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Original article (Orijinal araştırma)

Efficacy of *Nicotiana tabacum* L. (Solanaceae), *Allium sativum* L. (Amaryllidaceae) and soft soap for controlling *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae)[1]

Polyphagotarsonemus latus (Banks, 1904) (Acari: Tarsonemidae)'un kontrolünde *Nicotiana tabacum* L. (Solanaceae), *Allium sativum* L. (Amaryllidaceae) ve arap sabununun etkinliği

Rana AKYAZI[2*] Mete SOYSAL[2] Yunus Emre ALTUNÇ[2] Duygu AKYOL[2]

Abstract

The toxicity of bulb extract of garlic, *Allium sativum* L. (Amaryllidaceae), the leaf extract of tobacco, *Nicotiana tabacum* L. (Solanaceae), soft soap and the mixtures of soft soap with the garlic and tobacco extracts on *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae) were investigated in the Plant Protection Department of Ordu University between 2014 and 2018 under controlled conditions. The ovicidal and adulticidal effects were tested against 0-24-h-old eggs and newly emerged adult females (1-2 days old) of *P. latus*, respectively at five concentrations of each extract. The efficacies of the same solutions in controlling the *P. latus* population were investigated on potted Barbunia bean, *Phaseolus vulgaris* L., 1753 cv. Barbunia (Fabaceae) plants in a climatic room as well. The results demonstrated that the soft soap and the garlic bulb extract+soft soap mixture are promising for controlling *P. latus*. The ovicidal effects of soap at 10% and garlic+soap mixture at 20% were 100% and 97.8%, respectively. Additionally, the adulticidal effects of soap at 7.5 and 10% and garlic+soap mixture at 10% were not significantly different and ranged between 90-100%. Also, the soap (10%) and garlic+soap (20%) treatments were able to keep the mite population on bean plants below the economic threshold for 1 week.

Keywords: Adulticidal, garlic, indirect ovicidal activity, tobacco, yellow tea mite

Öz

Sarımsak, *Allium sativum* L. (Amaryllidaceae) yumru ekstraktı, tütün, *Nicotiana tabacum* L. (Solanaceae) yaprak ekstraktı, arap sabunu ile sarımsak ve tütün ekstraklarının sabun ile karışımlarının *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae)'a karşı toksik etkileri, Ordu Üniversitesi, Bitki Koruma Bölümü'nde, 2014-2018 yılları arasında araştırılmıştır. Ovisidal ve adultisidal etkiler, sırası ile *P. latus*'un 0-24 saatlik yumurtaları ve genç ergin dişilerine (1-2 günlük) karşı beş farklı konsantrasyonda test edilmiştir. Aynı solüsyonların *P. latus* popülasyonunu kontroldeki etkinlikleri ise saksılı fasulye, *Phaseolus vulgaris* L., 1753 cv. Barbunia (Fabaceae) bitkileri üzerinde, iklim odası koşullarında araştırılmıştır. Sonuçlar, arap sabunu ve sarımsak+sabun karışımının *P. latus* kontrolü açısından umut verici olduğu göstermiştir. Arap sabununun %10, sarımsak+sabun karışımının %20'lik konsantrasyonlarındaki ovisidal etkileri sırası ile %100 ve %97.8 olarak tespit edilmiştir. Sabunun %7.5 ve %10'luk konsantrasyonları ile sarımsak+sabun karışımının %10'luk konsantrasyonunun istatistiksel olarak aynı ve %90-100 arasında değişen adultisidal etkiye sahip oldukları da belirlenmiştir. Ayrıca, sabun (10%) ve sarımsak+sabun (20%) uygulamaları, fasulye bitkilerindeki akar popülasyonunu 1 hafta boyunca Ekonomik Zarar Eşiği altında tutabilmiştir.

Anahtar sözcükler: Adultisidal, sarımsak, ovisidal, tütün, sarı çay akarı

[1] This study was partly presented as a poster at the 15th International Conference of Acarology (ICA) (2-8 September 2018, Antalya, Türkiye) and the International Conference on Biopesticide 7 (ICOB7) (19-25 October 2014, Antalya, Türkiye) and published as an abstract in the abstract book.

[2] Ordu University, Faculty of Agriculture, Department of Plant Protection, 52200, Altınordu, Ordu, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: ranaakyazi@odu.edu.tr
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Introduction

Polyphagotarsonemus latus (Banks, 1904) (Acari: Trombidiformes: Prostigmata: Tarsonemidae) is an economically important pest mite on more than 60 plant families including Solanaceae, Cucurbitaceae, and Malvaceae (Grinberg et al., 2005). This species attacks growing young parts of the plants and is generally found on the undersides of young leaves. It can also inject toxic saliva into plant tissues (Rodriguez et al., 2017). Its feeding causes twisted, hardened and distorted growth in the terminal of a plant (Rogers et al., 2010; Rodriguez et al., 2017). Damaged leaves turn to coppery colors and bend. Also, the leaves of the heavily infested plants fall, they often fail to flower and produce bronze, cracked or blemished fruits (Gerson, 1992). Additionally, because individuals of this mite are microscopic (0.1-0.3 mm long), they can be noticed just when the symptoms occur (Venzon et al., 2008).

The control of *P. latus* is mostly based on the use of pesticides (Kousik et al., 2007). However, some common pesticides have unwanted various negative impacts on the non-target organisms and the environment (Aktar et al., 2009). So, there is an urgent need for the discovery of new non-toxic natural products as an alternative to conventional pesticides in the control of *P. latus.* Tobacco, *Nicotiana tabacum* L. (Solanaceae) used as an insecticide since 1960, garlic, *Allium sativum* L. (Amaryllidaceae) used for more than 7,000 years as a medicinal plant by humans (Pavela, 2016) and soft soap, which has been used as a pesticide since the 1700s (Olkowski et al., 1993), are among alternative natural pesticidal materials.

