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Orijinal araştırma (Original article)

Toxic and repellent effects of *Prunus laurocerasus* L. (Rosaceae) extracts against *Tetranychus urticae* Koch (Acari: Tetranychidae)[1]

Prunus laurocerasus L. (Rosaceae) ekstraktlarının *Tetranychus urticae* Koch (Acari: Tetranychidae)'ye karşı toksik ve repellent etkileri

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Summary

The toxic and repellent effects of leaves, flower and seed extracts of *Prunus laurocerasus* L. (Rosaceae) were investigated against *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions. Extracts at three different concentrations (1 %, 5 % (v/v), 10 % (w/v)) for ovicidal and repellent effects against eggs and adult females, respectively and at five different concentrations (1 %, 2.5 %, 5 %, 7.5 % (v/v), 10 % (w/v)) for the contact toxicity against female adults were evaluated. The results showed that seed extract was the most effective compared to flower and leaf extracts. It was found that at 5 % and 10 % concentrations of seed extract, the repellent effects were 92 % and 100 %, respectively within the first 72 hours. At 10% concentration of seed extract, the ovicidal and adulticidal effect were 96.56 % and 100 %, respectively. Moreover, further concentration response trials showed that LC_{50} and LC_{90} values for the contact toxicity of seed extract on eggs and adult females were LC_{50}=4.5 %, LC_{90}=9.4 % and LC_{50}=2.9 %, LC_{90}=9.1 %, respectively. The result indicated that seed extract of *P. laurocerasus* has good potential to be used to control *T. urticae*. However, the impact of these extracts on natural enemies of *T. urticae* should also be needed further studies.

Keywords: Acaricidal effect, cherry laurel, aqueous plant extracts, twospotted spider mite

Özet

Prunus laurocerasus L. (Rosaceae)'un yaprak, çiçek ve çekirdek ekstraktlarının *Tetranychus urticae* Koch (Acari: Tetranychidae)'ye karşı toksik ve repellent etkileri laboratuvar koşullarında araştırılmıştır. Ekstraktların yumurta ve ergin dişilere karşı sırası ile ovisidal ve repellent etkileri üç (% 1, % 5 (v/v), % 10 (w/v)), ergin dişilere karşı kontakt toksisiteleri ise beş (% 1, % 2.5, % 5, % 7.5 (v/v), % 10 (w/v)) farklı konsantrasyonda test edilmiştir. Sonuçlar çekirdek ekstraktının, çiçek ve yaprak ekstraktları ile kıyaslandığında en etkili ekstrakt olduğunu göstermiştir. Çekirdek ekstraktının 5 % ve 10 % konsantrasyonlarının ilk 72 saat içindeki repellent etkileri sırası ile % 92 ve % 100 olarak bulunmuştur. Çekirdek ekstraktının %10'luk konsatrasyonundaki ovisidal ve adultisidal etkileri ise sırası ile % 96.56 ve % 100 kadardır. Bununla birlikte, konsantrasyon tepki denemeleri çekirdek ekstraktının yumurta ve ergin dişi bireyler için LC_{50} ve LC_{90} değerlerinin sırası ile LC_{50}=4.5 %, LC_{90}=9.4 % ve LC_{50}=2.9 %, LC_{90}=9.1 %, olduğunu göstermiştir. Sonuçlar *P. laurocerasus*'un çekirdek ekstraktının *T. urticae* kontrolünde kullanılmak için iyi bir potansiyele sahip olduğunu işaret etmektedir. Fakat, ekstrakların *T. urticae*'nin doğal düşmanlarına etkilerinin belirlenmesi için çalışmaların devam etmesi gerekmektedir.

Anahtar sözcükler: Akarisidal etki, karayemiş, sulu bitki ekstraktları, iki noktalı kırmızı örümcek

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Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is a phytophagous mite that feeds on a wide variety of plant families worldwide. Its control is still largely based on the use of pesticides in view of their easy of application and rapidity of action. However, due to short life cycle, abundant progeny and arrhenotokous reproduction of red spider mites, they are able to develop resistance against to pesticides very rapidly. Also, the use of synthetic pesticides poses harmful effects on environment, human safety and nontarget organisms such as natural enemies, honeybees and wildlife. Therefore, there is need to develop nontoxic natural products that have potential to replace synthetic pesticides for controlling this pest.

Plant extracts are one of nonchemical control options. Some of them, Neem (Sundaram & Sloane, 1995; Martinez-Villar et al., 2005), *Tanacetum vulgare* L. and *Artemisia absinthium* L. (Asteraceae) (Chiasson et al., 2001), *Satoreja hortensis* L. (Lamiaceae) (Aslan et al., 2004), *Calotropis porcera* (Ait.) (Asclepiadecae), *Nerium oleander* L. (Apocynacae) (Islam et al., 2008), were reported as botanical acaricides (Derbalah et al., 2013). They contain secondary metabolites known from their repellent, antifeedant, ovicidal and killing action against arthropod pests (Smith, 1989; Tomczyk & Suszko, 2011).

