or migrate to other plants. In this study, the efficacy of two predatory mites, *P. persimilis* and *N. californicus* received from Koppert Biological Systems, Netherlands, were tested for the control of TSSM.

## 2. Materials and Methods

Strawberry plants of variety Royal Sovereign were raised in a greenhouse following the recommended practices of the department of

agriculture at Jagro Farms LTD, Radella for the experiments. One -month old plants infested with TSSM were selected for the experiment as per the protocol for experimentation given by Koppert Biological Systems, Netherlands. Four plants each were kept separately in three insect cages (03 replicates) per treatment and another three insect cages (Fig. 1a) with four infested plants.



Figure 1(a): Insect cages used for the experiment



Figure (1b): Strawberry plants used in the experiment

(Fig. 1b) each were prepared as control treatment. The TSSM counts were taken at the beginning of the experiment. Predators imported from Koppert Biological System, Netherlands by the Plant Quarantine Service of Department of Agriculture, Sri Lanka was used in the experiment with two treatments with

three insect cages each, one with *P. persimilis* [Spidex (Fig.2)] alone and another with *P. persimilis* (Spidex) and *N.*



Figure 2: *Phytoseiulus persimilis*



Figure 3: *Neoseiulus californicus*

*californicus* [Spical (Fig.3)] and a control with three insect cages. Experiment was conducted at the laboratory of the Plant Quarantine Unit, Department of Agriculture (DOA), Gannoruwa. The environment conditions in the laboratory were maintained at 22°C ambient temperature and relative humidity of 80% throughout the period. The biological agents were released with the recommended dose by the Koppert Biological Systems as four predators each per plant (i.e, 16 mites per cage). Strawberry plants

were maintained as per the recommended practices of the DOA except the pest control. The first releases of Spidex and Spical was done after taking the initial count of TSSM and the second release was done after two weeks. The number of TSSM, Spical and Spidex was first taken in the day after release and counts were taken in weekly interval. The number of spider mites were counted using randomly selected 10 leaves per treatment. This was repeated weekly, until five weeks after the last release (total of seven counts). The data obtained were statistically analyzed using the ANOVA procedure in SAS programme considering a split plot design with three replicates.

### 3. Results and Discussion

Experiment was conducted to find out the effect of predatory mites for the control of TSSM in strawberries singly and in combination. The results obtained are shown in the Table 1 shows that there was a significant decrease in TSSM population compared to the control plots in both treatments in main plots. Between the two treatments there was no significant difference in TSSM population. Statistical analysis shows that there was no significant control of TSSM until the 5<sup>th</sup> week after the first introduction of predator combination (Spidex and Spical) treatment whereas no significant difference among weekly counts in single predator (Spical) treatment. On the other hand, there was a significant decrease in TSSM in the control treatment from the 4<sup>th</sup> week onwards. In the control treatment, highest average insect count was observed in the 3<sup>rd</sup> week after introduction (132 TSSM/10 leaves) and a

gradual decrease thereafter. Therefore, it can be concluded that the decrease in TSSM counts in the control treatment, could be attributed to lack of food because of the very high population of TSSM in the 2<sup>nd</sup> Week and 3<sup>rd</sup> week due to continuous heavy sucking of leaf sap.

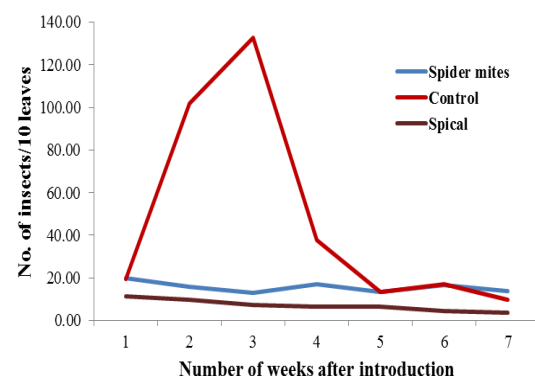
Table 2 shows the predator mites population counts against the TSSM counts, which shows a general decreasing tendency of predator mites similar to the TSSM counts. This could be attributed either to the uniqueness of the population or the controlled conditions provided in the experiment which was not conducive for their reproduction.

*Phytoseiulus persimilis* and *N. californicus* prefer TSSM and many other mite species and their reproduction rates reported to be higher when spider mites are the primary food source (Fraulo et al. 2008). In the present experiment, TSSM counts on both treatments were comparatively low. The population of the introduced predatory mites was decreasing over time due to their low reproductive rates.