Several studies have focused on the control of *P. latus* using garlic, tobacco and soft soap on different host plants except bean plants. Among these, Hossain et al. (2013) investigated the efficacy of garlic against this mite on jute, *Corchorus capsularis* L. and *Corchorus olitorius* L. (Malvaceae). Uraisakul (2003) and Mari et al. (2013) used the tobacco extract to control *P. latus* on chili, *Capsicum* spp. (Solanaceae). A study on the management of the *P. latus* population using soft soap on chili under both screen house and field conditions was conducted by David et al. (2009). Akyazi et al. (2019) investigated the effect of garlic and tobacco extracts, soft soap against *P. latus* on the tea plant, *Camellia sinensis* (L.) Kuntze (Theaceae). However, no study has yielded the efficiency of the soft soap, garlic, and tobacco extract against *P. latus* on bean plants. It is known that the different plant species have different leaf morphology. Leaf morphology may affect spray deposition (de Ruiter et al., 1990; Himel et al., 1990; Smith et al., 2000). Also, in particular, no study, to our knowledge, has considered the effect of the mixtures of soft soap with garlic and tobacco extracts on *P. latus* on any plant.

Additionally, although Almansour & Akbar (2013) and Akyazi et al. (2018) have illuminated the ovicidal effects of garlic and tobacco extracts, soft soap on *Tetranychus urticae* Koch., 1836 (Trombidiformes: Tetranychidae) eggs, a still-unanswered question is whether these treatments and their mixtures also have a toxic effect on *P. latus* eggs.

Also, few studies have focused on the adulticidal efficiency of the tested solutions in the current study on plant-parasitic mites. Previous studies such as Attia et al. (2002), Hincapie et al. (2008), Erdogan et al. (2012), Almansour & Akbar (2013), Nour El-Deen & Abdallah (2013), Akyazi et al. (2018) have almost exclusively focused on *T. urticae*. Also, it should be aware that the potential effect in the mixture may be less or greater than that of individual substances.

To fill these literature gaps, this paper identifies the effects of the aqueous garlic bulb and tobacco leaf extracts, soft soap, aqueous garlic bulb extract+soft soap, and aqueous tobacco leaf extract+soft soap mixtures against *P. latus* on bean plants, and their ovicidal and adulticidal effects on *P. latus* under controlled conditions.

Materials and Methods

Plant rearing as the host for *Polyphagotarsonemus latus* and the main material for test substrate

Barbunia bean, *Phaseolus vulgaris* L. cv. Barbunia (Fabaceae) plants were grown from seed in plastic pots in a plant growth room (25 ± 2°C, 70-80% RH, 16:8 h L:D photoperiod, 12.000 lux, daylight). The plants were used as a host for *P. latus*. Bean leaf discs (3 cm) served as a substrate for releasing the mites on potted bean plants and for adult oviposition to assess the ovicidal and adulticidal activities.

Mite origin and rearing

The original population of the *P. latus* was obtained from an organic tea plantation at the Rize Atatürk Tea and Horticultural Research Institute in 2014. The stock cultures of *P. latus* were maintained on potted bean plants in a climate room (25 ± 2°C, 70-80% RH, 16:8 h L:D photoperiod, 12.000 lux, daylight). Heavily infested bean plants were replaced with fresh ones for maintaining the mite population.

Zero-24-h-old eggs of *P. latus* were used to test the ovicidal efficacy of different solutions. To obtain eggs of the same age, five adult females of *P. latus* were transferred from the stock colony to each bean leaf disc and kept overnight for oviposition. After 24 h, adult females were removed from each disc. Discs including 10 eggs were used for ovicidal activity assessments.

The adulticidal assay was performed using 24-48-h-old adult females. For this purpose, 20 *P. latus* females taken from the stock culture were placed on each leaf disc. Mites were allowed to lay eggs for 24 h, then they were removed the disc. The leaf discs with the *P. latus* eggs were kept in a growth chamber (25 ± 2°C, 70-80% RH, 16:8 h L:D photoperiod, 12.000 lux, daylight). After egg hatching, the adult females from these experimental units were used for the adulticidal bioassays.

Larva and quiescent nymph stages were not included in the experiment because these stages generally lasted about 24 h or less under the study conditions.

Preparing stock solutions

Samsun Canik tobacco, Taşköprü garlic, and soft soap (5-10% KOH, 15-30% sunflower oil, distilled water) were used for preparing the stock solutions.

Tobacco was harvested using the priming method (the leaves are harvested as and when they are fully developed and matured) on 10 August 2013 in Vezirköprü, Samsun, Türkiye. The leaves were dried using the sun-curing method (leaves were spread out on racks and placed outdoor when the sun rises, at night or in rainy weather, the racks were brought indoor until the leaves were sun-cured).

The garlic was harvested when the stem turned yellow and fell over and the bottom few leaves turned brown on 15 July 2013 and 2018 in Çetmi village in Taşköprü, Kastamonu, Türkiye. The harvested garlic was cured in a warm, dry, shaded area with good ventilation by tying the stem together and hanging to dry for 3-4 weeks.

The impact of each solution on the different stages of *P. latus* was different. So, the content of each stock solution and their used concentrations were adjusted by preliminary trials so that the mortality rate was almost equally distributed (Table 1).

For preparing the aqueous garlic bulb extract stock solution, peel garlic cloves (50 g for ovicidal and acaricidal efficiency and 125 g for adulticidal efficiency) were crushed and mixed up with distilled water (500 ml) and then incubated into a tightly sealed glass jar at 25 ± 1°C for 24 h. After incubation, the product was separated using a fine muslin cloth and then filtered through Whatman No. 1 filter paper.

To obtain the aqueous tobacco leaf extract stock, the dried tobacco leaves (50 g) were mixed with 500 ml distilled water and incubated in a tightly sealed glass jar at 70°C for 24 h. After incubations, the obtained product was passed through firstly a fine muslin and then a filter paper (Whatman No. 1).

To the stock solution of soft soap, soft soap (50 g) was mixed with distilled water (500 ml), and then shaken into a tightly sealed glass jar at 25 °C for 24 h at 200 rpm until the soap was fully dissolved.

For the aqueous garlic bulb extract+soft soap mixture, 25 g soft soap was mixed up with the garlic stock solution (125 ml) and shacked until the soap was fully dissolved.