Many investigations have been performed on the ovicidal, toxic and repellency effects of some plant extracts on *T. urticae.* Yanar et al. (2011a) investigated the ovicidal activity of methanol extracts of nine plant species against *T. urticae* under laboratory conditions. They found that some of these plant extracts have a potential for ovicidal activity on *T. urticae* eggs. The extracts of different parts of 12 plant species were also evaluated for their acaricidal activity on *T. urticae* by Yanar et al. (2011b). Erdoğan et al. (2012) determined efficacy of pesticides extracted from *Allium sativum* L. (Alliaceae), *Rhododendron luteum* S. (Ericaceae), *Helichrysum arenarium* L. (Asteraceae), *Veratrum album* L. (Liliaceae) and *Tanacetum parthenium* L. (Asteraceae)] against this mite. Ghaderi et al. (2013) investigated the ovicidal activities of methanolic extracts of *Anisosciadium orientale* DC. (Apiaceae), *Scaligeria meifolia* Boiss. (Apiaceae), *Trigonella elliptica* Boiss. (Leguminosae) and *Ptelea viscosa* L. (Sapindaceae) against *T. urticae* under laboratory conditions. Derbalah et al. (2013) found that *Nigella sativa* Linn. (Ranunculaceae) (seeds) and *Artemisia cina* L. (leaf) (Asteraceae) extracts were toxic to eggs of *T. urticae* with LC_{50} values of 1850.92 and 2740.42 ppm. Additionally, aqueous extracts from *Sinapsis alba* L. (Brassicaceae), *A. sativum*, *Urtica dioica* L. (Urticaceae) (Dabrowski & Seredynska, 2007), water extract of *Artemisia judaica* L. (Asteraceae) (El-Sharabasy, 2010), aqueous solvent of *Nicotiana tabacum* L. (Solanaceae) and *Pegunum harmala* L. (Nitrariaceae) (Almansour & Akbar, 2013) were investigated for toxic effect on *T. utricae*. It found that all of them had toxic effects against *T. urticae* adult females too. The lethal effect of dichloromethane and ethanol extracts of *Melia azedarach* L. (Meliaceae), *P. harmala*, *N. sativa* and *Trigonella foenumgraecum* L. (Fabaceae) seeds was investigated on adult *T. urticae* under laboratory conditions by Elkertati et al. (2013).

At the same time, investigations show that several *Artemisia* spp. (Asteraceae) (Saber, 2004; (Dabrowski & Seredynska, 2007; El-Sharabasy, 2010), some *Capsicum* spp. (Solanaceae) (Antonious et al., 2006), wild tomato (Antonious & Snyder, 2006), *U. dioica* (Dabrowski & Seredynska, 2007), *Datura stramonium* L. (Solanaceae) (Kumral et al., 2010), *Mentha longifolia* L. (Lamiaceae), *Salvia officinalis* L. (Lamiaceae) and *Myrtus communis* L. (Myrtaceae) (Motazedian et al., 2012) have potential repellent action against *T. urticae* too.

Prunus laurocerasus is an evergreen species of cherry known insecticidal activity on arthropods (Rattan & Sharma, 2011). Thomas (2004) defined that the leaves of *P. laurocerasus* could be used as a pesticide. Furthermore, the toxicity effects of *P. laurocerasus* on *Plutella xylostella* L. (Insecta: Lepidoptera: Plutellidae) was determined as 50 % within seven days by Ertürk et al. (2004). But, there is no further research about effect of *P. laurocerasus* extracts on the phytophagous mites. The aim of this study was to identify the repellent effect of leaves, flower and seed extracts of *P. laurocerasus* against adults of *T. urticae* as well as the toxicity against eggs and adults of *T. urticae* under laboratory conditions.

Material and Method

Plant and mite rearing

Pinto bean plants, *Phaseoulus vulgaris* L. (Fabaceae), were used as a host plant for *T. urticae* and served as the test substrate. Plants were cultivated in a mixture of vermiculite and soil in plastic pots (26 x 14 cm) in the laboratory. Seeds were planted every two days in order to provide plants with primary leaves two or three days old for leaf discs. To reduce problems of leaf disc deterioration, young but fully expanded leaves were selected.

Tetranychus urticae was obtained from a stock colony maintained in the laboratory (Plant Protection Department, Ordu University, Ordu, Turkey) at 25 ± 2 °C and 70-80% relative humidity. The *T. urticae* colony was reared continuously on pinto bean plants by laying cut foliage containing abundant *T. urticae* on new plants at the 3-6 leaf stage. As the cut foliage dried, *T. urticae* moved to the fresh plants.

Plants and preparation of extracts

Leaves (pre-flowering) flowers and seeds of *P. laurocerasus* were collected during the spring and summer of 2011 and 2012.

Each plant material was dried under shade and powdered using a grinder. For extraction, powdered plant materials (50 g) were placed in an erlenmeyer flask, distilled water (500 ml) was added, and then it was shaken for 48 h in a horizontal shaker at 125 rpm in room temperature (25±2 ºC). The extracts were separated using fine muslin cloth and then filtered through Whatman No. 1 filter paper. This was called stock solution (% 10 w/v). The stock solution was dissolved in distilled water to obtain solutiones at 1 %, 2.5 %, 5 % and 7.5 % (v/v).

Experimental design for repellency

Extracts at three different concentrations (1 %, 5 % and 10 %) were evaluated for repellency. Experiments were performed using bean leaf discs 3 cm in diameter. Half of every disc was immersed for 5 seconds in each concentration of extract and after drying at room temperature; the other half was immersed for 5 seconds in distilled water. The treated leaf discs were placed underside up on water-soaked cotton in a plastic tray (15x11 cm). The wet cotton prevented escape and maintained leaf freshness. Adult female mites were released in the center of each disc to see where the red spider mites sattle down. The results were controlled after 2, 6, 24, 48, 72 and 96 hours by counting the number of adults present on each half of the leaf discs. Each treatment was replicated five times each with 10 adult mites.

Experimental design for ovicidal efficiency

Extracts at three different concentrations (1 %, 5 % and 10 %) were evaluated for ovicidal efficiency. Experiments were performed using 3 cm diameter bean leaf discs. Ten adult females of *T. urticae* were introduced on bean leaf discs for oviposition and kept overnight. After 24 hours the introduced mites were removed with the help of a fine brush. The eggs laid on leaf discs were counted. The leaf discs containing 25 eggs were used for assesment. For this purpose, some eggs were removed from discs to have 25 eggs on every one. The leaf discs were dipped for 5 seconds in extracts and allowed to dry at room temperature. Once dried, the treated leaf discs were placed underside up on water-soaked cotton on a plastic tray (15 x 11cm). The egg number on each disc was counted again. The discs that did not have 25 eggs were removed from the trial. The control discs were treated with distilled water. There were five replications for each concentration of each plant extract. Hatchability was determined for a period of 10 days after treatment. Those eggs that did not hatch after this period were regarded as non-viable.

