

Orijinal araştırma (Original article)

**Effects of soft soap and abamectin on the two spotted spider mite
Tetranychus urticae Koch (Acari: Tetranychidae) and predatory
mite *Phytoseiulus persimilis* A-H (Acari: Phytoseiidae)
under laboratory conditions**

Laboratuvar koşullarında arap sabunu ve abamectinin iki noktalı kırmızı örümcek *Tetranychus urticae* Koch (Acari: Tetranychidae) ve predatör *Phytoseiulus persimilis* A-H (Acari: Phytoseiidae)'e etkileri

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Summary

Soft soap at three dosages (3 ml/L, 5 ml/L and 7 ml/L) and abamectin (12.5 mg/100L) were applied to bean plants determine their residual effects on the predatory mite, *Phytoseiulus persimilis* A-H (Acari: Phytoseiidae) later released for the control of the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions. The contact toxicity of soft soap and abamectin were evaluated and classified according to IOBC (International Organization for Biological Control) standards. The soap at 3 ml/L and 5 ml/L did not suppress TSSM populations and could not provide satisfactory control when compared with soap at 7ml/L and abamectin. The efficacy of soap at 5 ml/L with *P. persimilis* gave sufficient control of TSSM when compared with soap at 3 ml/L with *P. persimilis*, and the predator alone.

Both the soap at 7 ml/L and abamectin in combination with the predatory mite gave satisfactory control of TSSM ,but in the abamectin treatment, the predatory mite population was not observed due to harmful effects of abamectin. However, use of soft soap at suitable dosage, with or without predatory mite, gave encouraging results for controlling TSSM but was moderately toxic at 5 ml/L and 7 ml/L *P. persimilis* in contact toxicity testing by causing 50.0 % and 42.3 % mortality, respectively, to adult of *P. persimilis*. In addition, abamectin at 12.5 and 25 mg/L (ppm) dosages was evaluated as harmful (T) to adults of *P. persimilis*. Separately, soft soap caused no phytotoxicity to host plants. However, its phytotoxicity needs to be further investigated under greenhouse and field conditions.

Key words: Soft soap, Abamectin, toxicity, *Tetranychus urticae*, *Phytoseiulus persimilis*

Özet

Laboratuvar koşullarında, fasulye bitkileri üzerinde yetiştirilen iki noktalı kırmızı örümcek *Tetranychus urticae* Koch (Acari: Tetranychidae) üzerine arap sabununun üç dozu (3ml/L, 5ml/L ve 7ml/L), abamectin (12.5mg/100L) ve *Phytoseiulus persimilis* A-H (Acari: Phytoseiidae)'in birlikte uygulamalarının etkileri belirlenmiştir. Arap sabunu ve abamectin'in kırmızı örümcek popülasyonuna kontakt toksisitesi değerlendirilmiş ve IOBC (Uluslararası Biyolojik Kontrol Organizasyonu)'ye göre sınıflandırılmıştır. Arap sabununun 3 ml/L ve 5 ml/L dozları *T. urticae* popülasyonunu kontrol altına almada yeterli olmazken, 7ml/L arap sabunu dozu ve abamectin yeterli kontrol sağlamıştır. Arap sabununun 5ml/L dozu ve *P. persimilis*'in birlikte uygulanması; TSSM popülasyonunu baskına almada 3ml/L arap sabun dozu ile *P. persimilis*'in birlikte ve avcı akarın tek başına uygulanmasına oranla daha başarılı olmuştur.

Avcı akar, arap sabunu (7ml/L dozu) ve abamectinin birlikte uygulamaları *T. urticae* (TSSM) popülasyonunu yeterli düzeyde baskı altına alabilmiştir. Abamectin uygulamaları avcı akar popülasyonu olumsuz etkilemektedir. Buna rağmen arap sabununun uygun dozu (5 ml/L and 7 ml/L), avcı ile birlikte veya tek olarak uygulandığında zararlı akar popülasyonunu baskı altına almada yeterli olmuştur. Söz konusu dozlar avcı akar *P. persimilis*'e orta derecede zararlı olmuştur. Arap sabununun (5 ml/L ve 7 ml/L dozları) kontakt toksisitesi IOBC kategorisine göre orta derecede zararlı (M) olarak değerlendirilmiş ve *P. persimilis* erginlerinde % 50.0 ve % 42.3 ölüm gözlenmiştir. Abamectin'inin 12.5 and 25 mg/L (ppm) dozları *P. persimilis* erginlerinde sırasıyla % 80.0 ve % 93.3 oranında ölüm meydana getirmiş, ve zararlı (T) olarak değerlendirilmiştir. Arap sabununun konukçu bitkiye herhangi bir olumsuz etkisi görülmemesine rağmen bu konunun sera ve tarla denemeleriyle de desteklenmesi gerektiği kanısına varılmıştır.

