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Original article (Orijinal araştırma)

Toxicity and repellency of sage (*Salvia officinalis* L.) (Lamiaceae) and rosemary (*Rosmarinus officinalis* L.) (Lamiaceae) extracts to *Neoseiulus californicus* (McGregor, 1954) and *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae)

Adaçayı (*Salvia officinalis* L.) (Lamiaceae) ve biberiye (*Rosmarinus officinalis* L.) (Lamiaceae) bitki ekstraktlarının *Neoseiulus californicus* (McGregor, 1954) ve *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae)'e karşı toksik ve repellent etkileri

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Abstract

In this study the toxicity and repellency of sage and rosemary extracts to two important predator mites were examined. In the toxicity experiments, leaf disc-spray tower method was used with 1, 3, 6 and 12% extract concentrations applied to egg, immature stage and adult stages of the predatory mites. All concentrations of plant extracts and control experiments were conducted with ten replicates with ten individuals in each replicate. The experiments include one control and four concentrations of each extract. For repellency experiments, 0.1, 1, 5 and 10 ml/L concentrations of plant extracts were used. In the toxicity experiments, the greatest effects of the sage and rosemary extracts were 33.3% and 33.3% toxicity to *Neoseiulus californicus* (McGregor, 1954) adults and 62.5% and 38.4% to *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae) adults, respectively. In the repellency experiments, the greatest effects of the sage and rosemary extracts were 43.7% and 77.7% repellency to *N. californicus* adults and 50.1% and 88.8% for *P. persimilis* adults, respectively. This study indicates the potential to use of sage extract in integrated pest management programs incorporating *N. californicus* and rosemary against *P. persimilis*.

Keywords: *Neoseiulus californicus*, *Phytoseiulus persimilis*, plant extract, repellency, toxicity

Öz

Bu çalışmada, adaçayı ve biberiye ekstraktlarının iki önemli predatör akara toksisitesi ve repellent etkisi incelenmiştir. Toksisite denemelerinde, predatör akarların yumurta, nimf ve ergin dönemlerine %1, 3, 6 ve 12 konsantrasyonları yaprak disk-ilaçlama kulesi yöntemi kullanılarak uygulanmıştır. Tüm ekstrakt ve kontrol denemelerinde konsantrasyonlar; on tekerrür ve her tekerrürde on birey olacak şekilde gerçekleştirilmiştir. Denemelerde bir kontrol ve dört konsantrasyon kullanılmıştır. Repellent etki denemeleri için, bitki ekstraktlarının 0.1, 1, 5 ve 10 ml/L konsantrasyonları kullanılmıştır. Toksik etki çalışmalarında, adaçayı ve biberiye ekstraktlarının en yüksek etkileri sırasıyla, *Neoseiulus californicus* (McGregor, 1954) erginlerinde %33.3 ve %33.3; *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae) erginlerinde ise %62.5 ve %38.4 olarak bulunmuştur. Repellent etki çalışmalarında ise adaçayı ve biberiye ekstraktlarının en büyük etkileri sırasıyla, *N. californicus* erginlerinde %43.7 ve %77.7; *P. persimilis* erginlerinde ise %50.1 ve %88.8 oranlarında bulunmuştur. Bu çalışma, entegre zararlı yönetim programlarında adaçayı ekstraktının *N. californicus*, biberiye ekstraktının ise *P. persimilis*'e dayalı potansiyel kullanım olanaklarının olduğunu bildirmektedir.

Anahtar sözcükler: *Neoseiulus californicus*, *Phytoseiulus persimilis*, bitki ekstraktı, repellent etki, toksik etki

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Introduction

Phytophagous mites are a major cause of economic losses for many plants produced in greenhouses (Cranham & Helle, 1985; Nachman & Zemek, 2002). *Phytoseiulus persimilis* Athias-Henriot, 1957 and *Neoseiulus californicus* (McGregor, 1954) (Acari: Phytoseiidae) are two important predator species used in greenhouses for the biological control of phytophagous mites (McMurtry & Croft, 1997; Raworth, 2001; Oliveira et al., 2007). Acaricides used to control phytophagous mites cause many negative effects including resistance development, side effects in non-target organisms and environmental pollution (Skirvin et al., 2002). Furthermore, the chemicals reduce egg production, preying and reproductive capacity in predatory mites (Kim & Yoo, 2002). However, using only the predators in the control of mites that cause significant economic losses in crops, usually cannot provide the desired control (Ibrahim & Yee, 2000; Fadini et al., 2004). For this reason, the use of *P. persimilis* and *N. californicus* along with some plant extracts in the control against plant parasitic mites in greenhouse cultivation has value (Schmutterer, 1997; Shi & Feng, 2004; Roobakkumar et al., 2010). However, the number of studies of use the plant extracts and phytoseiid predators in the literature is very limited. Choi et al. (2004) determined that essential oils of caraway seed, java citronell , lemon eucalyptus, pennyroyal, and peppermint can cause over 90% mortality of both *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) and *P. persimilis* adults. Miresmailli & Isman (2006) reported that the toxicity of rosemary essential oil is higher on *T. urticae* than *P. persimilis*. El-Sharabasy (2010) determined that the extract from *Artemisia judaica* L. (Compositae) is more toxic to *T. urticae* than *P. persimilis*. Vergel et al. (2011) reported mortality rates for garlic plant extract of 23.81% in *P. persimilis* and 9.82% in *N. californicus* at a concentration of 1.25 ml/L.

Sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) are two important medicinal and aromatic plants in the Lamiaceae. Sage and rosemary plants have antioxidant and antimicrobial properties due to the secondary metabolites they contain (Biljana et al., 2007). For this reason, these plants are both consumed and used in medicine in Turkey and around the world. Sage and rosemary plants are grown in many regions of Turkey. Recently, considerable work has been done on the insecticidal and acaricidal functions of many essences from both plants against many pests (Kawka &Tomczyk, 2002; Bozhüyük et al., 2016; Park et al., 2016; Tak et al., 2016). For this reason, sage and rosemary plants were used in the study for determine the possible effects on two natural enemies of phytophagous mites.

Compounds derived from these plants are recommended to be used in the control against insect and mite because of their new mechanism of action, non-target organisms, mammals and environmental effects (Isman, 2006). Another important point is that plant extracts are generally more suitable for use in combination with natural enemies than synthetic pesticides (Erdogan et al., 2012). However, all potential risks need to be addressed in order for these extracts to be used correctly. Therefore, it is absolutely necessary to conduct studies on the toxicity of plant extracts on natural enemies that are being considered for use in integrated pest management programs. In this study, the toxicity and repellency of different concentrations of ethanolic extracts derived from sage and rosemary on *N. californicus* and *P. persimilis* were determined.

Material and Methods

Origin and culture of predatory mites

Phytoseiulus persimilis was collected from vegetable fields in Turkey in 1993 and *N. californicus* was collected from an organic apple orchard in Turkey in 2008 (Sekeroglu & Kazak, 1993; Yorulmaz Salman & Ay, 2013). Predatory mites were cultivated without exposure to any pesticides in an insect rearing room (26±2ºC, 60±5% RH and 16:8 h L:D photoperiod). *Tetranychus urticae*, used to feed *P. persimilis* and *N. californicus*, were cultured on beans (*Phaseolus vulgaris* L. cv. Barbunia) in a controlled-environment room (26±2ºC, 60±5% RH and 16:8 h L:D photoperiod).

Preparation of plant extracts

Sage and rosemary plants were collected from production areas of the Agricultural Application and Research Centre, Faculty of Agriculture, Süleyman Demirel University during the 2016 growing period and extracts were obtained from leaves of the plants. The plant materials were dried at room temperature until they reached equilibrium moisture content. The sage and rosemary plants (100 g) were separately weighed and transferred to the Erlenmeyer flasks. For organic solvent, 500 ml of 99% ethanol, was added to the plant material and mixed 24 h on an orbital shaker. The ethanol in the mixture was removed with a rotary evaporator. The extracts were stored in glass tubes at +4ºC until used.