Experimental design for contact toxicity

Tests were conducted using the standard slide-dip method to compare the acaricidal toxicity of the *P. laurocerasus* extracts on *T. urticae* engorged female adults in the laboratory (FAO, 2004). Extracts at five different concentrations (1 %, 2.5 %, 5 %, 7.5 % and 10 %) were evaluated for contact toxicity. The adult female mites, which were uniform in size, color, and brightness, were selected using a zero size brush. Double-sided adhesives were cut into 2 cm-long pieces and stuck to one end of the slides. The dorsums of 10 mites were fastened onto double stick adhesive tape. Each treatment consisted of 5 slides. Each slide was checked under a microscope. Inactive or injured mites were removed. The slides were then dipped for 5 seconds in the extracts. The control slides were treated with distilled water. The slides were shaken gently to remove excess solution around the body of mites after dipping (Kovach & Gorsuch, 1986; Wang et al., 2012). Furthermore, any droplet of extract solutions remaining on the slides after dipping was removed with blotting paper. The mite number on each slide was counted again. The slides that did not have 10 adult females after dipping were removed from the trial. Mortality counts were made 1, 24, 48, 72 hours after the application. Mites which failed to respond with leg movements after being proded lightly with a fine brush were considered to be dead.

Statistical analysis

The repellent effect was calculated by using the following formula (% Repellent effect index) developed by Obeng-Ofori et al. (1997).

RI (%) = [NC-NT/(NC+NT)]×100

NC = The number of mites on control diet.

NT = The number of mites on treated diet.

RI = Repellent effect index

Datas were corrected for mortality in the controls using Abbott's formula (Abbott, 1925). The Kolmogorov-Smirnov and Levene's tests were applied to test normality and homogeneity of variance, respectively. Contact and repellent effects were analysed by three-way repeated measures ANOVA (between-subjects factors: dose and extract; within-subjects factors: time). Ovicidal effects were analysed by two-way ANOVA. The means compared with Tukey post-hoc test and the results were displayed in the form of letters. Variables were displayed as mean with 95% confidence interval (CI). The statistical analysis was performed using Minitab SPSS 23 and Minitab 17 statistical package program.

Dose response models, allowing for control were fitted using the SAS Probit procedure. Lethal concentrations (LC_{50} and LC_{90}) were estimated, along with 95% confidence. The alpha level was set at 5%.

Results and Discussion

The repellect effect of *Prunus laurocerasus* on *Tetranychus urticae* adult females

According to the analysis of variance, two-way interaction between dose and exposure time (Fig. 4) and main effect of extract (Fig. 5) were significant ($P< 0.001$), while three-way interaction between dose, exposure time and extract was not significant. The repellent effects of leaf, flower and seed extracts of *P. laurocerasus* on adult females of *T. urticae* were given in Figs 1-5. As shown in Fig. 1, at the lowest concentration (1%), all extracts had repellency rate ranging between 64 %- 88 % within the first 48 h and 28 % - 39 % after this time. At a concentration of 5 % (Fig. 2), leaf and flower extracts had repellency rate ranging between 84 %- 88 % and 76 %-88 % within the first 24 h, respectively and 64%- 68% and 60%-64% at 48 h-72 h, respectively. But, repellency of leaf and flower extracts was 44% at 96 h. Seed extract had a repellency rates of 92%- 100% within the first 72 h and 68% at 96 h. All extracts had high repellent effect at concentrations of 10% (Fig 3) especially within the first 72 h, and repellency rate of leaf, flower and seed extracts ranged between 68% and 100%. After this time, although repellency of leaf and flower extracts was 64% and 44%, respectively, repellency rate of seed extract was 84% at 96h. In agreement with Zhang et al., (2013), as processing time increased, the repellent activity gradually decreased. Additionaly, an increase in extract concentration could probably increase its repellent activity (Fig. 4), as was observed by Momen et al., (1997). When all data were compared over the repellency rates, seed extract was the most effective of all extracts (Figs 5-6).