For the aqueous tobacco leaf extract+soft soap mixture, 25 g soft soap was mixed up with the tobacco stock solution (125 ml) and shacked until the soap was fully dissolved.

Table 1. Stock solutions, stock contents and tested concentrations

Stock	Stock content	Ovicidal effect dose (%) (v/v) *	Adulticidal effect dose (%) (v/v) *	Acaricidal effect on bean plants dose (%) (w/v)
Aqueous garlic bulb extract 1	50 g garlic + 500 ml water (ovicidal and acaricidal)	1, 2.5, 5, 7.5, 10	-	10
Aqueous garlic bulb extract 2	125 g garlic + 500 ml water (adulticidal effect)	-	2.5, 6.25, 12.5, 18.75, 25	-
Aqueous tobacco leaf extract	50 g tobacco + 500 ml water	1, 2.5, 5, 7.5, 10	1, 2.5, 5, 7.5, 10	10
Soft soap	50 g soap + 500 ml water	1, 2.5, 5, 7.5, 10	1, 2.5, 5, 7.5, 10	10
Aqueous garlic bulb extract + soft soap	25 g soap + 125 ml garlic stock	2, 5, 10, 15, 20	2, 5, 10, 15, 20	20
Aqueous tobacco leaf extract + soft soap	25 g soap + 125 ml tobacco stock	2, 5, 10, 15, 20	2, 5, 10, 15, 20	20

* w/v for 10% of single solutions, 25% of garlic extract and 20% of mixture solutions.

Preparing required concentrations

Each treatment was evaluated at five concentrations for assessing ovicidal and adulticidal activities. The concentrations used for the ovicidal and adulticidal effects were chosen after preliminary bioassays to obtain mortality rates almost equally distributed between 0 and 100% (Table 1) (Tuncer, 2001). So, different concentrations of garlic extract were evaluated for ovicidal and adulticidal efficiencies. However, since some treatments had phytotoxic effects at high concentrations in the preliminary bioassay, their lower concentrations were used in the study.

To test the ovicidal and adulticidal effects, 1, 2.5, 5, 7.5 and 10 ml of each stock solution were diluted separately with distilled water to a total volume of 10 ml (final volume) to get five concentrations of each tested solution (Table 1).

However, the stock solutions were directly used to control the *P. latus* population on potted bean plants (Table 1). Due to the phytotoxic effect of the stock solution of garlic extract at 25% on bean leaf disc, the stock solution of garlic extract at 10% was used for the acaricidal effect experiment on potted bean plants. The other stock solutions and all tested concentrations had not any phytotoxic effect on bean leaf disc. So, they were used for the acaricidal effect experiment on potted bean plants.

Ovicidal effect on *Polyphagotarsonemus latus* eggs (0-24 h old)

The ovicidal effects of seven treatments including soft soap, garlic extract, tobacco extract and the mixtures of garlic and tobacco extracts with soap were investigated against *P. latus* eggs (0-24 h old). Each treatment was evaluated at five concentrations (Table 1). Five replicate bioassays with five concentrations and two controls (negative and positive) were performed. Distilled water and abamectin 18 g/L (25 ml/100 L water) were used as a negative and positive control, respectively. Abamectin is a natural fermentation product of the *Streptomyces avermitilis* (ex Burg et al., 1979) (Actinomycetales: Streptomycetaceae) (Siddique et al., 2013).

It has also translaminar or local systemic activity (Cloyd, 2016). It is used commonly and effectively against *T. urticae* on some vegetables. So, it was chosen as a positive control for the current study to test if it has also an effect on *P. latus*. A modified leaf disc dip method (de Silva et al., 2008) was used to evaluate the ovicidal activities of treatments. The discs with eggs were dipped in the solutions, distilled water or abamectin for 5 s. Treated leaf discs were dried at room temperature and placed underside up on moistened cotton in a plastic tray (15 x 11 cm). The number of hatched eggs in each treatment was recorded at 1- and 2-day intervals for 10 days following the application to be completely sure of the egg hatching rates in the treatments. Those eggs that did not hatch after this period were regarded as non-viable.

Adulticidal effect on *Polyphagotarsonemus latus* adult females (1-2 days old)

Adulticidal bioassay was performed by the leaf disc spraying method since the leaf disc dip method used to assess ovicidal efficacy was not appropriate for the adulticidal activity bioassays. Solutions at five different concentrations were evaluated for the adulticidal effect (Table 1). A single bioassay with five concentrations and two controls (negative and positive) was replicated five times. The negative control discs were treated with distilled water. Abamectin 18 g/L (25 ml/100 L water) was used for the positive control discs. A modified leaf disc spraying method described by Zalom et al. (2010) was used to assess the adulticidal activity of the tested solutions. For this purpose, ten adult female mites (1-2 days old) were gently transferred on each leaf disc by using a 5/0 size brush. The solutions were applied using a small hand sprayer held 15 cm away from the leaf discs resulting in a 3.08 ± 0.39 µl/cm^2 deposit. Dead mites were counted 1, 24, 48 and 72 h after solution applications (Akyazi et al., 2018).

Acaricidal effect on *Polyphagotarsonemus latus* population on potted bean plants

The method to detect the acaricidal effect on the *P. latus* population was spraying the potted bean plants infested by *P. latus*. In the experiment, there were seven treatments: soft soap, tobacco leaf extract, garlic bulb extract, the mixtures of garlic and tobacco extracts with soap, Abamectin 18 g/L (25 ml/100 L water) and untreated control. Five replicates were used per treatment. Each replicate contained six plants giving a total of 30 plants for each treatment. Seven days after planting bean plants, *P. latus* was released to the plants at about 15 cm in length and two-leaf-stage. The plants were infested with adult females of *P. latus* at a density of 4-5 mites/plant. Seven days after the release, the leaf sampling was started. The population of *P. latus* was evaluated weekly for 4 weeks. At each sampling date, 15 young leaves from the top of the plants were randomly examined for each treatment. All mites present on both sides of the leaves were counted using a stereomicroscope (Leica DM 2500). The spraying was done when the density of *P. latus* motile stages exceeds an average of 4 mites/leaf (Anonymous, 2008) using a hand-operated sprayer for each treatment. Untreated control plants were included. The experiment was conducted in a climate room (25 ± 2°C, 70-80% RH, 16:8 h L:D photoperiod, 12.000 lux, daylight).