Toxicity experiments

The method of El-Sharabasy (2010) was used for the determine of the toxicity of the plant extracts on *N. californicus* and *P. persimilis*. A preliminary study was conducted on eggs, immature stages and adult mites of the same age. Fifteen individual adult females were transferred to 3 cm bean leaf discs in separate 9 cm Petri dishes. In the egg experiments, eggs in the same age were used to determine the effect of plant extracts on egg hatch. Eggs were kept for 24 h to obtain immature stages of the same age for experiments to determine the effects of plant extracts on immature stages. In addition, female individuals of the same age, which matured a few days after the eggs were laid, were used in the experiments on adults.

In the toxicity studies, the leaf disc-spray tower method was used. For this purpose, 3 cm bean leaf discs were placed in 9 cm Petri dishes on moistened cotton. Due to the mobility of the mites and the stay in the leaf disc along the experiment, the leaf was surrounded by the Tangle Trap. Concentrations of 1, 3, 6 and 12% of plant extracts were applied to the mites of different life stages. Concentrations of plant extracts were determined by the concentrations of plant extracts applied to *P. persimilis* by El-Sharabasy (2010). Pure water was used as a control. TritonX100 (0.01%) was added to the purified water and the extracts. Each toxicity assay had one control and four extract concentrations replicated ten times.There were ten individuals in each replicate. Different concentrations of plant extracts were applied to the leaf surface at 2 ml at 100 kPa in the spray tower. After spraying, a large number of *T. urticae* were added as prey to leaf discs to prevent the predatory mites sticking to Tangle Trap. The toxicity of plant extracts was determined at 24, 48 and 72 h by dead-live counts of immature and adult stages of the predatory mites. For the egg hatch experiment, observations continued until all eggs in the control group had hatched. In the experiments, *T. urticae* were added to each Petri dish to feed predatory mites until the end of the assessment period.

Repellency experiments

To determine the repellency of the plant extracts on different life stages of the predatory mites, the methods of Miresmailli et al. (2006) and Nerio et al. (2009) were used. In the repellency experiments, the same age immature stages and adult individuals of predatory mites were used. Predatory mites were obtained according to the method described in the toxicity experiments of immature stage and adult individuals of the same age. Concentrations of 0.1, 1, 5 and 10 ml/L of plant extracts were applied to the predatory mites in the repellency experiments. For each concentration, the repellency experiments were carried out in four repetitions. In each repetition, 10 individuals were available. In the experiments, one half was transferred to a 9 cm Petri dish containing cotton with a base of 3 cm diameter bean leaf disks immersed in 0.01% TritonX100 solution (control portion) and the other half in plant extract solution. The predatory mites, for prevent their escape along the experiment the leaf was surrounded by the Tangle-Trap in repellent experiments. In addition, a large number of *T. urticae* individuals were added as prey in order to reduce the movement of predatory mites in the leaf disc. The immature stage or adult individuals of the predatory mites to be used in the middle part of the bean leaf disc dissection were transferred with a brush. In the repellency experiments, individuals in control and plant extract sections of leaf discs were counted after 24, 48 and 72 h.

Statistical analysis

The mortality percentages from the toxicity experiments were calculated using the Abbott formula (Abbott, 1925). The results from the repellency experiments are given using the repellency percent index according to Obeng-Ofori et al. (1997):

$$\text{Repellency (\%)} = [(Nc-Nt) / (Nc + Nt)] \times 100$$

where, Nc is the number of individuals moving to control direction on leaf surface, and Nt is the number of individuals in the direction of the application on the leaf surface.

The results were arcsin transformed (Zar, 1999). Toxicity and repellency were analyzed by three-way repeated measures ANOVA (there were three factors in experiments with immature and adult stages: extracts, concentrations and observation time). Ovicidal effects were analyzed by two-way ANOVA (for: extracts and concentrations). Tukey's test was used to determine the differences between the means.

Results

Toxicity bioassay results

The toxicity the plant extracts on *N. californicus* and *P. persimilis* adults are given in Table 1. The toxicity of extracts to adult and immature stages of predatory mites varied depending on the treatment, dose and time (for adults $F = 36.25$, $df = 6$, $p < 0.05$; for immature stages $F = 36.45$, $df = 6$, $p < 0.05$). There were interactions between these variables. It was found that the toxicity of the sage and rosemary extract to adults increased with time. Also, at all concentrations the toxicity of the rosemary extract to *N. californicus* was statistically higher than the toxicity of sage extract ($p < 0.05$). The highest toxicity to *N. californicus* adults was 33.3 and 62.5% for 12% sage and rosemary extracts, respectively ($F = 35.23$, $df = 6$, $p < 0.05$). For *P. persimilis* adults, the mortalities at all concentrations of sage and rosemary extracts were similar. The highest toxicity to *P. persimilis* was 33.3% for the sage extract and 38.4% for the rosemary extract ($F = 28.52$, $df = 6$, $p < 0.05$).

Table 1. Toxicity of the different concentrations of sage and rosemary extracts on the adults of *Neoseiulus californicus* and *Phytoseiulus persimilis* (mean±SE)

Time (h)	Concentration (ml/L)	Mortality (%)			
		*Neoseiulus californicus**		*Phytoseiulus persimilis**	
		Sage	Rosemary	Sage	Rosemary
24	1	2.0±0.25 bE	20.5±0.35 aD	10.4±0.88 aE	8.2±0.48 aE
	3	4.1±0.78 bD	28.5±1.57 aC	20.2±0.25 aC	22.2±0.56 aC
	6	12.5±0.88 bC	38.7±2.02 aB	25.3±0.68 bB	30.4±0.77 aB
	12	16.6±1.05 bB	51.0±0.35 aA	27.5±0.98 bA	31.6±0.26 aB
48	1	2.0±0.33 bE	25.6±0.78 aC	14.4±0.33 aD	10.2±0.25 bD
	3	6.3±0.45 bD	35.8±0.25 aB	22.6±0.57 aC	23.1±1.05 aC
	6	13.6±1.02 bC	59.1±0.56 aA	28.3±1.05 bA	34.5±1.05 aA
	12	19.1±0.88 bB	61.2±0.66 aA	30.5±0.33 bA	34.8±0.88 aA
72	1	2.2±0.28 bE	30.6±0.13 aC	18.2±2.05 aC	12.2±0.54 bD
	3	13.3±0.55 bC	40.8±0.55 aB	25.4±1.09 aB	25.6±0.33 aC
	6	15.5±0.45 bB	61.2±1.05 aA	29.1±0.35 bA	36.3±0.45 aA
	12	33.3±0.78 bA	62.5±1.08 aA	33.3±0.45 bA	38.4±1.09 aA

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

The toxicity of different concentrations of the plant extracts on *N. californicus* and *P. persimilis* immature stages are given in Table 2. The toxicity of the rosemary extract in all concentrations of *N. californicus* immature stages was statistically greater than the toxicity of the sage extract ($p < 0.05$). The highest toxicity to *N. californicus* immature stages was 37.5 and 67.3% for 12% sage and rosemary extracts, respectively. The toxicity of the sage extracts to *P. persimilis* was statistically higher than the toxicity of the rosemary extract at all concentrations ($F = 36.83$, $df = 6$, $p < 0.05$). The highest toxicity to *P. persimilis* was 50.2% for the sage extract and 44.6% for the rosemary extract ($F = 27.56$, $df = 6$, $p < 0.05$).