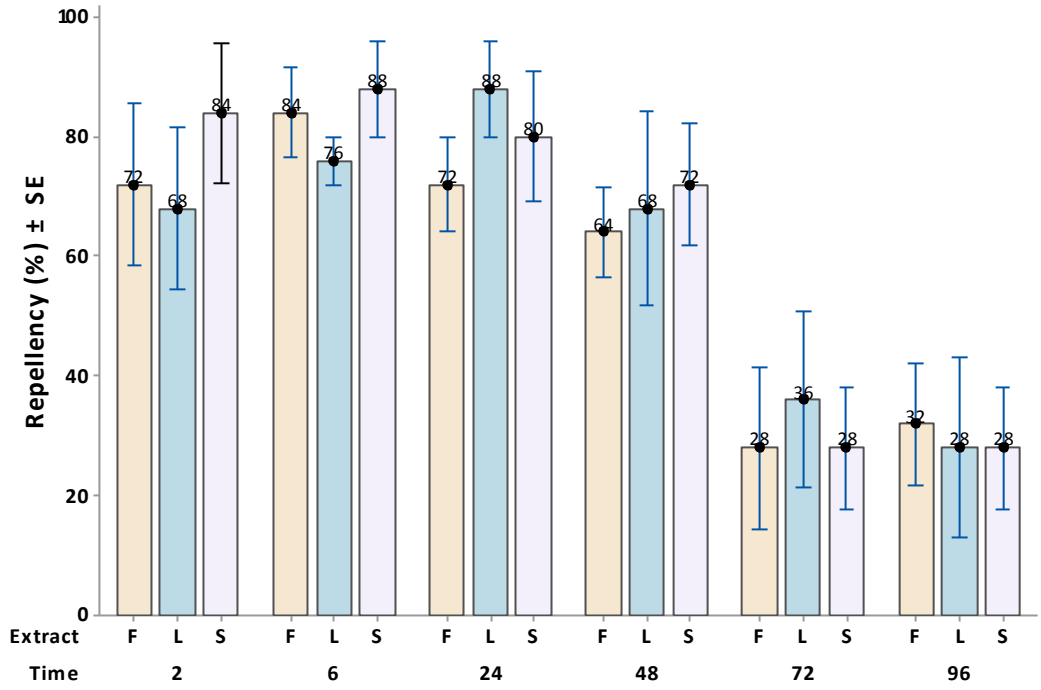

Figure 1. The repellent effects (Mean ±SE) of leaf (L), flower (F) and seed (S) extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females at 1% concentration at different counting times (hour).

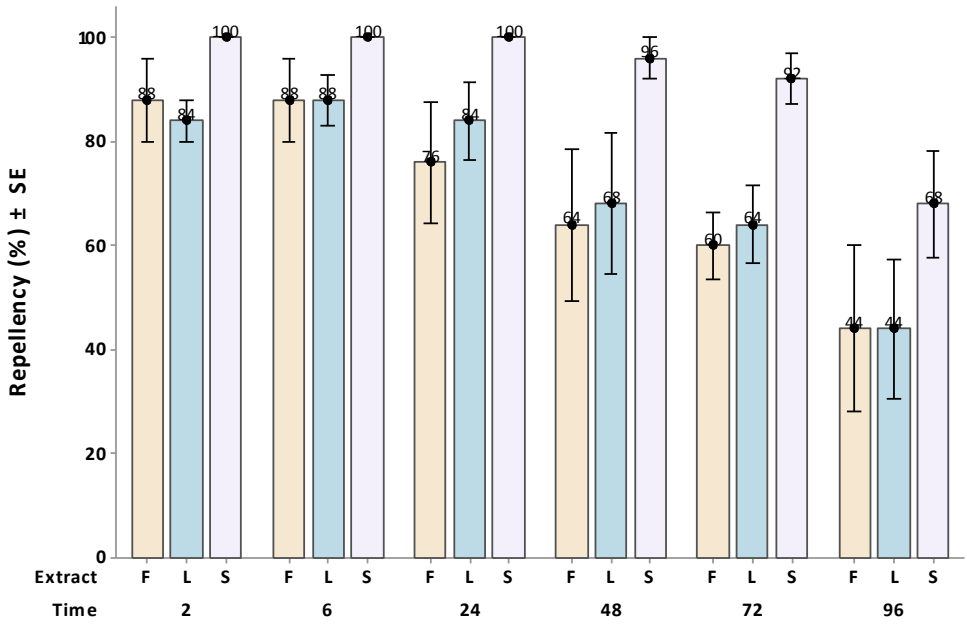

Figure 2. The repellent effects (Mean ±SE) of leaf (L), flower (F) and seed (S) extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females at 5% concentration at different counting times (hour).

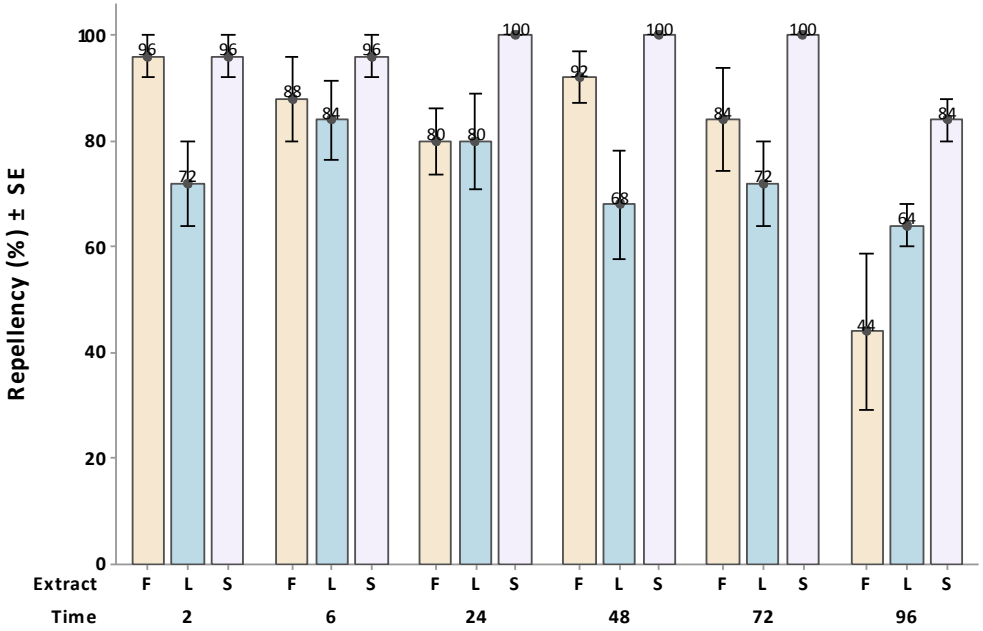

Figure 3. The repellent effects (Mean ±SE) of leaf (L), flower (F) and seed (S) extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females at 10 % concentration at different counting times (hour).