Anahtar sözcükler: Arap sabunu, Abamectin, toksisite, *Tetranychus urticae*, *Phytoseiulus persimilis*.

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Introduction

Pesticides cause many problems, including pest resistance; increased costs; poisoning of wildlife and beneficial natural enemies and other non-target organisms; and death and injury to humans. For these reasons, public concern has focused on the role of pesticides and encouraged the use of appropriate alternatives such as insecticidal soap, horticultural oil, plant extracts and pheromones within the strategy of IPM (Henn et al. 1991). In addition, Weinzierl (1998) reported that soaps are made from the salts of fatty acids; which have been known for centuries as contact insecticides and that dried residues on plant surfaces have no residual effect on natural enemies.

The two-spotted spider mite (TSSM) *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important and highly polyphagous pest on cultivated areas in Turkey (Ay 2005; Alzoubi & Çobanoğlu 2007). Considerable research efforts have been devoted to finding alternative strategies for the suppression of *T. urticae*. Phytoseiid predators such as *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) are the most important biological control agents of phytophagous mites in integrated pest management programs of outdoor and greenhouse crops (Helle & Overmeer 1985; Van Lenteren & Woets 1988; McMurtry & Croft 1997). Despite the effectiveness of phytoseiid predators for biological control of spider mites on their host plants, the predators alone may not be able to maintain spider mite populations below the economic injury level for an extended period of time (Field & Hoy 1986; Ibrahim & Yee 2000). Thus, biological control of *T. urticae* must be accomplished in the presence of chemical applications. In the presence of chemical applications, biological control of spider mites may be achieved by the selective or less toxic pesticides (Hoy & Ouyang 1986; Zhang & Sanderson 1990; Spollen & Isman 1996)

Some natural pesticides, including soft soap, were tested under laboratory conditions for insecticidal effects on spider mites [*Tetranychus cinnabarinus* (Boisduval) (Acarina: Tetranychidae)] and other small insects. The soft soap was considered most effective for controlling mites. However, it was not highly toxic to the eggs of spider mites and the population increased very rapidly and frequent application was necessary for controlling the mite population (Madanlar et al. 2000). The effects of soft soap on *T. cinnabarinus*, on cucumbers grown under greenhouse conditions were also investigated. Alternative pesticides were studied in greenhouses for both integrated pest management and organic agriculture and promising results were obtained with soft soap (Madanlar et al. 2000). The mortality of the spider mite population was 100% on cucumber plants in plastic greenhouses; beside this, it was not harmful to the predatory mite *P. persimilis* (Madanlar et al. 2002; Bulut & Madanlar, 2004). No significant differences were found between natural (such as soft soap) and conventional pesticides in respect to yield and fruit properties such as total soluble solids, titratable acidity and pH of fruit juice, fruit firmness and fruit colour of cucumber (Koçar et al. 2003). Spider mites caused significant injury but their injury level decreased with application of potassium soap (Soft soap) 1-2 times a week and organic vegetable production could be successful if alternative pesticides and also commercially biological control agents were used (Güncan et al. 2006). Soft soap (4%) was used with *Neoseiulus cucumeris* (Oudemans) (Acarina: Phytoseiidae) for the control of thrips (Oetting and Latimer, 1995). There was no significant difference regarding the effects on productivity of cucumbers of natural and conventional pesticide treatments (Madanlar et al. 2002). The side-effects of soft soap were determined for *Phytoseiulus persimilis* on detached bean leaves. Pre-adult death was 18% and egg fertility was decreased by 33%. It was therefore evaluated as 'harmless' to the predatory mites (Bulut & Madanlar, 2004).