Table 2. Toxicity of the different concentrations of sage and rosemary extracts on *Neoseiulus californicus* and *Phytoseiulus persimilis* immature stages (mean±SE)

Time (h)	Concentration (ml/L)	Mortality (%)			
		*Neoseiulus californicus**		*Phytoseiulus persimilis**	
		Sage	Rosemary	Sage	Rosemary
24	1	8.1±0.75 bE	18.7±0.33 aF	15.5±0.35 aF	5.7±0.25 bF
	3	18.3±1.75 bC	22.9±1.55 aE	30.1±0.28 aC	15.2±0.25 bE
	6	20.4±0.25 bC	29.1±0.15 aD	41.2±0.22 aB	32.4±0.35 bC
	12	28.5±0.44 bB	37.5±0.45 aC	48.3±0.55 aA	36.1±1.08 bC
48	1	10.2±0.55 bE	20.7±0.58 aE	20.2±0.64 aE	9.1±1.22 bF
	3	20.4±1.15 bC	29.1±0.75 aD	33.3±0.98 aC	18.2±0.45 bD
	6	22.4±2.02 bC	35.4±0.63 aC	46.4±0.45 aA	33.3±0.22 bC
	12	30.6±0.33 bB	45.8±0.48 aB	49.6±0.69 aA	40.1±0.75 bB
72	1	16.6±0.28 bD	22.9±0.35 aE	25.2±0.58 aD	12.1±0.78 bE
	3	27.0±0.75 bB	33.3±0.75 aC	35.7±0.75 aC	20.4±0.85 bD
	6	33.3±0.48 bA	39.5±2.05 aC	48.4±0.67 aA	35.3±0.98 bC
	12	37.5±0.88 bA	67.3±1.05 aA	50.2±1.09 aA	44.6±1.05 bA

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

The results for different concentrations of the sage and rosemary extracts on *N. californicus* and *P. persimilis* egg development are given in Table 3. As the concentration both plant extracts increased, the effect on *N. californicus* hatch also increased. The greatest effect on *N. californicus* egg hatch was 51.1% with 12% rosemary extract ($F = 10.58$, $df = 4$, $p < 0.05$). The greatest effect on *P. persimilis* egg hatch was 18.6% at 12% for both sage and rosemary extracts ($F = 14.12$, $df = 4$, $p < 0.05$).

Table 3. Egg hatching effect of plant extracts with different concentration on *Neoseiulus californicus* and *Phytoseiulus persimilis* eggs (mean±SE)

Concentration (ml/L)	Mortality (%)			
	*Neoseiulus californicus**		*Phytoseiulus persimilis**	
	Sage	Rosemary	Sage	Rosemary
1	1.2±0.22 bD	11.6±0.25 aD	2.1±0.55 aD	2.1±0.78 aD
3	4.1±0.55 bC	18.6±0.65 aC	6.8±1.12 aC	4.16±0.46 aC
6	10.4±1.05 bB	37.2±0.78 aB	12.4±0.98 aB	10.4±0.15 aB
12	16.6±0.88 bA	51.1±0.15 aA	18.6±0.45 aA	18.6±0.33 aA

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

Repellency bioassay results

Repellency of the different concentrations of the sage and rosemary extracts to *N. californicus* and *P. persimilis* adults is shown in Table 4. The repellency of plant extracts to adult and immature stages of predatory mites varied depending on the dose and time. Similarly, to the toxicity experiment, there were interactions between these explanatory variables. The repellency of sage and rosemary extracts to *N. californicus* adults showed that the rosemary extract was statistically more repellent than the sage extract at 5 and 10 ml/L ($p < 0.05$). The highest repellency to *N. californicus* adults was 43.7 and 77.7% at the 10 ml/L for the sage and rosemary extracts, respectively ($F = 35.85$, $df = 6$, $p < 0.05$). Repellency of the plant extracts to *P. persimilis* adults were similar to that for *N. californicus* adults. Especially at 5 and 10 ml/L, both extracts had increasing repellency to *P. persimilis* adults. The highest repellency to *P. persimilis* was 50.1 and 88.8% at 10 ml/L for the sage and rosemary extracts, respectively ($F = 33.24$, $df = 6$, $p < 0.05$).

The repellency of the different concentrations of the sage and rosemary extracts to *N. californicus* and *P. persimilis* immature stages is shown in Table 5. The highest repellency to *N. californicus* immature stages was 38.8 and 44.1% for the sage and rosemary extracts, respectively ($F = 35.84$, $df = 6$, $p < 0.05$). For *P. persimilis* immature stages, the highest repellency of plant extracts was at 10 ml/L. At 10 ml/L, the repellency of the sage extract was statistically higher than that of the rosemary extract to *P. persimilis* immature stages ($p < 0.05$).

Table 4. Repellency of different concentrations of sage and rosemary extracts on *Neoseiulus californicus* and *Phytoseiulus persimilis* adults (mean±SE)

Concentration (ml/L)	Time (h)	Repellency (%)			
		*Neoseiulus californicus**		*Phytoseiulus persimilis**	
		Sage	Rosemary	Sage	Rosemary
0.1	24	6.25±0.43 bD	13.5±0.85 aE	8.32±1.33 bF	15.3±0.36 aF
	48	18.7±0.38 aC	13.5±0.75 bE	16.4±0.75 bE	29.7±0.45 aE
	72	12.5±0.55 bC	18.9±0.33 aE	27.9±0.45 bD	40.2±0.55 aD
1	24	21.2±0.78 aB	15.3±0.48 bE	22.6±0.69 aD	12.5±1.45 bF
	48	21.2±1.05 aB	15.3±1.65 bE	31.1±0.58 aC	17.3±1.13 bF
	72	9.09±1.38 bD	33.3±1.89 aD	37.5±1.14 aB	33.3±0.78 bE
5	24	8.1±0.89 bD	43.5±1.15 aC	23.7±1.45 bD	43.5±0.65 aD
	48	24.3±0.33 bB	53.8±0.36 aB	25.5±0.88 bD	53.8±0.54 aC
	72	29.7±0.45 bB	48.7±0.64 aC	33.3±0.95 bC	58.6±0.35 aC
10	24	42.8±1.45 bA	66.6±0.55 aA	45.9±0.45 bA	63.3±1.98 aB
	48	37.5±1.15 bA	72.2±1.15 aA	50.1±0.65 bA	68.2±1.65 aB
	72	43.7±0.75 bA	77.7±1.25 aA	50.1±1.05 bA	88.8±0.35 aA

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

Table 5. Repellency of different concentrations of sage and rosemary extracts on *Neoseiulus californicus* and *Phytoseiulus persimilis* immature stages (mean±SE)

Concentration (ml/L)	Time (h)	Repellency (%)			
		*Neoseiulus californicus**		*Phytoseiulus persimilis**	
		Sage	Rosemary	Sage	Rosemary
0.1	24	5.5±0.56 bE	11.7±0.59 aE	8.2±0.66 bF	11.1±0.48 aE
	48	11.1±0.89 aD	11.7±0.46 aE	16.4±0.95 aE	11.1±0.65 bE
	72	11.1±0.78 aD	11.7±0.75 aE	25.5±0.35 aD	27.7±0.52 aC
1	24	10.5±1.56 aD	12.4±0.68 aE	21.3±0.75 aE	23.0±1.54 aD
	48	14.2±0.98 aC	12.1±1.55 aE	24.4±1.56 bD	28.2±1.78 aC
	72	14.2±1.45 bC	17.0±0.52 aD	27.7±1.35 bD	33.3±1.45 aB
5	24	18.7±1.05 bC	23.0±1.45 aC	57.1±0.85 aA	26.0±0.95 bC
	48	31.2±1.89 aB	28.2±1.85 bB	40.4±0.47 aB	21.3±0.35 bD
	72	35.2±0.55 aA	33.3±1.09 aB	45.5±0.69 aB	31.2±0.75 bB
10	24	27.7±0.36 aB	10.0±1.45 bE	33.3±0.58 bC	43.5±0.33 aA
	48	27.7±0.76 bB	39.5±0.45 aA	36.9±0.78 aC	38.8±0.89 aA
	72	38.8±0.85 bA	44.1±0.78 aA	47.7±1.84 aB	38.8±1.15 bA

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

Discussion

Plants can protect themselves from herbivores and pathogenic attack using secondary metabolites (Miresmailli & Isman, 2014; Pavela, 2016). This feature of plants gives suggests that these metabolites can be used to control pests. However, for invertebrate pest control it is also important to know the effects of the herbal substances on the natural enemies. This study demonstrates toxicity and repellency of sage and rosemary extracts to two major predators (*P. persimilis* and *N. californicus*), which are widely used in the control against phytophagous mites. The toxicity to both predatory mites increased with increasing extract concentration and exposure time. The effect of rosemary extract on *N. californicus* adults was greater than the sage extract. For *P. persimilis* adults, however, the effect of both extracts was similar. The toxicity of the rosemary extract was much higher for *N. californicus* immature stages than that of the sage extract. However, the toxicity of the rosemary extract to *P. persimilis* immature stages was less than the sage extract. In addition, the toxicity of plant extracts to the immature stages at higher concentrations was greater than on the adults. The reason for this might be that the immature stages have not yet completed their development and therefore, the toxicity of plant extracts was greater due to the lower amount of chitin in their bodies. The effect of the extracts on *N. californicus* egg hatch was greater with the rosemary extract than the sage extract. Whereas, the ovicidal effects of rosemary and sage extracts on *P. persimilis* were similar. It was observed that the effects of the sage extract on *N. californicus* and *P. persimilis* were moderate and similar. However, the effect of the rosemary extract on *P. persimilis* was much less than on *N. californicus*. This suggests that the active components of the rosemary extract are more effective on *N. californicus* than *P. persimilis*. Another possibility is that *P. persimilis* is more resistant to the active components or essences in the rosemary extract than *N. californicus*. However, in order to reach a definite judgment, the toxicity of all the active components of rosemary plant extract need to be determined for *P. persimilis*.