Data analysis

The data on ovicidal efficacy was corrected using Abbott's formula (Abbott, 1925). The Kolmogorov-Smirnov and Levene's tests were applied to test normality and homogeneity of variance, respectively. Two-way ANOVA was used for analyzing data sets. Means were compared by letters with Duncan's multiple range test (DMRT). The significance level was set at 0.05 (5%). Minitab 17 statistical package program was used for the statistical analysis.

The data on adulticidal efficacy were also corrected using Abbott's formula (Abbott, 1925). Data normality was tested by the Kolmogorov-Smirnov test. Levene's and Bartlett's tests were applied to test the homogeneity of variances. If the assumption fitted, the variables were analyzed by one-way ANOVA. Minitab 17 software program was used for all calculations and the comparison of the groups in letters was displayed by Tukey's test. The alpha level was also preferred as 0.05 (5%).

Probit analysis was not applied for the ovicidal and adulticidal data because the dead effects of some treatments did not show a distribution between 20% and 80% (Simon, 2014).

The non-continuous data sets of effect against *P. latus* population on bean plants were analyzed with Kruskal Wallis and Ranks were compared by letters with Dunn multiple comparison test. The significance level was set at 5%. The SPSS 24 statistical package program was used for the statistical analysis.

Results

Assessment of ovicidal efficacy

The results of the ovicidal bioassay of the tested solutions were given in Table 2. In some cases, mortality was lower than the control. According to the single solution data, at a concentration of 1%, the ovicidal effect of tobacco leaf extract (T) (28.3%) was greater than the efficacy of garlic bulb extract (G) (-6.9%) and soft soap (S) (-5.0%). However, at a concentration of 2.5%, the toxic effects on *P. latus* eggs of T (39.9%) and G (28.8%) were the not significantly different and the effect of T was higher than the effect of S (15.4%). There were also no significant differences between the effects of G (53.3%) and T (66.2%) at a concentration of 5%. Also, the ovicidal effect of S (31.3%) was lower than the other two extracts at that concentration. S (62.1%), G (75.7%), and T (75.9%) had no significantly different ovicidal effects at 7.5%. Notable, the ovicidal efficacy of S increased to 100% at a concentration of 10%. Additionally, there were no significant differences between its effect and the effect of T (86.1%). Also, the effect of G (84.9%) was not significantly different from T (86.1%) but lower than S (100%).

Table 2. Ovicidal effects of tested solutions on the *Polyphagotarsonemus latus* eggs (0-24 h old)

Dose single (%)	Ovicidal effect of a single solution (%, mean ± SE)			Dose mixture (%)	Ovicidal effect of mixture solution (%, mean ± SE)		Ovicidal effect of abamectin (%, mean ± SE)
	T	G	S		T+S	G+S	
1	28.3 ± 4.9 Ca	*-6.9 ± 2.9 Db	*-5 ± 6.1 Eb	2	*-7.2 ± 4.9 Db	14.8 ± 6.9 Da	*-9.2 ± 2.3 b
2.5	39.9 ± 2.1 Cab	28.8 ± 2.2 Cbc	15.4 ± 2.4 Dc	5	24.6 ± 2.3 Cc	51.1 ± 2.1 Ca	*-9.2 ± 2.3 d
5	66.2 ± 4.5 Bb	53.3 ± 1.4 Bbc	31.2 ± 2.6 Cd	10	41.9 ± 6.1 Bcd	84.1 ± 3.2 Ba	*-9.2 ± 2.3 e
7.5	75.9 ± 3.6 ABab	75.7 ± 1.5 Aab	62.1 ± 2.9 Bbc	15	55.6 ± 3.1 Ac	89.1 ± 3.2 ABa	*-9.2 ± 2.3 d
10	86.1 ± 4.5 Aab	84.9 ± 4.3 Ab	100 ± 0.0 Aa	20	69.0 ± 1.6 Ac	97.8 ± 2.2 Aab	*-9.2 ± 2.3 d

Means followed by the same uppercase or lowercase letters for doses and extracts, respectively, are not significantly different ($P < 0.05$) (SE: Standard Error). T, tobacco; G, garlic; S, soft soap; T+S, tobacco+ soft soap; and G+S: garlic+ soft soap.

* Negative mortality values indicate lower mortality than in the control.

According to the data of binary mixtures of tested extracts with soap (Table 2), the ovicidal effect of the garlic extract+soft soap mixture (G+S) (14.8%) was higher than the effect of tobacco extract+soft soap mixture (T+S) (-7.2%) at 2%. At 5%, the effect of T+S and G+S were significantly different and again the toxic efficacy of G+S (51.1%) was higher than that of T+S (24.6%). Also, the G+S (84.11%) showed significantly greater efficacy than the T+S (41.9%) at 10% as well. At 15%, there were significant differences between the ovicidal effect of T+S (55.6%) and G+S (89.1%). It was clear that the significantly high level of egg mortality was caused by the G+S (97.8%) at 20%. Also, the effect of T+S (69.0%) was lower than the effect of G+S.

In conclusion, it would appear that the ovicidal effects of all tested solutions generally increased with increasing concentration. However, there were no significant differences between the effect of 7.5 and 10% of T. The G extract also had no significantly different ovicidal effects at 7.5 and 10%. However, the ovicidal effect of S at 10% was the highest in its other concentrations tested. The effects of T+S at 15 and 20% were also not significantly different. Also, the mean effects of 15 and 20% of G+S were not significantly different (Table 2).

A comparison of the effect of the mixture and single solutions revealed that there were no significant differences between the effects of T at 1% and G+S at 2%. T at 2.5% and G+S at 5% did not have significantly different ovicidal effects. The effect of G+S at 10% was the highest in the tested solutions at 5% (single) and 10% (mixture). The ovicidal effects of the T and G at 7.5% and G+S at 15% were not significantly different. There were also no significant differences between the ovicidal effects of S at 10%, T at 10% and G+S at 20%. The effects of these three solutions ranged from 86 to 100% (Table 2).