The aim of this study was to investigate the use of soft soap with different dosages alone or combination with releasing predatory mites to control the TSSM, and also to compare soap with abamectin, a natural compound isolated from soil micro-organism, *Streptomyces avermitilis* Burg (Actinobacteria: Actinobacteridae). In addition, one of the goals was to determine the phytotoxicity of the soap applications.

Materials and Methods

Chemicals – Soft Soap (ABC[®] liquid 25%) and Abamectin (Agrimec[®] 18^{EC}, Bayer) were applied. Abamectin is a naturally derived acaricide/insecticide-isolated from the fermentation of the soil micro-organism, *Streptomyces avermitilis*. The experimental dosage of abamectin was 12.5 mg/ 100 L (half of the label dosage) whereas; soap was applied at three dosages (3 ml/L, 5 ml/L and 7 ml (soap)/ L water (Bulut & Madanlar, 2004).

Source of mites – The *T. urticae* population was obtained from a culture kept for five years on bean plants (*Phaseolus vulgaris* L.) at Ankara University, Turkey. *Tetranychus urticae* was reared on bean plants (*P. vulgaris* cv. Barbunia) at 25±1 °C and 65± 5% RH under a 16-h light regime. The predatory mite, *P. persimilis* was collected from vegetable plants in Hatay-Samandag/ Turkey in 2004. It was reared under laboratory conditions on bean plants infested by *T. urticae* under the same conditions as mentioned above. The experiments were conducted as a split-plot design with four replications and two plants for each replication.

Effects of chemicals and predatory mite on the two-spotted spider mite - The experiments were carried out under laboratory conditions at 25±1°C and 60±5% RH under a 16-h light regime. The method used in this research was the spraying of the bean plants infested by mite populations (Helle & Overmeer 1985). Bean seedlings were reared in pots and at 4-leaf stage (4 true-leaf stage), the plants were infested with *T. urticae* TSSM (20 females per plant). After five days of infestation with spider mites, soft soap and abamectin were applied directly to infested plants with a hand sprayer, with special attention to producing uniform coverage of the plants. In treatments which consisted of chemicals with predatory mite, chemicals were applied after five days of infestation with TSSM. In order to determine the side effects of chemicals on the predatory mite, three female *P. persimilis* were released per plant three days after treatment by soap and abamectin. The release ratio of predators was taken from a table of the impact of released biological control agents on selected pests (Crowder 2007). The experiments in the current study consisted of ten treatments; four treatments (soap at three dosages, abamectin) as chemical controls, four treatments as a combination of chemicals with *P. persimilis*, one treatment as a biological control only (predatory mite released after five days of infestation with TSSM), and one treatment as a control which was applied with water (without chemical or predator).

The number of predatory mites and TSSM (eggs, immature and adult stages) were counted on 5 cm² of bean leaf. The leaf samples were taken as 4 leaves per treatment; before application, and five, seven and ten days thereafter. The corrected efficiency percentage was calculated according to the Henderson-Tilton formula (Henderson & Tilton 1955). The experiments were in a Split-Plot design with four replications and two plants in each replication.

Contact toxicity test for predatory mite – The Leaf-Spray method, which is accepted by the IOBC/WRPS Working Group on 'Pesticides and Beneficial Arthropods', was used (Helle & Overmeer 1985). The detached bean leaf was placed on wet cotton wool in a Petri dish (9 cm diameter) and ten female adults of the predatory mite were transferred to the leaf which was surrounded with vaseline to prevent the escape. Adults and nymphs of *T. urticae* were transferred to the leaf as prey after the spraying of chemicals. The experimental dosages of abamectin were 12.5 (2.25 ppm) and 25 mg/ 100 L (4.5 ppm), the label dosage. Soap was applied as three dosages (3 ml/L, 5 ml/L and 7 ml/L). Corrected mortality was evaluated 24/ h after application with Abbott's formula (Abbott 1925). Three replicates and one control were used for each dosage. The classification of the direct side-effects of the chemical was

evaluated according to the IOBC category (International Organization for Biological Control) for laboratory tests against natural enemies; <30 % mortality is harmless or slightly harmful (N), 30-79 % mortality is moderately harmful (M) and > 79 % mortality is considered as harmful (T) (Boller et al. 2006).

Phytotoxicity test - Phytotoxicity of soap dosages was evaluated at 5 days after soap applications; the phytotoxicity scale for the soap was from 0-3 (0= no visible injury; 1= slight injury, 25 % injury; 2= moderate injury, 50 % injury; and 3= severe injury, more than 75 % injury) (Cloyd & Cycholl 2003).