There have been many studies on the toxicity of plant extracts to phytophagous mites (Mateeva et al., 2003; Liu et al., 2004; Rasikari et al., 2005; Antonious et al., 2006; Shi et al., 2006; Wang et al., 2007; Moneim et al., 2011; Topuz & Madanlar, 2011). Yorulmaz Salman et al. (2014) found that the extracts of sage and rosemary were toxic to *T. urticae* with nymph and adult mortality of 79 and 62%, respectively, for sage extract and 58 and 82% for rosemary extract, respectively. In the present study, *P. persimilis* was more resistant than *N. californicus* when exposed to the same extracts. Hence, it is concluded that *P. persimilis* for the control of two spotted spider mite can be used in an integrated management programs using sage and rosemary extracts. However, the number of studies in which the effects of plant extracts on natural enemies have been identified, especially in integrated management programs, is rather limited. Vergel et al. (2011) reported that the garlic plant extract resulted in a mortality rate of 23.81% for *P. persimilis* and 9.82% for *N. californicus* at 1.25 ml/L. Bernardi et al. (2013) reported that azadirachtin, a plant-based preparation, was highly toxic to *T. urticae*, while the toxicity to *N. californicus* and *Phytoseiulus macropolis* (Banks, 1904) (Acari: Phytoseiidae) were much lower, so the combined use of azadirachtin and predatory mites was suitable. In our study, it was also shown, based on the published reports, that the toxicity of the plant extracts to *T. urticae*, were higher than the two predatory mites in the Phytoseiidae. This indicates the potential for combined use of plant extracts and predatory mites in integrated pest management programs.

When the results of repellency were evaluated, it was determined that the repellency of the rosemary extract to *N. californicus* and *P. persimilis* adults was higher than that of the sage extract. Also, the repellency of the rosemary extract to *N. californicus* immature stages was higher than that of the the sage extract. In the case of *P. persimilis* immature stages, the repellency of the sage and rosemary extracts were varied according to the concentration and exposure time. The extracts of sage and rosemary showed repellency at certain rates to both predatory mites. There are many studies in which plant extracts have been found to repel phytophagous mites (Antonious & Snyder, 2006; Antonious et al., 2006; Kumral et al., 2010; Mozaffaria et al., 2012). However, there are no studies reporting repellency of plant extracts on predatory mites in the Phytoseiidae. This study is very important because it is the first study to determine the repellency of sage and rosemary extracts on the Phytoseiidae. It is also thought that the toxic and repellency of plant extracts and use of natural enemies combined, should be evaluated together in integrated management programs. The repellency of the sage and rosemary extracts to *N. californicus* and *P. persimilis* needs to be considered if these extracts are to be used in integrated control programs that use these predatory mites.

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Original article (Orijinal araştırma)

Pleiotropic effects of propargite on life-table parameters of susceptible and resistant strains and reciprocal *F1* hybrids of *Tetranychus urticae* Koch, 1836 and their implications for population growth[1]

Tetranychus urticae Koch 1836'nin hassas ve dirençli ırklarının ve karşılıklı *F1* çapraz melezlerinin yaşam tablosu parametreleri üzerine propargite'in pleiotropik etkileri ve bunların popülasyon gelişimi üzerine çıkarımlar

Riaz SHAH[2*]

Abstract

Demographic toxicological studies or life-table response experiments have been proposed as a more reliable approach for predicting pesticide impact in the field. Life-table parameters of the susceptible, propargite-resistant and reciprocal *F1* hybrids of *Tetranychus urticae* Koch, 1836 (Prostigmata: Tetranychidae) were studied in the presence and absence of propargite residues at LC_{50} and LC_{99} of susceptible strain at Lincoln University, New Zealand. The life history data of all individuals were analyzed using the age-stage, two-sex life table. Treatment with LC_{50} of the susceptible strain did not affect the duration of developmental time of any strain. LC_{99} of the susceptible strain, however, prolonged the developmental time of the propargite-resistant strain by approximately 2 d. The intrinsic rate of increase (r_m), R_o and total progeny production of the propargite-resistant strain and $S♂ \times R♀$ hybrid treated with LC_{50} of the susceptible strain were higher compared to that of the susceptible strain and $R♂ \times S♀$ hybrid. Population projections were used to study the effects of relatively small differences in the life-table parameters of strains/hybrids of *T. urticae*. For the untreated control groups, the susceptible strain gave the highest population projection after 10 generations. In groups treated with LC_{50} of the susceptible strain, the projected population size showed that the number of adult females of the propargite-resistant strain superseded that of the susceptible strain. The hybrid $S♂ \times R♀$ increased most from treatment with the LC_{50} of the susceptible strain. The differential success of different strains could, therefore, change resistance frequency throughout a growing season at a location.

Keywords: Intrinsic rate of increase, life-table parameters, population projection, propargite, resistance, *Tetranychus urticae*

Öz

Demografik toksikolojik çalışmalar ya da yaşam tablosu tepki denemeleri, tarlada insektisit etkisini öngörmek için daha güvenilir bir yaklaşım olarak önerilmektedir. *Tetranychus urticae* Koch, 1836 (Prostigmata: Tetranychidae)'nin hassas, propargite karşı dirençli ve karşılıklı *F1* çapraz melezlerinin yaşam tablosu parametreleri, Lincoln Üniversitesi (Yeni Zelanda)'nde hassas ırkın LC_{50} ve LC_{99}'unda propargite kalıntılarının varlığında ve yokluğunda çalışılmıştır. Tüm bireylerin yaşam tablosu verileri, yaş-evre, iki cinsiyetli yaşam tablosu kullanılarak analiz edilmiştir. Duyarlı ırkın LC_{50} değerleri, herhangi bir ırkta gelişim zamanını etkilememiştir. Bununla birlikte, hassas ırkın LC_{99}'u, propargite dirençli ırkın gelişim süresini yaklaşık iki gün uzatmıştır. Duyarlı ırkın LC_{50} değerleri, propargite dirençli ırkın ve $S♂ \times R♀$ hibridinin kalıtsal üreme yeteneği (r_m), R_o ve toplam döl verimi, hassas ırk ve $R♂ \times S♂$ hibritine kıyasla daha yüksek bulunmuştur. Popülasyon tahminleri, *T. urticae*'nin ırkları / melezlerinin yaşam tablosu parametrelerindeki nispeten küçük farklılıkların etkilerini incelemek için kullanılmıştır. Uygulama yapılmayan kontrol grupları için hassas ırk 10 dölden sonra en yüksek popülasyon tahminini vermiştir. Duyarlı ırkın LC_{50} değeri uygulanan gruplarda, tahmin edilen popülasyon büyüklüğü, propargite dirençli ırkın ergin dişi sayısının, hassas ırkın yerine geçtiğini göstermiştir. Hibrid $S♂ \times R♀$, hassas ırkın LC_{50}'si ile uygulamadan en fazla artmıştır. Bu yüzden, farklı ırkların kademeli başarısı, bir bölgede yetiştirme sezonu boyunca direnç sıklığını değiştirebilir.