Additionally, it was observed that unhatched eggs treated with T (10%), G (10%) or G+S (10%, 15%, 20%) lost their original shape, then seemed as if they melted 10 days after treatment (Figure 1).

Figure 1. Treated with a) T and b) G at 10%, G+S at c) 10%, d) 20% concentrations ten days after treatment and, e) healthy *Polphagotarsonemus latus* eggs.

Another finding was that the ovicidal efficacy of abamectin was even lower compared with control. Its toxic effect on *P. latus* eggs was the lowest in all treatments (Table 2). However, we observed that young larvae died shortly (0-24 h) after hatching (Figure 2).

Figure 2. Hatched eggs treated with Abamectin and dead neonate larvae of *Polyphagotarsonemus latus* from abamectin sprayed eggs.

Assessment of adulticidal efficacy

The adulticidal bioassay results of tested solutions were given in Table 3. According to the single solution data, 72-h after exposure, there were no significant differences between the adulticidal effects of G at 2.5 and 6.25%, T and S at 1% and 2.5%. However, the effects of G at 12.5% and T at 5%, G at 18.75%, and T at 7.5% were also not significantly different but were lower than the effect of S at 5 and 7.5%. No adult mites exposed to the S at a concentration of 10% were found to survive 72 h after treatment. Also, there were no significant differences between the effects of S at 7.5% and 10% (97.8 and 100%, respectively) and abamectin (100%). However, it was observed that G at 25% had a phytotoxic effect on bean leaf disc.

According to the results for binary mixtures of tested extracts with soap 72-h after exposure, the adulticidal effects of G+S and T+S mixtures at 2%, and also 5, 10 and 15% were not significantly different. The G+S showed significantly greater efficacy than the T+S (66.2%) at 20%. The significantly high level of adult mortality (89.3%) was caused by the G+S at 20% (Table 3).

A comparison of the effect of the mixture and single solutions revealed that there were not any significant differences between the effect of all single and mixture solutions at the lowest two dose levels of each solution. The effect of S (80.2-97.8%) was the highest in the tested single solutions at 5, 7.5, 12.25 and 18.75% and mixture solutions at 10 and 15%. However, the effects of the S (100%) at 10% and G+S (89.3%) at 20% were not significantly different but were higher than the effects of tested other single and mixture solutions at the same concentrations including the 25% of single solutions (Table 3).

It can be also concluded that the adulticidal effects of all tested solutions generally increased with increasing extract concentration. However, there were no significant differences between the effect of 18.7 and 25% of G and also between the effect of T or S at 7.5 and 10% (Table 3).

We also found that all the adult mites died 1 h after application with abamectin. This is an important finding in the understanding of the adulticidal efficacy of abamectin against *P. latus*. Additionally, there were no significant differences between the effect of S at 7.5 and 10% 72-h after exposure and abamectin. The G+S mixture at 20% after 72 h of treatment also had no significantly different adulticidal effect to abamectin (Table 3).

Table 3. Adulticidal effects of the tested solutions against *Polyphagotarsonemus latus* adult females (1-2 days old)

Time (h)	Effects of single solutions (%) (Mean ± SE)					Effects of mixture solutions (%) (Mean ± SE)		
	Dose (%)	G	Dose (%)	S	T	Dose (%)	T+S	G+S
1	2.5	0 ± 0.0 Ca i	1	0 ± 0.0 Da iii	0 ± 0.0 Da ii	2	0 ± 0.0 Da i	2 ± 2.0 Da i
	6.25	2 ± 2.0 Ca ii	2.5	2 ± 2.0 Da ii	4 ± 2.5 Da i	5	2 ± 2.0 Da i	8 ± 3.5 Da ii
	12.25	4 ± 2.5 Cb ii	5	38 ± 4.9 Ca ii	4 ± 2.5 Db ii	10	14 ± 7.5 CDb ii	12 ± 3.8 Db ii
	18.75	28 ± 3.8 Bb ii	7.5	62 ± 8.6 Ba ii	20 ± 5.5 Cb ii	15	26 ± 2.5 Cb ii	32 ± 2.0 Cb ii
	25	24 ± 2.5 Bc iii	10	76 ± 4.0 Ba ii	38 ± 3.8 Bc ii	20	58 ± 4.9 Bb ii	68 ± 3.8 Bab iii
	Abamectin	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i
24	2.5	2 ± 2.0 Da i	1	6.0 ± 4.0 Ca ii,iii	6 ± 4.0 Ca i,ii	2	4 ± 2.5 Da i	10 ± 3.2 Ca i
	6.25	2 ± 2.0 Da ii	2.5	13.8 ± 6.9 Ca i,ii	8 ± 3.7 Ca i	5	4 ± 4.0 Da i	20 ± 8.4 Ca i,ii
	12.25	16 ± 4.0 Cab i	5	36.4 ± 4.8 Ba ii	8 ± 2.0 Cb i,ii	10	22 ± 7.4 CDab i,ii	22 ± 4.9 Cab iii
	18.75	34 ± 4.0 Bb i,ii	7.5	86.0 ± 5.1 Aa i	40 ± 8.9 Bb i	15	36 ± 5.1 Cb i,ii	46 ± 5.1 Bb i
	25	36 ± 2.5 Bc ii	10	91.8 ± 3.8 Aa i	58 ± 7.4 Bbc i	20	66 ± 8.1 Bb i	80 ± 5.5 Aab ii
	Abamectin	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i
48	2.5	1.8 ± 3.9 Da i	1	12.4 ± 4.0 Ca i,ii	10 ± 4.5 Ca i,ii	2	8.0 ± 5.8 Da i	14 ± 5.1 Ca i
	6.25	4.0 ± 2.5 Da i,ii	2.5	26.7 ± 7.6 Ca i	10 ± 3.6 Ca i	5	9.8 ± 5.7 Da i	22 ± 10.2 Ca i,ii
	12.25	18.2 ± 1.8 Cb i	5	65.3 ± 3.9 Ba i	10 ± 0.0 Cb i,ii	10	24.2 ± 7.8 CDbi	26 ± 5.1 BCb i,ii
	18.75	36.4 ± 4.7 Bb i,ii	7.5	94.0 ± 2.5 Aa i	44 ± 10.3 Bb i	15	40.9 ± 5.6 Cbi	48 ± 4.9 Bb i
	25	40.7 ± 2.7 Bd i,ii	10	100 ± 0.0 Aa i	62 ± 5.8 Bc i	20	67.1 ± 7.6 Bbci	86 ± 5.1 Aab i,ii
	Abamectin	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aai	100 ± 0.0 Aa i
72	2.5	5.8 ± 5.3 Ca i	1	17.3 ± 5.6 Ca i	12 ± 5.8 Ca i	2	10 ± 5.5 Da i	8.7 ± 7.4 Ca i
	6.25	10.2 ± 3.2 Ca i	2.5	34.5 ± 8.3 Ca i	12 ± 2.0 Ca i	5	12 ± 6.8 Da i	28.7 ± 12.6 BCa i
	12.25	20.2 ± 2.9 Cb i	5	80.2 ± 1.9 Ba i	14 ± 2.5 Cb i	10	29.3 ± 5.3 CDb i	32.2 ± 7.1 BCb i
	18.75	40.4 ± 5.1 Bb i	7.5	97.8 ± 2.2 ABa i	48 ± 9.7 Bb i	15	39.1 ± 7.3 Cb iii	47.6 ± 4.7 Bb i
	25	46.9 ± 2.0 Bc i	10	100 ± 0.0 Aa i	66 ± 5.1 Bbc i	20	66.2 ± 7.9 Bb i	89.3 ± 3.2 Aa i
	Abamectin	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i	Abamectin	100 ± 0.0 Aa i	100 ± 0.0 Aa i