Data were analyzed with ANOVA by using the computer program Cohort Software (Costat, CoHort software, Monterey, CA, USA) and means were separated according to Duncan's Multiple Range Test (DMRT) at P= 0.05. Data in the form of percentages were transformed to arcsine values for ANOVA.

Results

Effect of chemicals and predatory mite on the two-spotted spider mite – The density of the *Tetranychus urticae* (TSSM) population decreased gradually over time with soft soap at 7 ml/L and abamectin, while there was increasing density of TSSM population seven and ten days after application of soap at 3 ml/L and 5 ml/L (Figure 1). Therefore, the effect of soap at 3 ml/L and 5 ml/L on the TSSM population decreased seven days after application, while for 7 ml/L the efficacy reached 100 % five days after application (Table 1). In addition, there was no significant difference between soap at 7 ml/L and abamectin over time. The efficacy of soap at 3 ml/L (55.6 %) was less than soap at 5 ml/L and 7 ml/L (60.3 % and 100 %, respectively), and abamectin (100 %), after ten days. There was no significant difference between soap at 3 ml/L and 5 ml/L with *P. persimilis* after five and seven days but there was a significant difference ten days after application (Table 1). The efficacy of soap at 7 ml/L and abamectin with *P. persimilis* showed no significant difference to soap at 5 ml/L with *P. persimilis* after ten days. The treatment with *P. persimilis* only showed a significant difference with chemical treatments. The efficacy of *P. persimilis* (93.3 %) alone was greater than soap applications at 3 ml/L and 5 ml/L (55.6 %, 60.3 % respectively) ten days after application. The efficacy of soap at 3 ml/L with *P. persimilis* showed no significant difference to the efficacy of *P. persimilis* alone over time (Table 1).

Contact toxicity test for the predatory mite - The toxicity of soap at 5 ml/L and 7 ml/L was categorized as moderately harmful (M) according to IOBC standards, causing 50.0 % and 42.3 % mortality to adult of *P. persimilis*, while the toxicity of soap at 3 ml/L was evaluated as slightly harmful (N) by causing 30 % mortality. Abamectin at 12.5 and 25 mg/L (Ppm) caused 80.0 % and 93.3 % mortality, respectively, to adult of *P. persimilis* and was evaluated as harmful (T) (Table 2).

Phytotoxicity test - All the applied dosages of soap were not phytotoxic to bean plants under laboratory conditions (Table 2).