Fraulo and Liburd (2007) have reported that the release of *N. californicus* could control TSSM in strawberry throughout the growing season if released when TSSM populations are lower at the early periods of the season. They also indicated that when released at the appropriate ratio of between 1:5 and 1:10 predator: prey and when TSSM population are “low”, or less than 70 TSSM per trifoliolate, regardless of calendar date, *N. californicus* is able to maintain populations of TSSM below damaging levels throughout a growing season (using the

recommended dosage of 1–2 *N. californicus* / m<sup>2</sup>). Further, Hassel et al. (1976) indicated that sharp decline in the late releases to levels significantly below the earlier releases at lower prey densities, demonstrated that *N. californicus* is an effective and voracious predator at high prey density if the 1:10 predator: prey ratio stays unmodified. Since yield data was not collected in the current experiment, it was not possible to conclude whether TSSM population would maintain at below damaging levels throughout the growing season.

The population dynamics of both TSSM and Spical, when used alone, is given in Fig. 4, which also clearly shows a decreasing population of Spical and sustaining population of TSSM. Fig. 4 also shows the TSSM count in control treatment fluctuated and reached the population level of the treatment, which could be attributed to death of TSSM due to lack of food as the leaves dried up after five weeks.



**Figure 4:** Population dynamics of TSSM and *N. californicus* (Spical) in the treatment where Spical used alone over control

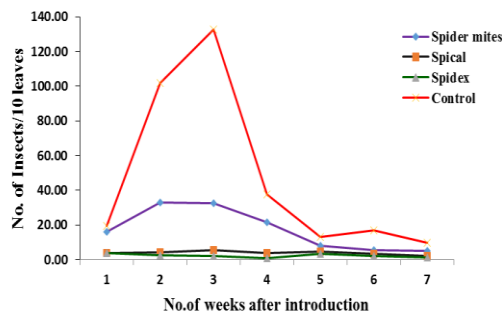
Therefore, results show that Spical alone cannot control the TSSM even at low populations under

tropical conditions at Gannoruwa. García and Molina (2015) indicated opposite results and surmised using combinations of bio-control agents; *P. persimilis*, *N. californicum*, resulted in the reduction of TSSM numbers; but no treatment was better than the release of *P. persimilis* alone ( $p < 0.05$ ). However, in the present study, *P. persimilis* was not applied alone. In this treatment, the TSSM population was increased during 2<sup>nd</sup> and 3<sup>rd</sup> week after introduction, probably be due to high egg laying of the TSSM population, which could be considered incidental.

Predatory mite *P. persimilis* (Spidex) is identified as a specialist predator (Walzer and Schausberger, 1999) and is a highly voracious and effective predator on a number of horticultural and ornamental crops and serves as a key tool for managing spider mites in greenhouses for several decades (Van Lenteren, 2012). The use of *P. persimilis* in open-field crops are not nearly as common as in greenhouse production systems due to environmental and agronomic characteristics as well as a lack of research on how to effectively implement *P. persimilis* in these systems. McMurtry and Croft (1997) surmised that *P. persimilis* has been used very successfully in field-grown strawberries in California and Florida for a number of years (despite the reported limitations of *P. persimilis* on tomatoes due to defensive glandular trichomes) where their research in experimental plots and commercial fields have demonstrated excellent, season-long TSSM control when *P. persimilis* is applied correctly.

Stenseth (1979) has shown that *P. persimilis* is an effective predator of TSSM when temperature is as low as 15° C and relative humidity is 60-90%, with improved performance at a higher temperature and humidity. *P. persimilis*, like many other predatory mites, is sensitive to humidity, which can be a limitation to their success as biological control agents in field-grown crops. As humidity decreases below 60%, successful egg hatch declines steadily. In the present study, the laboratory conditions were maintained at 22°C temperature and 80% of relative humidity respectively throughout the season and therefore, *P. persimilis* made effective control of TSSM though their population was decreased over time.