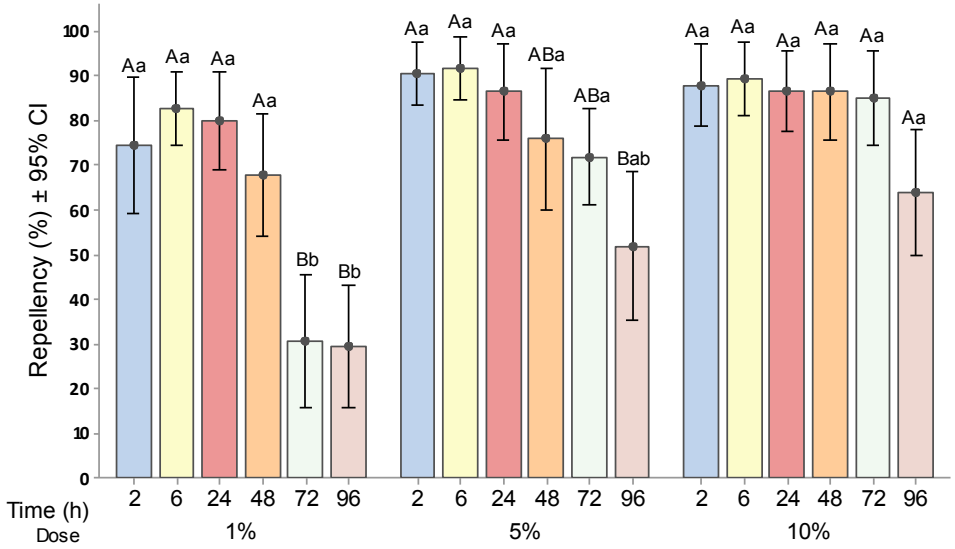

Figure 4. The repellent effects (Mean ± 95 % CI) of the extracts of different parts of *Prunus laurocerasus* against *Tetranychus urticae* adult females at different counting times and concentrations. Different upper letters represent statistically differences between times in the same dose and different lower letters represent statistically significant differences between doses in the same time according to Tukey's test (P<0.05) (CI: Confidence Interval).

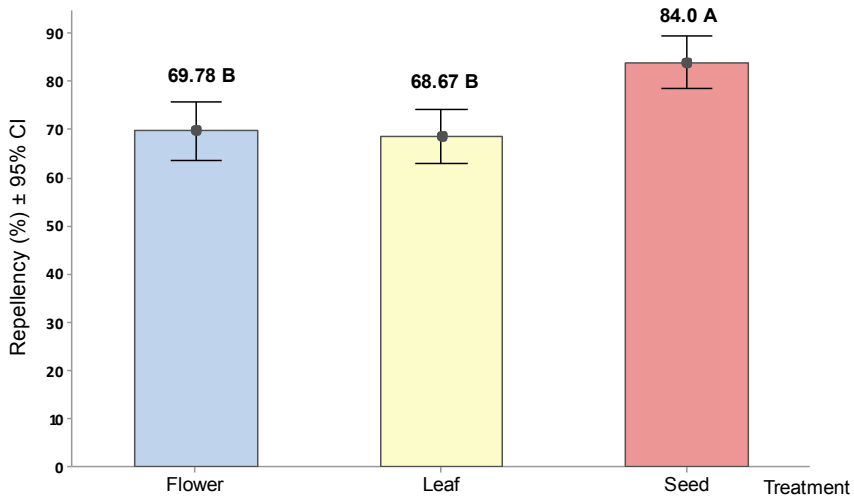

Figure 5. The repellent effects (Mean ± 95 % CI) of leaf, flower and seed extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females. Different upper letters represent statistically differences between treatment according to Tukey's test (P<0.05) (CI: Confidence Interval).

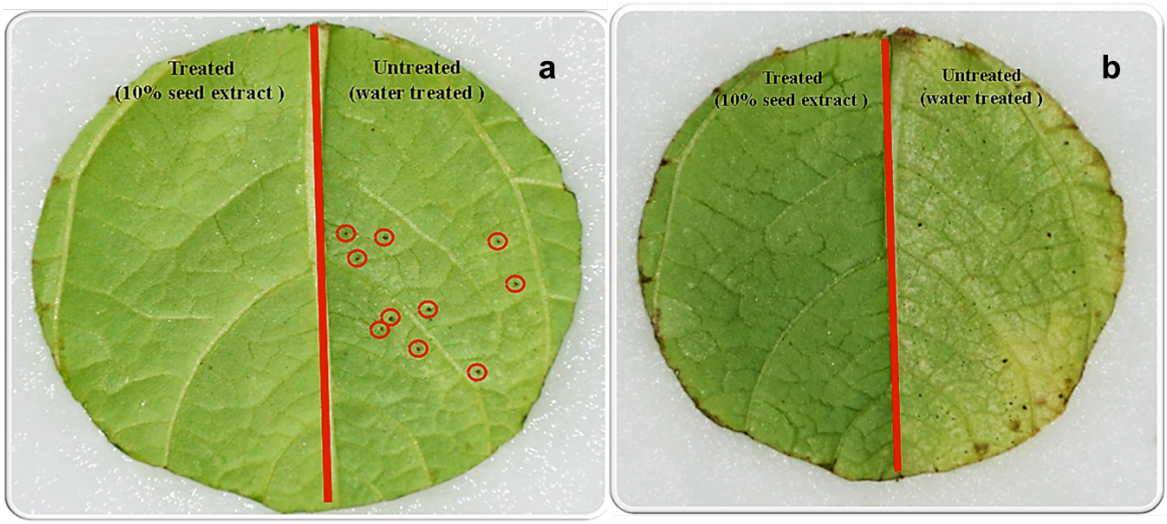

Figure 6. The repellent effect of a 10% *Prunus laurocerasus* seed extract on adult females of *Tetranychus urticae* (shown in circles) after 72 hours (a) (There is no mites on half of the leaf disc treated with a 10% seed extract), and feeding damage only on the untreated half of the leaf disc after 6 days (b).