Anahtar sözcükler: Kalıtsal üreme yeteneği, yaşam tablosu parametreleri, popülasyon tahmini, propargite, direnç, *Tetranychus urticae*

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Introduction

Insecticide resistance is an example of evolutionary change where the insecticide acts as a powerful selective sieve (Crow, 1957). The rate of change in allele frequency in a population under the influence of selection pressure is a function of the initial allele frequency, dominance, population structure and the relative fitness of the various genotypes (Roush & McKenzie, 1987). However, resistant genotypes must be at some fitness disadvantage in the absence of the pesticide, otherwise resistance alleles would be very common prior to selection (Crow, 1957). Studies carried out on diverse groups of arthropods have reported some deleterious effects of resistance on their life history characteristics. For example, Schulten (1968) reported reduced fertility, fecundity and development rates for an organophosphorus-resistant strain of *Tetranychus urticae* Koch, 1836 (Prostigmata: Tetranychidae). Kono (1987) also reported lower survival rate, egg production and intrinsic rate of increase (r_m) for the dicofol-resistant strain of *T. urticae*. Kasamatsu & Ogawa (1992) studied the reproductivity of fenpropathrin-resistant and susceptible strains of *T. urticae* at 20, 25 and 30ºC and the lower r_m values for the resistant strain at each temperature suggested a lower fitness value of that strain.

Other insect pests have also been found to have fitness related costs associated with insecticide resistance. Reduced relative fitness of resistant genotypes in insecticide-free environments is characteristic of many insect species (Sayyed et al., 2008). Udeaan & Judge (1990) reported significantly longer larval period in the phosphine-resistant strain of *Trogoderma granarium* Everts, 1898; and Trisyono & Whalon (1997) also found slower larval development, reduced fecundity and shorter oviposition periods in a *Bacillus thuringiensis* Berliner, 1915 resistant strain of Colorado potato beetle, *Leptinotarsa decemlineata* Say, 1824.

Demographic toxicological studies or life-table response experiments have been proposed as a more reliable approach than the lethal dose estimates for predicting pesticide impact in the field since they show effects of pesticides on survivors, thus providing a measure of impact on the population growth rate (Robertson & Worner, 1990; Robertson & Preisler, 1992; Stark & Banks, 2003). Differences in the biological parameters affecting the net replacement rate (R_o) and the intrinsic rate of population increase (r_m) are of particular interest to insecticide resistance management (Haubruge & Arnaud, 2001). Although life-table bioassays impose some limitations, Stark & Banks (2003) stated that the population growth rate approach should be adopted more widely, if we are to improve our knowledge about toxicant impacts on arthropods.

Kheradmand et al. (2007) also emphasized the importance of the life-table parameters for analyzing and understanding the impact of an external factor on the growth, survival rate, reproduction and increase rate of an arthropod population. These parameters influence population growth rates of an insect in the current and next generations (Frel et al., 2003). The susceptibility of an individual to insecticides may vary greatly with sex and developmental stage, therefore, stage differentiation and the male population should be taken into consideration (Chi & Liu, 1985; Chi, 1988).

Roush & Daly (1990) suggested that in any study of the fitness of resistant arthropods the susceptible-resistant hybrid (heterogeneous) strain should be included because of the high frequency of heterogeneous individuals occurring in populations during the early development of resistance. In addition, a study of the life-table parameters of pesticide susceptible and resistant strains and susceptible-resistant reciprocal *F1* hybrids ($R^{♂} \times S^{♀}$ and $S^{♂} \times R^{♀}$) in the presence and absence of the pesticide residues is required. All such strains are continuously exposed to pesticides in the field; therefore, comparisons of the different life-table parameters in the presence and absence of pesticide residues may better explain the dynamics of resistance and would assist the development of more appropriate sampling plans and resistance management programs.

The objectives of the studies reported in this paper were, 1) to determine the effects of propargite on life-table parameters of the susceptible and propargite-resistant strains and susceptible-resistant reciprocal *F1* hybrids of *T. urticae* in the presence and absence of propargite residues, and, 2) to explore the impact of any differences in these parameters on the frequency of propargite-resistant individuals in the population.

Material and Methods

Mite source

A susceptible strain of *T. urticae* was collected from wild hosts from the Lincoln University organic production area. No pesticide of any type had been applied in this area for about 20 years. A resistant strain of *T. urticae* was air freighted from a glasshouse in Auckland, New Zealand where there had been intensive use of miticides including propargite. Both strains were reared on French dwarf bean (*Phaseolus vulgaris* cv. Tendergreen) in separate controlled temperature rooms at 21±3ºC, 60±15% RH and a 16:8 h L:D photoperiod at Lincoln University New Zealand. Bean plants were grown in 15 cm diameter plastic pots in a glasshouse and supplied to the colonies when required. The colony of the resistant strain was sprayed with 0.05% propargite (Omite 30WP; Uniroyal Chemicals, Frensno, CA, USA) twice a month to eliminate any heterozygotes and narrow the response of the strain to the miticide. At LC_{95}, the resistance ratio (RR_{95}) for propargite-resistant, $R^{\male} \times S^{\female}$ and $S^{\male} \times R^{\female}$ were 1200, 28 and 78 times, respectively (Shah et al., 2002).

Backcrossing of susceptible and propargite-resistant strains of *Tetranychus urticae*

To obtain the reciprocal *F1* hybrids, thirty newly emerged adult females of both susceptible and propargite-resistant strains of *T. urticae* were transferred separately to single whole bean leaves. Each leaf was placed in a Petri dish on moist cotton wool with the lower surface facing upward. One adult male from the opposite strain was released on to each leaf. After 24 h of oviposition the mites were removed from the leaves and the eggs counted. The emerging *F1* hybrid, and the susceptible and propargite-resistant adult females were used in the following life-table parameter study.

Life-table parameter study of the susceptible and propargite-resistant strains, and reciprocal *F1* hybrids of *Tetranychus urticae*

Construction of the fertility life-table and determination of developmental time

Fifteen adult females from the susceptible and propargite-resistant strains and the $R^{\male} \times S^{\female}$ and $S^{\male} \times R^{\female}$ *F1* hybrids were transferred to bean leaves within a 10 mm diameter circle of Tracktrap® (used to prevent the escape of mites). As nothing was known about the genetic status of propargite-resistant individuals the symbols (*R* and *S*) are used for convenience. After 24 h of oviposition all adult mites were removed and the eggs were sprayed under a Potter tower at 69 kpa with propargite 30WP using either the LC_{50} (0.006% ai) or LC_{99} (0.032% ai) of the susceptible strain (Shah et al., 2002). Control groups were treated with water only.

Each Petri dish with a cohort size of four to six eggs per dish was considered one replicate giving a total of about 70 mites per strain or hybrid. The Petri dishes were maintained under a 16:8 h L:D photoperiod at 22.4±1.5ºC with 50±5% RH. Leaves were changed every second day after the eggs had hatched. The life-table data for all individuals for each developmental stage including the chrysalis stage were recorded at 12 h intervals until all had died.

Fertility life-tables were constructed for the susceptible and propargite-resistant strains and *F1* hybrids. The following life-table parameters were calculated using the methods of Carey (1993): adult longevity; mean total progeny production per female; pre- and post-ovipositional periods; birth rate (*b*); death rate; age structure; number of eggs/female/d and percentage of mites reaching adulthood and sex ratio (expressed as the percentage females).

Calculation of age-stage, two-sex life table

The life history raw data of all individuals (males, females and those dying before the adult stage) were analyzed according to the age-stage, two-sex life-table theory (Chi & Liu, 1985; Chi, 1988). The age-stage-specific survival rate (s_{xj}) (with x = age in days and j = stage); the age-stage-specific fecundity (f_{xj}); the age-specific survival rate (l_x); the age-specific fecundity (m_x); and the population growth parameters [the intrinsic rate of increase (r); the finite rate of increase ($\lambda = e^r$); the gross reproductive rate (GRR); the net reproductive rate (R_0) and the mean generation time (T)] were calculated accordingly. The age-specific survival rate includes both male and female, and is calculated according to Chi and Liu (1985) as:

$$lx = \sum_{j=1}^{k} s_{xj} \quad (1)$$

and

$$mx = \frac{\sum_{j=1}^{k} s_{xi} f_{xj}}{\sum_{j=1}^{k} s_{xj}} \quad (2)$$

where, k is the number of stages.

The intrinsic rate of increase is estimated by using:

$$\sum_{x=0}^{\omega} e^{-r(x+1)}\, lxmx = 1 \quad (3)$$

with age indexed from 0 to ω (maximum age).

The GRR is calculated as GRR = Σm_x.

Data analysis and population parameters (r, λ, GRR, R_0 and T) for group-reared life table based on matrices N and F_{total} were calculated by using the TWOSEX-MSChart program (Chi, 2018).