Means followed by the same uppercase and lowercase letters or Roman numerals for doses, extracts and times, respectively, are not significantly different (P < 0.05). T, tobacco; G, garlic; S, soft soap; T+S, tobacco+ soft soap; and G+S: garlic+ soft soap.

Assessment of acaricidal efficacy to control of *Polyphagotarsonemus latus* on barbunia bean plants

For four weeks, the acaricidal effects of G, T, S, T+S and G+S were also investigated against the *P. latus* population on barbunia bean plants (Table 4). In the S treatment plants, mite density exceeded the ET in the first (10 mites/leaf) and third week (9.5 mites/leaf) of the experiment. After spraying in those weeks, the population was maintained below damaging levels for 1 week. During the application period, the highest weekly mean number of *P. latus* in the S treatment group was 9.5 mites/leaf.

Table 4. Densities of *Polyphagotarsonemus latus* active stages (mean ± SE) on bean plants during the 4-week experimental period

Treatment	Mean mite (all motile stages) / leaf ± SE											
	Week 1*			Week 2			Week 3			Week 4		
	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank
Untreated Control	6.9 ± 1.3	5	39.9	56.4 ± 6.7 a	57	91.3	128 ± 27 a	45	93.3	47.7 ± 5.6 a	9	94.0
Abamectin	12.1 ± 1.9	11	62.3	0.0 ± 0.0 b	0	12.5	0.0 ± 0.0 b	0	8.0	0.5 ± 0.2 b	0	25.0
S	10.0 ± 1.6	9	55.0	0.9 ± 0.3 bc	1	24.0	9.5 ± 2.1 bc	1	31.5	2.6 ± 0.7 b	0	46.5
G	9.0 ± 1.7	12	48.9	10.8 ± 2.0 cd	8	55.0	19.5 ± 2.7 cd	10	47.5	26.7 ± 5.5 ac	3	83.2
T	10.0 ± 1.7	9	54.9	26.4 ± 3.4 ad	28	74.7	19.3 ± 2.9 cd	16	47.6	1.3 ± 0.6 b	0	31.4
T+S	12.5 ± 2.2	9	63.5	39.7 ± 10.7 ad	25	76.9	47.0 ± 12.6 ac	30	64.1	4.3 ± 1.1 bc	4	49.9
G+S	8.4 ± 1.6	7	46.5	3.6 ± 1.4 bc	2	36.7	65.6 ± 10.3 ad	52	79.0	2.9 ± 1.1 b	1	40.9
P-value	0.314			0.000			0.000			0.000		

Means followed by the same letters within columns are not significantly different (P<0.05).

***** Mite densities before the spraying. T, tobacco; G, garlic; S, soft soap; T+S, tobacco+ soft soap; and G+S: garlic+ soft soap.

The G applications were not able to maintain the mite density below the ET. The mite density was always above the ET despite the three times of spraying during the experiment. In this treatment group, the highest mite level was 26.7 mites/leaf.

The T and T+S applications were also not able to maintain the mite density below the ET except the last week of the experiment. However, we observed that both of them had a phytotoxic effect on bean plants. We think that the decrease in mite density is due to the deterioration of plant quality as a result of the phytotoxic effect. The highest mite levels were 47.0 mites/leaf in the T treatment group and 26.4 mites/leaf in the T+S treatment group during the experiment.

In the G+S treatment plants, the mite density exceeded the ET two times (week 1 and 3). G+S treatments were able to keep the population below the ET for 1 week after application. The highest population density (65.0 mites/leaf) was detected on the third week of the experiment.

In the unsprayed control plants, the mite population was above the ET during the entire experiment. In this treatment group, the highest mite level was 128 mites/leaf.

However, in the sprayed control plants, the mite population was above the ET just in the first week of the experiment (pre-infestation period). After spraying with abamectin at that time, the population was maintained below the ET during the remaining experimental period. The highest mite level was 0.5 mites/leaf in this treatment group during the experiment after the pre-infestation period.

It was also clear in the results that the *P. latus* egg population followed almost the same pattern of variation as the motile stage population during the experimental period (Table 5).