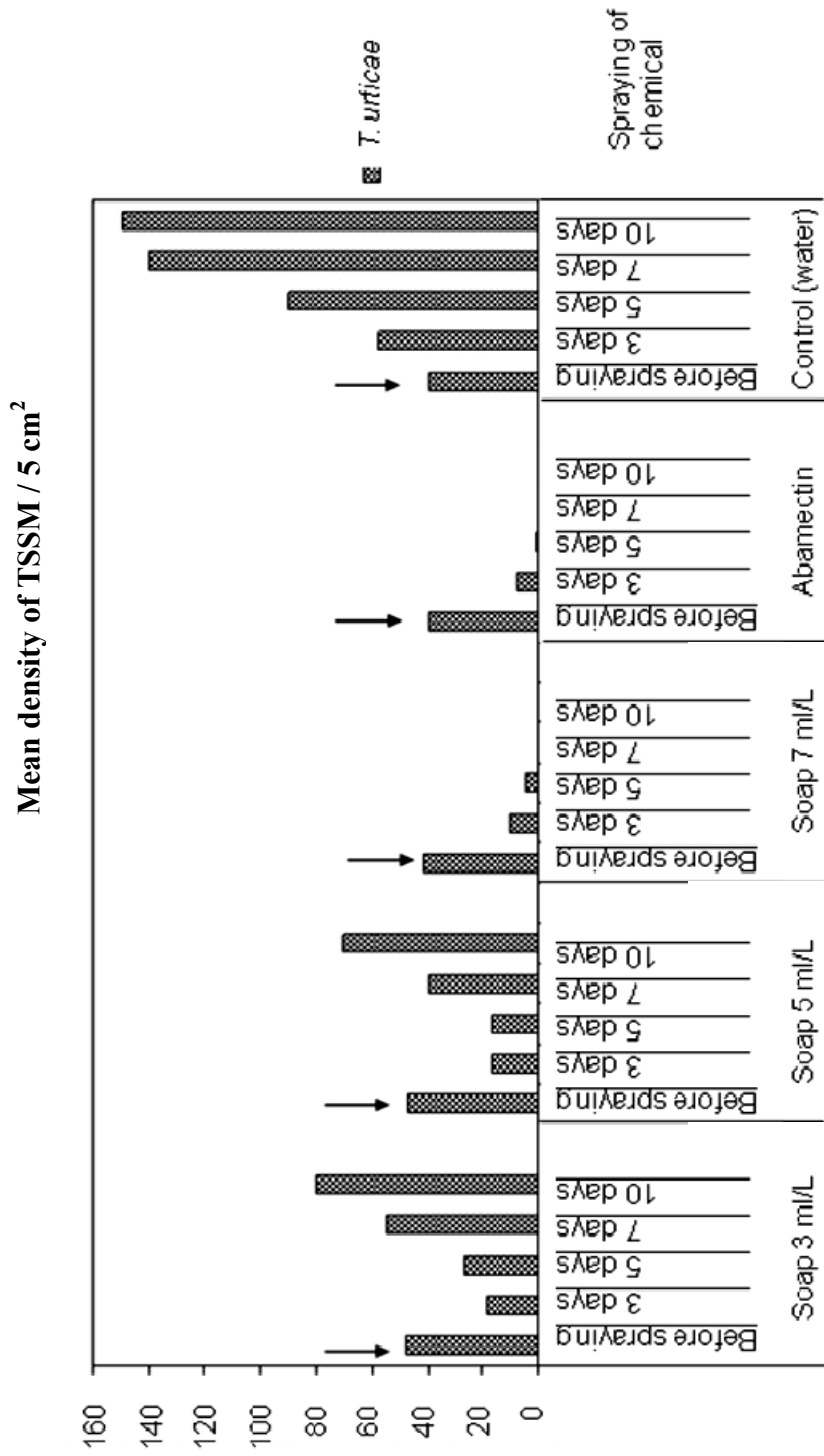


Figure 1. Mean density of *Tetranychus urticae* (TSSM) on 5 cm² of bean leaf exposed to chemicals (soft soap and Abamectin) treatments under laboratory conditions.

Table 1. Mean mortality (%) percentage for chemical treatments and the predatory mite *Phytoseiulus persimilis* on *Tetranychus urticae* (TSSM) on bean leaf exposed to chemical (Soft Soap and Abamectin) treatments under laboratory conditions (Mean±St. Error)*

| Treatment | | After 5 days | After 7 days | After 10 days |
|--|--|---------------------------|---------------------------|----------------------------|
| Chemical treatments | Soap ³ (soap at 3 ml/L) | 75.0± 4.03 c | 67.85± 1.53 d | 55.55± 3.95 d |
| | Soap ⁵ (soap at 5 ml/L) | 83.92± 1.68 b | 75.68± 2.13 c | 60.28± 1.80 c |
| | Soap ⁷ (soap at 7 ml/L) | 95.66± 1.53 a | 100± 0 a | 100± 0 a |
| | Abamectin (at 12.5 mg/100L) | 98.88± 0.45 a | 100± 0 a | 100± 0 a |
| Combination of chemicals with predatory mite | Soap ³ + <i>P. persimilis</i> | 84.87± 2.0 b | 92.09± 0.96 b | 89.22± 1.48 b |
| | Soap ⁵ + <i>P. Persimilis</i> | 89.11± 1.43 b | 91.83± 1.06 b | 99.86± 0.13 a |
| | Soap ⁷ + <i>P. Persimilis</i> | 99.73± 0.26 a | 100± 0 a | 100± 0 a |
| | Abamectin + <i>P. persimilis</i> | 98.99± 0 a | 100± 0 a | 100± 0 a |
| Predatory mite treatment | <i>P. persimilis</i> | 85.55± 2.68 b | 89.28 ± 1.77 b | 93.33± 1.36 b |
| | | P= 0.0121, df= 24, F=9.36 | P= 0.0042, df= 24 F= 9.56 | P= 0.0000, df= 24, F= 4.95 |

*Means with different letters within a column are significantly different ($P= 0.05$. Duncan's Test); St.error: Standard error.