The means and standard errors of the population parameters were estimated by using the Bootstrap procedure (Meyer et al., 1986; Huang & Chi, 2013). In the bootstrap procedure, a sample of n individuals from the cohort with replacement was taken randomly and the $r_{i\text{-}boot}$ for this bootstrap sample was calculated as:

$$\sum_{x=0}^{\omega} e^{-r_{i\,boot}(x+1)}\, lxmx = 1 \quad (4)$$

where the i-boot represents the i^{th} bootstrap, and l_x and m_x are calculated from the n individuals selected randomly with replacement. Generally, the data on the same individual are repeatedly selected. This procedure was repeated m times (m = 10,000) and computed the mean of these m bootstraps as:

$$r_B = \frac{\sum_{i=1}^{m} r_{i-boot}}{m} \quad (5)$$

The variance ($VARr_B$) and standard error (SEr_B) of these m bootstraps were calculated as:

$$VARr_B = \frac{\sum_{i=1}^{m} (r_{i-boot} - r_B)^2}{m-1} \quad (6)$$

$$SEr_B = \sqrt{VARr_B} \quad (7)$$

The same methods are used for the corresponding estimates of the finite rate of increase (λ), GRR, R_0 and mean generation time (T).

ANOVA was applied to the life-table data obtained from life history and multiple comparisons were made using $LSD_{(\alpha=0.05)}$ to determine significant differences between stage durations using Quattro Pro (Corel Corp., 1996; version 6.02). The two-sex life-table bootstrap-values of the TSSM were also compared using $LSD_{(\alpha=0.05)}$.

To establish the possible long term (for at least one season with average of 10 generations) influence of comparatively small differences in the parameter values, population numbers were projected over 10 generations (each generation was assumed to receive the same dose of propargite) using the equation:

$$N_t = (\lambda)^t N_0 \qquad (8)$$

where, t is the mean generation time, λ is the finite rate of increase and N_0 is the initial population (100 females). The relative increase or decrease per generation in the number of treated females in relation to the control mites was calculated by dividing the number of females present in the treated generation by that of untreated generation. All calculations were performed using Quattro Pro.

Results

Life-table parameters of the susceptible and propargite-resistant strains of *Tetranychus urticae*

The various life-table parameters of susceptible and propargite-resistant strains and the reciprocal *F1* hybrids of *T. urticae* are given in Tables 1 and 2. The duration of each life stage including the chrysalis stages and the developmental time from egg to adult did not differ significantly among the untreated strains and hybrids (Table 1). The effect of treating eggs of strains and hybrids with the LC_{50} of susceptible strain on the developmental time from egg to adult was also nonsignificant. However, the developmental time of the propargite-resistant strain increased by about 2 d when the eggs were treated with the LC_{99} of susceptible strain. The development time of the propargite-resistant strain with this treatment was significantly greater ($P > 0.001$) than that of both strains and hybrids either untreated or treated with the LC_{50} of the susceptible strain, requiring more time to complete their development from egg to adult. All the newly emerged larvae of susceptible and both hybrid strains died as a result of treatment with the LC_{99} of susceptible strain. Developmental times for males were slightly but not significantly shorter than that for females for all strains and hybrids.

As a consequence of the higher mortality, the calculated parameters, the intrinsic rate of increase (r_m), R_o and total progeny production (Table 1), of the susceptible strain and the $R^{♂} \times S^{♀}$ hybrid treated with LC_{50} of the susceptible strain were lower compared with the control parameters. These parameters also decreased for the propargite-resistant strain treated with LC_{99} of the susceptible strain. In contrast, due to an increased birth rate and slightly decreased mortality, r_m, R_o and total progeny production of the propargite-resistant strain and $S^{♂} \times R^{♀}$ hybrids treated with LC_{50} of the susceptible strain, increased. Changes (either increase or decrease) in these parameters also resulted in a corresponding inverse change in doubling time.

The percentage of females (sex ratio) in both the strains and hybrids increased with treatment by propargite, possibly indicating that males of both the strains and hybrids were more susceptible to propargite than females. However, percentage of propargite-resistant strain females treated with LC_{50} of susceptible strain decreased slightly. The life expectancy of the susceptible strain, propargite-resistant strain and the reciprocal *F1* hybrids treated with LC_{50} of susceptible strain and the propargite-resistant strain treated with LC_{99} of susceptible strain were lower than the control. The largest difference was 5.64 d for the susceptible strain.

Table 1. Duration (d, mean±SE) of developmental stages of susceptible and propargite-resistant strains and reciprocal *F1* hybrids of *Tetranychus urticae* at 22.4°C

Treatment	Egg stage	Larval stage	Proto-chrysalis	Proto-nymph	Deuto-chrysalis	Deuto-nymph	Teliochrysalis		Total duration[a]		Pre-oviposition	Post-oviposition	Adult longevity		Total life span	
							♀	♂	♀	♂			♀	♂	♀	♂
Susceptible strain (LC_{50})	5.27a (0.07)	0.93a (0.09)	0.89b (0.06)	0.96a (0.03)	0.58a (0.05)	0.69a (0.06)	1.71b (0.06)	1.12a (0.08)	11.02a (0.08)	10.37a (0.15)	1.10a (0.08)	1.60a (0.40)	8.54b (1.13)	7.52b (0.98)	19.56b (1.24)	17.89b (1.21)
Control	5.20a (0.12)	1.00a (0.08)	0.70ab (0.12)	0.90a (0.10)	0.70a (0.12)	0.70a (0.10)	1.50b (0.08)	1.00 (0.29)	10.70a (0.09)	10.25a (0.14)	1.20a (0.18)	1.10a (0.40)	8.90b (1.17)	7.32b (0.88)	19.6b (1.33)	17.57b (1.22)
Resistant strain (LC_{50})	5.33a (0.06)	0.83a (0.09)	0.87b (0.06)	0.73a (0.07)	0.87a (0.08)	0.83a (0.05)	1.44b (0.11)	1.34a (0.15)	10.97a (0.12)	10.78a (0.25)	1.40b (0.14)	2.00b (0.35)	9.17b (0.92)	8.14b (1.08)	20.14b (1.40)	18.92b (1.10)
Control	5.50a (0.08)	0.90a (0.10)	0.60a (0.10)	1.00b (0.08)	0.70a (0.12)	1.20b (0.12)	1.20a (0.12)	1.00 (0.08)	11.10a (0.10)	10.83a (0.17)	1.30b (0.20)	2.50b (0.94)	9.30b (0.94)	7.92b (1.12)	20.4b (1.11)	18.75b (1.13)
$R♂ \times S♀$ (LC_{50})	5.17a (0.06)	0.97a (0.06)	0.93b (0.07)	0.77a (0.07)	0.78a (0.06)	0.83a (0.06)	1.57b (0.12)	1.15a (0.15)	11.05a (0.16)	10.60a (0.26)	1.40b (0.21)	1.20a (0.34)	6.00a (0.76)	4.63a (0.93)	17.05ab (1.33)	15.23ab (0.85)
Control	5.20a (0.12)	1.10a (0.10)	0.70ab (0.12)	1.10b (0.10)	0.60a (0.10)	0.90a (0.06)	1.79b (0.13)	1.20 (0.15)	11.29a (0.16)	10.80a (0.12)	1.80c (0.18)	1.60a (1.12)	9.00b (0.84)	8.21b (1.31)	20.29b (1.14)	19.01b (1.06)
$S♂ \times R♀$ (LC_{50})	5.25a (0.09)	1.04a (0.06)	0.89b (0.08)	0.86a (0.06)	0.77a (0.09)	0.96a (0.07)	1.71b (0.09)	1.25a (0.16)	11.46a (0.15)	10.97a (0.23)	1.30b (0.18)	2.30b (0.28)	8.77b (0.74)	7.15b (1.22)	20.23b (1.33)	18.12b (1.03)
Control	5.20a (0.12)	1.00a (0.08)	0.80b (0.12)	0.90a (0.10)	0.70a (0.12)	0.75a (0.11)	1.30a (0.20)	1.25 (0.25)	10.65a (0.31)	10.50a (0.50)	2.20c (0.49)	1.40a (0.49)	5.30a (0.85)	4.22a (0.79)	15.95a (1.01)	14.72a (0.76)
Resistant strain (LC_{90})	5.13a (0.06)	0.97a (0.06)	1.23c (0.11)	0.83a (0.18)	1.04b (0.03)	2.09c (0.1)	2.02c (0.14)	1.75b (0.15)	13.04b (0.21)	12.63b (0.24)	2.10c (0.36)	2.00b (0.49)	8.23b (0.77)	7.78b (1.24)	21.27b (1.42)	20.41b (1.13)
Control	5.50a (0.08)	0.90a (0.10)	0.60a (0.10)	1.00a (0.08)	0.70a (0.12)	1.20b (0.12)	1.20a (0.12)	1.00a (0.08)	11.10a (0.10)	10.83a (0.17)	1.30b (0.20)	2.50b (0.94)	9.30b (0.87)	7.92b (1.12)	20.4b (1.11)	18.75b (1.03)