Table 5. The densities of *Polyphagotarsonemus latus* eggs (mean ± SE) on bean plants during the 4- week experimental period

Treatment	Mean mite (egg stage)/leaf ± SE											
	Week 1*			Week 2			Week 3			Week 4		
	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank	Mean ± SE	Median	Rank
Untreated Control	13.6 ± 2.9 a	11	35.3	150 ± 23 a	183	94.0	214 ± 31 a	226	92.4	42.6 ± 10.2 a	21	90.4
Abamectin	19.8 ± 3.3 ab	19	51.4	0.0 ± 0.0 b	0	17.5	0.0 ± 0.0 b	0	12.5	1.0 ± 0.5 b	0	38.1
S	25.5 ± 4.5 ab	23	59.6	1.1 ± 0.5 b	0	25.9	11.0 ± 4.3 bc	4	38.2	2.8 ± 0.7 bc	2	56.9
G	14.7 ± 3.4 a	11	37.6	16.8 ± 3.2 cd	14	59.8	27.0 ± 5.8 acd	19	59.9	24.4 ± 6.6 ac	12	85.2
T	19.8 ± 2.4 ab	19	53.6	20.1 ± 3.4 ac	18	63.5	8.2 ± 1.9 bd	5	40.9	0.2 ± 0.2 b	0	26.7
T+S	36.4 ± 5.6 b	33	75.8	68.8 ± 15.8 ac	46	82.0	41.6 ± 18.7 bce	8	47.5	0.2 ± 0.2 b	0	28.2
G+S	23.4 ± 3.8 ab	20	57.8	2.0 ± 1.2 bd	0	28.3	102 ± 19 ae	71	79.6	4.2 ± 1.9 b	0	45.5
P-value	0.314			0.000			0.000			0.000		

Means followed by the same letters within columns are not significantly different ($P < 0.05$),

*****Mite densities before the spraying. T, tobacco; G, garlic; S, soft soap; T+S, tobacco+ soft soap; and G+S: garlic+ soft soap.

Discussion

About a hundred species of plants have been determined to have the potential to suppress phytophagous mites (Peng-ying et al., 2011). Among these, *N. tabacum* (Almansour & Akbar, 2013) and *A. sativum (*Attia et al., 2012) are well known for their acaricidal properties. Soft-bodied mites including the two-spotted spider mite are susceptible to soap applications as well (Cloyd, 2018).

However, to our knowledge, no study has tested the G, T, S, and their mixtures on *P. latus* eggs. Also, only a few studies have demonstrated their ovicidal effect on mites. Among them, the effect of aqueous extracts of *N. tabacum* on *T. urticae* eggs was researched by Almansour & Akbar (2013). The mortality rate of eggs ranged between 16.2-90.0% at 0-100%. They also showed that the LC_{50} value of tobacco extract against the egg stages of *T. urticae* was 0.25 g/100 ml. A recent study by Akyazi et al. (2018) declared that the LC_{50} values of S+T mixture, S, S+G mixture, G and T extracts were 12.5, 7.4, 5.2%, 3.8%, 3.4%, respectively for *T. urticae* eggs. The same solutions had the LC_{90} values ranging from 6.9 to 38.3%.

Also, to our knowledge, no prior study has also examined the effect of T+S and G+S mixtures on *P. latus* eggs. However, it should be aware that the potential effect in the mixture may be less (antagonistic) or greater (synergistic) than that of individual substances (Larsen et al., 2003). This finding is consistent with Akyazi et al. (2018) showing that the ovicidal efficiency of soft soap on *T. urticae* can be increased by the addition of the garlic extract. Until the current study, a still-unanswered question is whether these mixtures have the same effect on *P. latus.* In agreement with Akyazi et al. (2018), our result also showed that the toxicity of garlic extract on *P. latus* egg can be generally increased by the addition of soft soap. However, the tobacco showed a lower toxic effect when mixed with the soft soap.

To our knowledge, no study has yielded the ovicidal efficacy of abamectin on *P. latus* eggs. In the current study, abamectin has been reported as having extremely low toxicity against the egg stage of *P. latus*. However, we observed that young larvae died shortly after hatching (Figure 2). This effect has been evaluated as an indirect ovicidal effect by Ismail et al. (2007). Based on this, it can be concluded that abamectin has indirect ovicidal properties against *P. latus* under laboratory conditions. Previous studies have almost exclusively focused on *T. urticae*. For example, Kumar & Singh (2004) reported it did not have any ovicidal effect on *T. urticae* eggs. Consistent with the results of our study, Ismail et al. (2007) found the abamectin effectively lacked of ovicidal action and had a clear toxic effect against newly hatched larva of *T. urticae.*

Only a few studies have shown the adulticidal effects of tested solutions on plant pest mites. Also, previous studies have almost exclusively focused on *T. urticae.* Among examples of previous research, the adulticidal effect of aqueous extract of tobacco was investigated by Almansour & Akbar (2013). They found that the adult mortality rate was about 82% at the highest dose tested. LC_{50} values of the same treatment against the adult stage of *T. urticae* was 0.12 g/100 ml. Hincapie et al. (2008) declared that the mortality rate of water extract of garlic bulb on *T. urticae* adult females was less than 40%. They used the leaf disc-dipping method. The toxic effect of the garlic steam distillate on the adult females of *T. urticae* was investigated by Attia et al. (2012). They found that the LC_{50} and LC_{90} values of garlic were 7.49 and 13.5 mg/L, respectively. It was also reported that the garlic application caused significant mortality at low concentrations. Erdogan et al. (2012) also found that the efficiency of the ethanol extract of garlic against *T. urticae* adults on bean leaf disc at 12% was 39.5%. Nour El-Deen & Abdallah (2013) tested the adulticidal effect of garlic seed extract against *T. urticae* using the leaf dip method under laboratory conditions. They found that the LC_{50} value of garlic was 8.4 ml/L while the LC_{90} value was 1678.3 ml/L. Akyazi et al. (2018) investigated the effectiveness of the soft soap, garlic bulb, tobacco leaf extracts and their mixtures against the adult females of *T. urticae.* It was found that the garlic extract had the lowest toxic effect in the tested solutions. However, its mixtures with soft soap and tobacco extract gave higher toxic effects in combination. They also found that the LC_{50} and LC_{90} values of the same treatments ranged between 0.07-9.35 and 0.28-40.4%, respectively against *T. urticae* adults. Our results showed that the toxicity of garlic extract on *P. latus* adults can be increased by the addition of the soft soap only at its concentration of 10%. The adulticidal effect of soft soap did not change when mixed with the garlic extract in combinations at that concentration. The adulticidal effect of tobacco extract generally did not change when mixed with the soap in combinations. However, the soft soap showed a lower toxic effect when mixed with the tobacco extract at 5, 7.5 and 10%. A literature review revealed that no study to date has examined the efficacy of tested solutions on *P. latus* adults.