High repellent effect of *P. laurocerasus* may be explained by the cyanide content of the plant based on EMEA (2000) and Dursun (2010) giving the content of this plant. According to Selmar (2010), some repellent effects were detected for a few intact cyanogenic glucosides; in most cases, the repellent effect for herbivores was due to the HCN liberated from cyanogenic glucosides. Moreover, carbonyl compounds produced during cyanogenesis, e.g. benzaldehyde, were also deterrents. Consequently, the repellent effect of cyanogenic plant is attributed mainly to the process of cynogenesis and formation of the decomposition products of cyanogenic glucosides (Selmar, 2010).

Although there is no research for repellent effect of *P. laurocerasus* extracts on phytophagous mites, some researchers investigated repellent efficacy of other plant extracts. Saber (2004) found that *Artemisia monosperma* Del. (Asteraceae) had repellency effects against females of *T. urticae*. The investigation of Antonious et al. (2006) suggests that methanolic extracts from accessions PI-596057

(*Capsicum baccatum* L.), PI-195299 (*C. annuum* L.), and Grif- 9270 (*C. annuum*) (Solanaceae) may have a great potential for repelling *T. urticae*. Antonious & Snyder (2006) found that the hexane leaf extracts of the *Lycopersicon hirsutum f. glabratum* Mueller, C.H. (Solanaceae) accessions (PI-251304, PI-134417, PI-134418, and PI-126449) exhibited strong repellency. Dabrowski & Seredynska (2007) showed the repellent action by water extract from *A. sativum*, *U. dioica*. and *S. alba* for *T. urticae*. Kumral et al. (2010) found repellent activities of the ethanol extracts obtained from both leaf and seed in the *D. stramonium* against adult *T. urticae*. El-Sharabasy (2010) evaluated the potential of crude extracts of *A. judaica* L. for repellent effect against adult females and immature stage of *T. urticae.* They found ethanolic leaf extraction was more effective as repellent effect against adult females and immature stage of *T. urticae*, followed by acetone, petroleum ether and aqueous extraction. Motazedian et al. (2012) found that *M. longifolia*, *S. officinalis* and *M. communis* essential oils have repellency effect against *T. urticae*.

The results also showed that at concentrations of 1%, the ovicidal effects of leaf and flower extracts were 12.59 % and 13.77 %, respectively (Table 1). The ovicidal effect was 24.37 % at the same concentration of seed extract. At concentrations of 5 % of the leaf, flower and seed extracts, the ovicidal effect was 24.41 %, 28.01 % and 40.37 %, respectively. Increasing the extract concentration by 10 % in leaf, flower and seed extracts increased their ovicidal effects to 55.57 %, 79.22 % and 96.56 %, respectively. We have demonstrated that the significantly high levels of egg mortality (96.56%) were caused by the seed extract of *P. laurocerasus* at 10 % concentration. During a 10 day observation period after treatment, the unhatched eggs treated with seed extract (5 % and especially 10 %) lose their original shape, then became orange in color (Fig. 7).

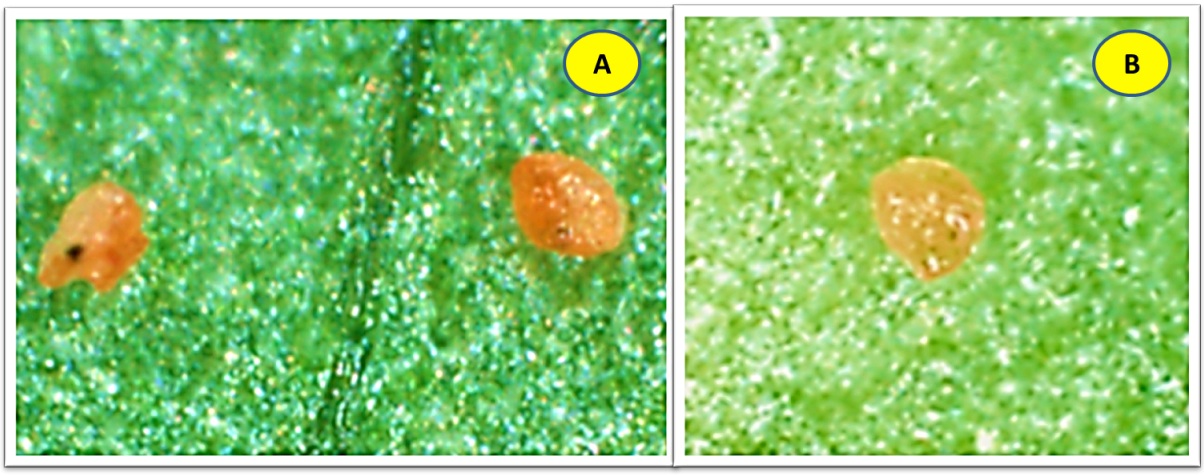

Figure 7. Ten days after treatment, unhatched *Tetranychus urticae* eggs treated with seed extracts at 10% (A) and %5 (B) concentrations.

The tests also showed that the seed extract is the most potent giving a LC_{50} and a LC_{90} of 4.5 % and 9.4 %, respectively followed by the flower extract with a LC_{50} and a LC_{90} of 6.6 % and 12.2 %, respectively and finally by the leaf extract with a LC_{50} and a LC_{90} of 8.9 % and 17.3 %, respectively against *T. urticae* eggs (Table 3).