[a] From egg to adult. Within columns data followed by the same letter are not statistically different ($LSD_{(\alpha=0.05)}$)

Table 2. Life-table parameters (mean±SE) of susceptible and propargite-resistant strains and reciprocal *F1* hybrids of *Tetranychus urticae* at 22.4°C

Treatment	Birth rate (*b*)	Death rate (*d*)	*b*/*d*	Age structure (%)			GRR offspring	Eggs/ female /d	R_o offspring	r_m/d	Total progeny	Generation time	λ/d	Percent females	Life expectancy	% reaching adulthood
				Egg	Immature	Adult										
Susceptible strain (LC_{50})	0.209a (0.04)	0.033a (0.004)	6.29a (2.3)	76.94c (4.31)	17.48a (3.56)	5.58b (1.2)	105.05h (5.56)	5.08c (1.31)	27.97e (1.04)	0.177bc (0.014)	40.30b (8.84)	18.82ab (1.25)	1.193ab (0.042)	70.45ab (5.63)	14.36a (1.22)	66.67b (3.28)
Control	0.203a (0.04)	0.012a (0.004)	16.4d (2.4)	77.47c (4.96)	16.34a (3.96)	6.19b (1.65)	85.96g (5.32)	5.6bc (1.21)	35.08g (2.31)	0.214e (0.013)	49.80b (18.02)	16.63a (1.05)	1.243bc (0.042)	66.32ab (4.21)	20.00b (1.68)	85.71c (6.98)
Resistant strain (LC_{50})	0.199a (0.03)	0.015a (0.004)	13.58c (3.1)	75.86c (5.21)	17.42a 2.58)	6.72b (1.98)	66.49e (6.21)	5.24c (1.41)	32.60f (1.26)	0.194de (0.009)	48.00b (9.58)	17.92ab (1.22)	1.213c (0.042)	62.26ab (3.35)	16.98a (1.39)	80.30bc (6.35)
Control	0.194a (0.03)	0.016a (0.004)	12.46c (3.3)	76.46c (5.43)	16.89a (3.21)	6.64b (1.76)	75.25f (6.52)	4.75bc (1.02)	30.19f (1.99)	0.188d (0.012)	36.80b (16.94)	18.12ab (1.04)	1.204bc (0.042)	65.38ab (6.33)	20.00b (1.59)	78.57bc (5.68)
$R^{♂} \times S^{♀}$ (LC_{50})	0.163a (0.03)	0.019a (0.004)	8.55b (1.21)	69.77a (3.95)	20.74b (2.54)	10.09d (1.24)	53.98d (6.31)	4.12b (0.95)	13.86b (0.92)	0.153ab (0.015)	24.70ab (6.69)	17.23ab (1.31)	1.163a (0.042)	65.38ab (5.67)	16.75a (1.33)	79.10bc (4.39)
Control	0.178a (0.03)	0.013a (0.004)	13.44c (3.12)	73.61b (4.21)	19.23ab (3.36)	7.16b (1.22)	65.86e (5.41)	4.44bc (1.12)	21.50cd (1.03)	0.174bc (0.021)	40.00b (15.52)	17.65ab (1.15)	1.194ab (0.042)	51.32a (3.69)	21.85b (1.27)	67.47b (5.36)
$S^{♂} \times R^{♀}$ (LC_{50})	0.184a (0.04)	0.023a (0.003)	7.87ab (1.98)	73.78b (5.64)	17.75a (3.85)	8.47c (1.23)	48.74c (6.12)	4.35bc (0.86)	18.38c (0.84)	0.161b (0.012)	38.10b (6.38)	18.14ab (1.53)	1.187a (0.042)	64.86ab (4.06)	16.90a (1.15)	71.15b (3.45)
Control	0.135a (0.04)	0.014a (0.003)	9.5b (2.64)	63.6a (4.58)	24.73c (3.34)	11.66d (1.98)	22.72a (4.12)	2.79a (0.75)	7.09a (0.86)	0.148a (0.023)	18.50a (7.50)	15.23a (0.96)	1.144a (0.042)	46.03a (5.03)	19.71b (1.09)	70.80b (4.66)
Resistant strain (LC_{90})	0.175a (0.04)	0.028a (0.003)	6.31a (1.54)	75.49c (6.7)	20.2b (3.58)	4.31a (1.21)	35.46b (3.56)	3.03a (0.56)	17.13c (1.31)	0.148a (0.022)	24.90ab (5.58)	19.25b (1.22)	1.161a (0.042)	90.32b (6.68)	15.30a (1.62)	44.93a (3.36)
Control	0.194a (0.04)	0.016a (0.003)	12.46c (3.1)	76.46c (6.87)	16.89a (3.46)	6.64b (1.33)	75.25f (5.62)	4.75bc (1.03)	30.18f (1.99)	0.188d (0.012)	36.80b (16.94)	18.12ab (1.04)	1.204bc (0.042)	65.38ab (6.33)	20.00b (1.59)	78.57bc (5.68)

Within columns data followed by the same letter are not statistically different ($LSD_{(\alpha=0.05)}$)

Age-stage, two-sex life table

Age-stage-specific survival rate and stage mortality

The age-stage-specific survival rates (s_{xj}) of TSSM show the probability that a newborn will survive to age *x* and develop to stage *j,* the survivorship and stage differentiation and the variable developmental rate (Figure 1). If untreated, there is 0.6, 0.4, 0.4 and 0.6 probability that a newborn egg of the susceptible strain, propargite-resistant strain, $R♂ \times S♀$ hybrid and $S♀ \times R♂$ hybrid of *T. urticae*, respectively, survived to the female adult stage. The probability remains the same if treated with LC_{50} of susceptible strain. There is 0.2, 0.4, 0.4 and 0.2 probability that a newborn egg of the susceptible strain, propargite-resistant strain, $R♂ \times S♀$ hybrid and $S♀ \times R♂$ hybrid of *T. urticae*, respectively, survived to the male adult stage. The probability decreased if treated with LC_{50} of susceptible strain. The lowest survival rate of a newborn egg to male adult stage is related to TSSM receiving LC_{90} of susceptible strain.