Walzer and Schausberger (1999) examined intra- and interspecific predation of adult females and immature stages of the generalist *N. californicus* and the specialist *P. persimilis*. Adult females and immature of both predators exhibited higher predation rates on larvae than on eggs and protonymphs. *N. californicus* fed more inter- than intra-specifically. Predation on *P. persimilis* by *N. californicus* was more severe than *vice versa*. *P. persimilis* had higher predation rates and was more prone to cannibalism than *N. californicus*. In our experiment even though we did not monitor cannibalism, the lack of population increase of Spical of Spidex in treatments could be attributed to the cannibalism between predator mites.



**Figure 5:** Population dynamics of TSSM, *P. persimilis* and *N. californicus*.

A significant reduction of TSSM population was achieved after 5<sup>th</sup> week of introduction when Spidex and Spical used in combination (Fig.5). Population dynamics of TSSM and introduced predatory mites (Fig. 5) in controlled environment show that there is adequate control of TSSM when both predators are introduced as recommended by the Koppert Biological systems. However, there was no population increase in both predatory species of mites indicating that there is no possibility of surviving these predators in the field for augmentative releases in subsequent seasons, if introduced to field. Therefore, predatory mites need to introduce at the beginning of each season for an effective control of TSSM. This will be a costly affair for the farmers unless they are large scale cultivators.

#### 4. Conclusion

Results indicated that generalist predator mite *N. californicus* was not effective in controlling TSSM, when introduced alone, whereas the combine application of *P. persimilis* and *N. californicus* at the rate of four predators per plant was effective in TSSM control at low

population levels. However, these two predators were not multiplying under 22°C and RH of 80%. Therefore, predatory mites need to introduce at the beginning of each season for an effective control of TSSM.

#### 5. Acknowledgement

Authors wishes to place on record their deepest gratitude to Koppert Biological Systems, The Netherland, for providing the predatory mite free of charge and guiding the authors in setting up laboratory experiments. Further they wish to thank the Management of Jagro Farms Ltd, Radella for providing us strawberry plants for the experimental purpose.

**Conflicts of Interest:** The authors declare that there are no conflicts of interest regarding the publication of this paper.

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**Table 1: Weekly average count of TSSM mites found on 10 leaves/treatment**

Treatments	Main Plot Means	TSSM count/10 leaves						
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
<b>Main Plot</b> Spical and Spidex	129.33 <sup>b*</sup>							
Spical alone	110.00 <sup>b</sup>							
Control	331.66 <sup>a</sup>							
<b>Sub Plot</b> Spical and spidex		16.00 <sup>b*</sup>	33.00 <sup>a</sup>	32.66 <sup>a</sup>	21.66 <sup>a</sup>	8.00 <sup>b</sup>	5.66 <sup>b</sup>	5.00 <sup>b</sup>
Spical alone		20.00 <sup>a</sup>	16.00 <sup>a</sup>	13.00 <sup>a</sup>	17.00 <sup>a</sup>	13.33 <sup>a</sup>	16.66 <sup>a</sup>	14.00 <sup>a</sup>
Control		19.66 <sup>b</sup>	101.66 <sup>a</sup>	132.66 <sup>a</sup>	37.66 <sup>b</sup>	13.33 <sup>b</sup>	17.00 <sup>b</sup>	9.66 <sup>b</sup>
<i>S.E.D. (main plot)</i>		37.11						
<i>S.E.D. (sub plot)</i>		16.96						

\*Means denoted by the same letter are not significantly different.

**Table 2: Changes in average predator count in relation to TSSM counts in 10 leaves/treatment**

Period	Spical alone treatment		Spical with Spidex treatment			
	TSSM	Spical	TSSM	Spical	Spidex	
Week 1	20.00 <sup>a*</sup>		11.33	16.00 <sup>a</sup>	4.00	4.0
Week 2	16.33 <sup>a</sup>		9.67	33.00 <sup>a</sup>	4.33	2.66
Week 3	13.00 <sup>a</sup>		7.33	32.66 <sup>a</sup>	5.66	2.00
Week 4	17.00 <sup>a</sup>		6.67	21.66 <sup>a</sup>	3.66	1.00
Week 5	13.33 <sup>a</sup>		7.89	8.00 <sup>b</sup>	4.66	3.33
Week 6	16.66 <sup>a</sup>		4.67	5.66 <sup>b</sup>	3.33	2.00
Week 7	14.00 <sup>a</sup>		1.00	5.00 <sup>b</sup>	2.33	1.33
<i>SED</i>	<i>11.9</i>			<i>7.9</i>		

\*Means denoted by the same letter are not significantly different.