Although there is no research about ovicidal effect of *P. laurocerasus* extracts on mites, Gençsoylu (2007) showed that leaf and root extracts from *Asphodelus aestivus* Brot. (Asphodelaceae) inhibit the egg hatching of *Tetranychus cinnabarinus* Boisduval (Prostigmata: Tetranychidae). Methanol extracts of nine plant species were evaluated for their ovicidal activity against *T. urticae* in a bioassay under laboratory conditions by Yanar et al. (2011b). They found that leaf and flower bud extracts of *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) exhibited 63.26 % and 43.46 % mortality, respectively on eggs of two-spotted spider mite at 10 % extract concentration. *Xanthium strumarium* L. (Asteraceae) fruit and leaf extract caused 59.64 % and 57.45 % egg mortality, respectively in the same study. Also, Ghaderi et al. (2013) observed that the ovicidal activity of methanolic extracts of *S. meifolia*, *A. orientale*, *T. elliptica* and *P. viscosa* against *T. urticae* eggs were 45.84 %, 41.40 %, 40.11 % and 37.66 %, respectively.

Table 1. Ovicidal effect of different concentration of leaf, flower and seed extracts of *Prunus lausocerasus* against *Tetranychus urticae* eggs after a 10 days

Treatment	Dose (%)	Mean Ovicidal Effect (%)		±SE
Flower	1	13.77	aBC	8.29
	5	28.01	aB	5.49
	10	79.22	abA	2.71
	Control	7.20	aC	0.09
Leaf	1	12.59	aBC	6.55
	5	24.41	aB	6.07
	10	55.57	bA	9.05
	Control	7.20	aC	0.09
Seed	1	24.37	aBC	7.09
	5	40.37	aB	4.43
	10	96.56	aA	0.86
	Control	7.20	aC	0.09

Different lower letters represent statistically differences between extracts in the same dose and different upper letters represent statistically differences between doses in the same extract according to Tukey's test ($P<0.05$) (SE: Standard Error)

In agreement with Salman et al. (2013), it was observed that the percentage effect values of the extracts on *T. urticae* adults increased depending on the increases of counting time and concentration (Table 2). The significantly highest mortality rate (100%) occured at the 10 % concentration of the seed extract after 72 hours in all extracts. The effect of the flower and leaf extract were 72.50 % and 37.50 %, respectively at same dose and time. As a result, it can be said that the especially seed and flower extracts were seen to be more effective than the leaf extracts against *T. urticae* adult females.

LC_{50} values at 1, 24, 48 and 72 h exposure of to the seed extract were lower than flower and leaf extract (Table 3). At the same time, LC_{90} values at 1, 24, 48 and 72 h exposure to the seed extract were lower than flower and leaf extract too (Table 3). The datas obtained showed that the seed extracts of *P. laurocerasus* were most toxic against adult females.

In parallel with the result of our study, some aqueous extracts have acaricidal effect on *T. urticae.* Aqueous extracts from three plant species (*S. alba*, *A. sativum*, *U. dioica*) were tested against *T. urticae* by Dabrowski & Seredynska (2007). The high and significant mortality of *T. urticae* females were observed on leaves treated by *U. dioica.* After 6 days, mortality increased to 87 % and 96 %, at the 0.3 % and 0.5 % concentrations respectively. *Alium sativum* extracts caused only 48-57 % mite mortality and only between 34-41 % by *S. alba* extracts. El-Sharabasy (2010) evaluated the potential of water extract of *A. judaica* L. for toxic effect against adult females of *T. urticae.* They found that LC_{50} value was 103.2 gm/ml, after 72 hours. Almansour & Akbar (2013) investigated the toxic effect of aqueous solvent of *N. tabacum* and *P. harmala* on larval, nymphal and adult stages of *T. utricae*. The mortality rate varied among 20.1 - 88.2, 18.2 - 83.3, 17.7 - 82.3 % in *N. tabacum* while, in *P. harmala* it varied among 12.7 - 77, 13.7 - 75.8, 11.8 - 73.5 % in larval, nymphal and adult stages, respectively.

Table 2. The contact toxicity of different concentrations of leaf, flower and seed extracts of *Prunus laurocerasus* on *Tetranychus urticae* adult females at different counting times

		Contact Toxicity								
Time (h)	Dose (%)	Flower			Leaf			Seed		
		Mean (%)		±SE	Mean (%)		±SE	Mean (%)		±SE
1	0	0.00	Aa ii	0.00	0.00	Aa ii	0.00	0.00	Ba ii	0.00
	1	0.00	Aa ii	0.00	0.00	Aa iii	0.00	0.00	Ba ii	0.00
	2.5	2.00	Aa iii	2.00	4.00	Aa ii	2.45	2.00	Ba ii	2.00
	5	2.00	Aa iii	2.00	4.00	Aa ii	2.45	2.00	Ba iii	2.00
	7.5	6.00	Aa iii	4.00	12.00	Aa ii	5.83	24.00	Ba iii	6.78
	10	16.00	Ab iii	5.10	12.00	Ab ii	2.45	82.00	Aa i	5.83
24	0	2.00	Ba ii	2.00	2.00	Aa ii	2.00	2.00	Ca ii	2.00
	1	4.00	Ba ii	2.45	2.22	Aa iii	2.22	8.00	BCa i,ii	2.00
	2.5	8.00	Aba ii,iii	3.74	4.00	Aa ii	2.45	8.00	BCa ii	3.74
	5	10.22	Aba ii,iii	3.17	8.22	Aa ii	2.07	12.22	BCa ii,iii	1.96
	7.5	14.22	Aba iii	5.06	18.22	Aa i,ii	3.63	30.20	Ba ii,iii	11.3
	10	28.22	Ab ii,iii	5.66	18.44	Ab ii	5.89	87.78	Aa i,ii	1.96
48	0	8.00	Ba i,ii	2.00	8.00	Aa i,ii	2.00	8.00	Ca i,ii	2.00
	1	12.67	Aba ii	5.89	6.22	Aa ii	4.06	17.78	Ca ii	5.67
	2.5	17.33	ABab ii	2.57	2.22	Ab ii	2.22	23.78	BCa i	3.84
	5	17.33	Aba ii	2.57	4.44	Aa ii	2.72	16.89	Ca ii	5.78
	7.5	30.44	ABab ii	4.12	15.11	Ab ii	4.30	43.56	Ba ii	6.15
	10	34.67	Ab ii	3.76	24.00	Ab i,ii	4.24	93.56	Aa i,ii	2.64
72	0	18.00	Ba i	5.83	18.00	Aai	5.83	18.00	Da i	5.83
	1	28.61	Bab i	6.33	19.72	Abi	4.90	43.06	Ca i	3.78
	2.5	37.50	Ba i	6.43	21.39	Aa i	3.50	37.22	CDa i	7.11
	5	68.33	Aa i	3.89	23.61	Ab i	5.52	69.40	Ba i	10.10
	7.5	68.06	Aa i	2.56	30.6	Ab i	12.8	69.44	Ba i	5.27
	10	72.50	Ab i	3.30	37.50	Ac i	6.43	100.00	Aa i	0.00