Table 2. Mean of the percentage corrected efficacy for chemical treatments (Soft Soap and Abamectin) under laboratory conditions and IOBC toxic category of chemicals in direct toxicity test on predatory mite and phytotoxicity of soap to host plant (bean)

| Chemicals | Mortality (%) | IOBC category | Phytotoxicity category |
|------------------------|---------------|---------------|------------------------|
| Soap ⁷ | 50.0 | M | 0 |
| Soap ⁵ | 43.3 | M | 0 |
| Soap ³ | 30.0 | S | 0 |
| Abamectin ¹ | 93.3 | T | - |
| Abamectin ² | 80.0 | T | - |

Soap³: at 3 ml/L; Soap⁵: at 5 ml/L; Soap⁷: at 7 ml/L; Abamectin¹: at 25 ml/ 100 L; Abamectin²: at 12.5 ml/ 100 L.

Discussion

The effect of soap at 3 ml/L and 5 ml/L was insufficient to suppress the TSSM population and could not provide satisfactory control when compared with abamectin and soap at 7 ml/L, which suppressed TSSM populations.

The predatory mite could develop well under the residual effect of soap at different dosages. Its population increased over time and controlled the TSSM population 10 days after application of soap at 5 ml/L. At 3 ml/L of soap, the population of the predator increased, accompanied by an increasing TSSM population after ten days. Several studies have indicated that, predatory mites failed to control higher spider mite populations (Kim & Paik 1996; Ibrahim & Yee 2000; Naher & Haque 2007). In addition, the predator could also reproduce well, especially five days after application of soap at 7 ml/L. Henn et al. (1991) mentioned that soaps degrade rapidly in sunlight, air and moisture, and this is very important because rapid breakdown means less persistence in the environment and reduced risks to non-target organisms.

In the current study, populations of the predatory mite were not observed in abamectin treatments due to its harmful effects. Bostanian & Akalach (2006) and Ersin & Madanlar (2006) reported that the contact toxicity of abamectin and insecticidal soap were very high for *P. persimilis* under laboratory conditions. In addition, results of the leaf spray experiment (contact toxicity) in the current study indicated that abamectin is extremely toxic to *P. persimilis*.

The combination of soap at 5 ml/L with *P. persimilis* gave control against TSSM when compared with the combination of soap at 3 ml/L with *P. persimilis* and with *P. persimilis* alone. Therefore, the effect of soap at 5 ml/L enhanced the *P. persimilis* effect. Spider mite populations can develop resistance to chemical applications; if the TSSM population developed resistance to soap, the combination of soap at 7 ml/L with *P. persimilis* would be suitable to control resistant spider mites. The predator would repress spider mite populations and there would be synergy between soap at 7 ml/L and *P. persimilis*. For these reasons, the combination of soap at 7 ml/L with predator is a better choice in comparison with abamectin and predator combined because abamectin is extremely toxic.

Soap at 7 ml/L without the predator provided satisfactory control when compared with abamectin and caused no phytotoxicity to host plants. Madanlar et al. (2000) reported that neem oil, Neem Azal T/S, soft soap and tobacco gave promising results against greenhouse pests under laboratory conditions. Henn et al. (1991) stated that soaps have antifeeding effects on pests. Although soaps may not cause death for hours or days, they often cause immediate paralysis or cessation of feeding so insecticidal soaps are promising alternatives for use in insect management. Weinzierl (1998) mentioned that soaps could be used effectively to kill soft-bodied pests such as aphids, thrips, scale crawlers, whitefly, leafhopper nymphs and mites. Therefore, it is necessary to investigate the phytotoxicity of soap treatments under greenhouse and field conditions. However, Henn et al. (1991) reported that soaps and nicotine sulphate might be toxic to some ornamentals.

The use of soap, with or without the predatory mite, gave encouraging results for controlling spider mites but was moderately toxic at 5 ml/L and 7 ml/L to the predatory mite. Therefore, it seems that control of TSSM populations can be successfully achieved by combining soap at suitable dosage with *P. persimilis* releases, or soap alone, under laboratory condition. However, it cannot be overemphasised that soaps degrade rapidly in sunlight and this is very important because *P. persimilis* is mainly released on greenhouse plants. It is therefore necessary that to try these experiment in greenhouses.

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