To our knowledge, no study has also yielded the adulticidal efficacy of abamectin against *P. latus* on bean leaf discs. In the current study, abamectin has been reported as having high toxicity against the adult stage of *P. latus*. After a 1 h exposure to abamectin, no mites were alive. Also, the effect of S at 7.5 and 10% and the G+S at 10% after 72 h of treatment had no significantly different adulticidal effect than abamectin. On this basis, we conclude that especially the S and the G+S mixture have the potential in the control *P. latus* adults.

According to the data on acaricidal efficacy on bean plants, both S and G+S treatments were able to keep the population below the ET for 1 week after each application. Although the mite density increased to 65.6 mites/leaf 1 week after the G+S application, a single G+S application at that time was able to reduce the mite density by 2.9 mites/leaf. However, the G applications were not able to maintain the mite density on bean plants below the ET. T and T+S had phytotoxic effects on bean plants. So, the main conclusion that can also be drawn is that especially S but also G+S are promising for controlling *P. latus* on the bean plants. Many studies have shown that some plant extracts have a toxic effect on *P. latus* (Ponte, 1996; Palaniswamy & Ragini, 2000; Al-Ani et al., 2008; Venzon et al., 2008; Mari et al., 2013; Hossain et al., 2013; Hasyim et al., 2017). There are also reports on the effectiveness of tobacco on different mite species (McIndoo, 1943; Madanlar et al., 2000; Almansour & Akbar, 2013; Akyazi et al., 2018). However, a literature review revealed that garlic has also acaricidal properties on some mite species (Hincapie et al., 2008; Attia et al., 2012; Nour El-Deen & Abdallah, 2013; Akyazi et al., 2018, 2019). Also, a series of studies such as Madanlar et al. (2000, 2002), Koçar et al. (2003), Güncan et al. (2006), Çobanoğlu & Alzoubi (2013), Skorupska (2013), Akyazi et al. (2018, 2019) have indicated soft soap toxicity to several mite species. However, to date, the influences of garlic, tobacco, and soap against *P. latus* on bean plants have been insufficiently examined. Most early studies, as well as current work, have almost exclusively focused on the influences of garlic, tobacco and soap against *P. latus* on different plants especially pepper. For example, the effects of tobacco extract on chili (Uraisakul, 2003; Mari et al., 2013), garlic extract on jute (Hossain et al., 2013) and soft soap on chili (David et al., 2009) against *P. latus* were researched. In a recent study by Akyazı et al. (2019), the effectiveness of soft soap, garlic and tobacco extracts in the control of *P. latus* was investigated on tea plants under field conditions. It is known that the different plant species have different leaf morphology. Also, leaf morphology may affect spray deposition (de Ruiter et al., 1990; Himel et al., 1990; Smith et al., 2000). So, before this study, a still-unanswered question was the potential of G, S and T against *P. latus* on a bean plant.

Also, as far as we know, no study to date has also examined the effect of their mixtures on the *P. latus* population on bean plants. As mentioned before, the interactions may result in either a weaker or stronger combined effect than the toxicity and mode of action of each compound (Larsen et al., 2003). To fill this literature gap, this paper also identified the effect of T+S and G+S mixtures on the *P. latus* population on bean plants. The result showed that the toxicity of garlic extract against *P. latus* on bean plants can be increased by the addition of soft soap. Given that the G applications were not able to maintain the mite density below the ET. The mite density was always above the ET despite the three times spraying during the experiment. In the S and G+S treated plants, the mite density exceeded the ET two times. Both of these treatments were able to keep the population below the ET for 1 week after each application on these dates. However, the effect of tobacco extract against *P. latus* on bean plants did not generally change when mixed with the soap in combinations. However, the effectiveness of soap alone was higher than its effectiveness in the mixture with tobacco. Also, the T+S at 20% had a phytotoxic effect on bean plants.

Our results cast a new light on the high efficacy of abamectin against *P. latus* on bean plants. A single abamectin application after the pre-infestation period maintained the mite population below the economic threshold during the experiment. Also, to our knowledge, no study has yielded the effect of abamectin on the *P. latus* population on bean plants. However, Goldsmith & James (2002), Montasser et al. (2011), and Singh & Singh (2013) reported that the abamectin gave satisfactory control against this mite on pepper plants.

We need to consider which ingredients are responsible for the toxic effects of the solutions tested in the current study. Consistent with Singh et al. (2001), the toxic effect that has been attributed to garlic are thought to be related to the high organosulfur substances content. Also, the water extracts of tobacco have been used for garden insect control as early as 1690. Consistent with previous studies (George et al., 2000; Ntalli & Menkissoglu-Spiroudi, 2011), the nicotine in tobacco can have a toxic effect. Soaps are prepared from animal fats or vegetable oils and sodium or potassium salts of fatty acids. So, it is assumed that they may kill mites in different ways (Cloyd, 2018). Abamectin has both acaricidal and insecticidal properties (Lewis et al., 2016). The toxic effect of abamectin may be attributed to its binding effect to the glutamate-gated chloride channels causing hyperpolarization of the nerve and muscles cell membrane (Montasser et al., 2011). The affected pest becomes paralyzed, stops feeding, and dies after a few days (El-Ashry & Ramadan, 2021).

In summary, this study has shown that especially soft soap (10%) but also garlic+soft soap mixture (20%) may be non-toxic alternative control options to current pesticides used to control *P. latus.* However, further work is required to determine the efficiency of these natural products under greenhouse and field conditions. Another area for future research would be to investigate the effect of the same solutions on natural enemies for pest mites.

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