Different upper letters represent statistically differences between doses in the same time and the same extract, different lower letters represent statistically differences between extracts in the same time and the same dose and different Roman Numerals (i, ii, iii) represent statistically differences between times in the same dose and the same extract according to Tukey's test ($p<0.05$)
Three-way interaction between dose: extract: time is significant ($p<0.05$) (SE: Standard Error)

Table 3. LC_{50} and LC_{90} values (with 95 % CI) of flower, leaf and seed extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females after 1, 24, 48, 72 h and *Tetranychus urticae* eggs after a 10 days

Activity	Treatment	LC_{50} (%)	95% CI for LC_{50}	LC_{90} (%)	95% CI for LC_{90}	Slope ± SE	χ^2
Ovicidal	Flower	6.6	(0.059-0.073)	12.2	(0.110-0.140)	22.86 ± 2.55	80.08
	Leaf	8.9	(0.073-0.117)	17.3	(0.137-0.263)	15.27 ± 3.18	23.01
	Seed	4.5	(0.036-0.054)	9.4	(0.081-0.114)	26.47 ± 3.47	58.08
Contact	Treatment	LC_{50} (%)	95% CI for LC_{50}	LC_{90} (%)	95% CI for LC_{90}	Slope ± SE	χ^2
1 h	Flower	15.7	(0.125-0.277)	22.8	(0.170-0.450)	18.03 ± 5.45	10.95
	Leaf	18.7	(0.136-0.460)	29.2	(0.199-0.804)	12.17 ± 4.31	7.96
	Seed	8.5	(0.074-0.100)	11.1	(0.097-0.149)	50.36 ±13.14	14.70
24 h	Flower	17.9	(0.128-0.503)	30.8	(0.203-0.990)	9.97 ± 3.75	7.07
	Leaf	14.9	(0.119-0.268)	22.7	(0.168-0.467)	16.53 ± 5.16	10.25
	Seed	7.8	(0.071-0.086)	11.8	(0.106-0.142)	31.88 ± 5.40	34.85
48 h	Flower	14.5	(0.104-0.444)	29.9	(0.193-1.126)	8.35 ± 3.30	6.38
	Leaf	13.9	(-)	20.9	(-)	18.13 ± 9.86	3.38
	Seed	6.7	(0.058-0.076)	11.9	(0.104-0.146)	24.58 ± 3.93	39.09
72 h	Flower	4.0	(0.018-0.055)	13.9	(0.109-0.214)	12.95 ± 3.03	18.33
	Leaf	12.8	(0.093-0.406)	26.5	(0.172-1.114)	9.37 ± 3.86	5.89
	Seed	2.9	(0.014-0.039)	9.1	(0.077-0.117)	20.69 ± 3.61	32.90

SE: Standard Error CI: Confidence Interval h: Hour

In the present study, it was determined over 50 % ovicidal activity at 10% concentrations of leaf, flower and seed extracts. Additionaly, at the same concentrations of the leaf, flower and seed extracts, the toxic effects on *T. urticae* adult females were 37.50 %, 72.50 % and 100%, respectively. The high acaricidal effect of *P. laurocerasus* may be explained by high concentrations of cyanogenic glycosides such as amygdalin, prunasin based on Poulton & Li (1994). EMEA (2000), Dursun (2010) and Wishhart & Media (2014) giving informations about the content of this plant. Acording to EMEA (2000) the leaves of *P. laurocerasus* contain cyanogenic glycosides. Poulton & Li (1994) indicated that *Prunus* seeds contain the cyanogenic diglucoside (R)-amygdalin. Whereas there is no research about cyanogenic glycosides content of *P. laurocerasus* flowers, Wishhart & Media (2014) reported that all parts of the plant contain hydrogen cyanide, also known as prussic acid. The toxicity of the plant is attributed to hydrocyanic acid, a poison that gives almonds their characteristic flavor, liberated from cyanogenic glycosides (EMEA, 2000; Ubalua, 2010). Acording to Robertson (2014), this toxin is found mainly in the leaves and seeds. When the leaves and seeds of the plant distilled with water, as it was done in the present study, yield a distillate of hydrocyanic acid (Felter & Lloyd, 1898).

Seed extract had the highest contact and ovicidal effect in the extracts. We believe that the differences among the effects of the leaf, flower and seed extracts of *P. laurocerasus* may be attributed to the difference in their chemical composition. Our hypothesis is supported by Dursun (2010) who reported that while mean amygdalin, HCN contents in the leaves of *P. laurocerasus* were 0.28, 0.32 g/kg respectively, mean amygdalin and HCN contents in its seeds were 94.35 and 5.64g/kg, respectively.

When all data were compared, it can be concluded that the ovicidal effect of seed extract and the contact toxicity of flower and especially seed extracts of *P. laurocerasus* at 10 % concentration has potential to be used in the control of two-spotted spider mite eggs and adults. Additionally, the repellent effect of seed extracts of *P. laurocerasus* was found to be promising for practical application.

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