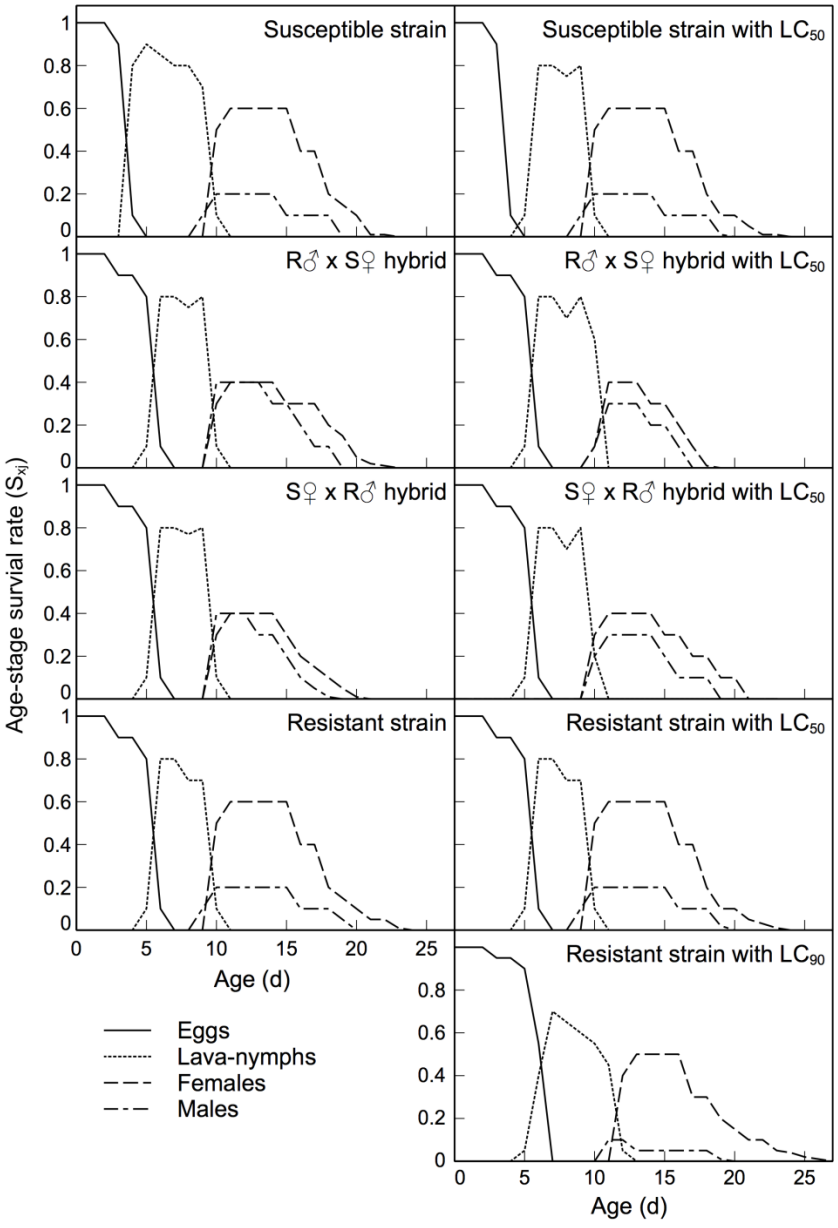

Figure 1. Age-stage-specific survival rate (S_{xj}) of susceptible and resistant strains and *F1* hybrids of *Tetranychus urticae*.

Age-specific survivorship, age and age-stage-specific fecundity

The age-specific survivorship (l_x), mean number of offspring produced by TSSM individuals of the age *x* and stage *j* per d with the age-stage-specific fecundity (f_{xj}) and age-specific fecundity (m_x) of TSSM of strains and hybrids are shown in Figure 2. The start of oviposition of the first female occurred at the age of 10.70, 11.10, 11.29 and 10.65 for the strains and hybrids. Treatment of the resistant strain with LC_{90} of susceptible strain did delay the start of oviposition by 2 d (13.03 d). The highest daily fecundity [peak of *f* (*i*, female)], when untreated, of TSSM for the strains and hybrids was 2.5, 3.0, 3.0 and 2.0 eggs, at the age of 18, 19, 19 and 16, respectively. If treated with LC_{50} of susceptible strain there was a decrease in daily fecundity except the resistant strain. The highest daily fecundity for the resistant strain three times lower when treated with LC_{90} of susceptible strain.

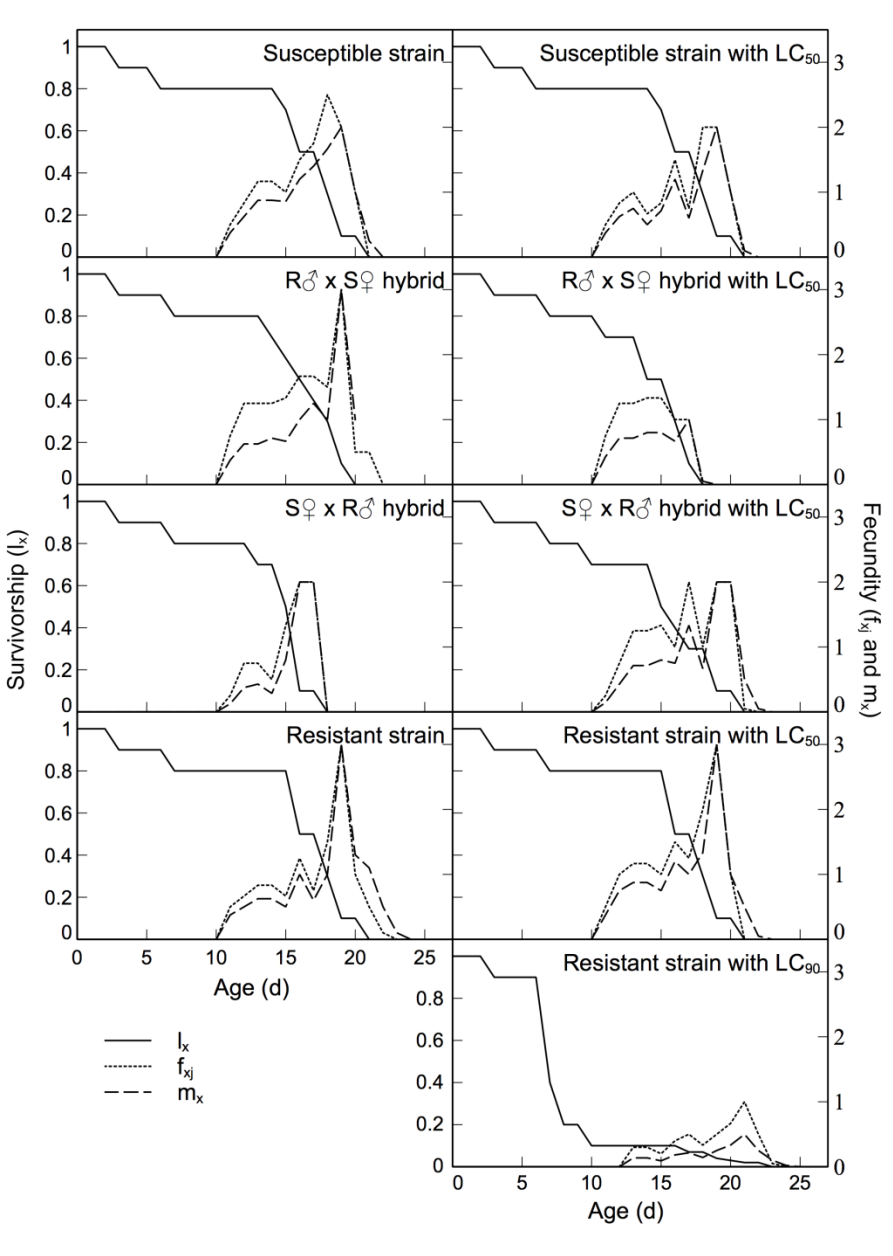

Figure 2. Age-specific survivorship (l_x), age-stage fecundity of female (f_{xj}) (offspring), and age-specific fecundity (m_x) of susceptible and resistant strains and *F1* hybrids of *Tetranychus urticae*, using the age-stage, two-sex life table.

Population projections of the susceptible and propargite-resistant strains and reciprocal *F1* hybrids of *Tetranychus urticae*

Population projections of the number of adult females of *T. urticae* are shown in Figure 3 and the increase or decrease (relative to the control group) in the number of adult females of the susceptible and propargite-resistant strains and *F1* hybrids over 10 generations (using Equation 8) are shown in Figure 4. For the untreated control groups (Figure 3), the susceptible strain gave the highest population projection followed by the propargite-resistant strain, the $R^{\male} \times S^{\female}$ and $S^{\male} \times R^{\female}$ hybrids, respectively. In groups treated with LC_{50} of the susceptible strain (Figure 3), the projected population size after 10 generations showed that the propargite-resistant strain superseded the susceptible strain followed by the $S^{\male} \times R^{\female}$ hybrid, the propargite-resistant strain treated with LC_{99} of susceptible strain and the $R^{\male} \times S^{\female}$ hybrid. Figure 4 clearly shows that the hybrid $S^{\male} \times R^{\female}$ increased most (from treatment with the LC_{50} of the susceptible strain), followed by the propargite-resistant strain, as their numbers increased at each generation (in relation to that of the respective control). The number of adult females decreased with each generation in the susceptible strain and the $R^{\male} \times S^{\female}$ hybrids, as a result of treatment with LC_{50} of the susceptible strain, compared with their untreated group.

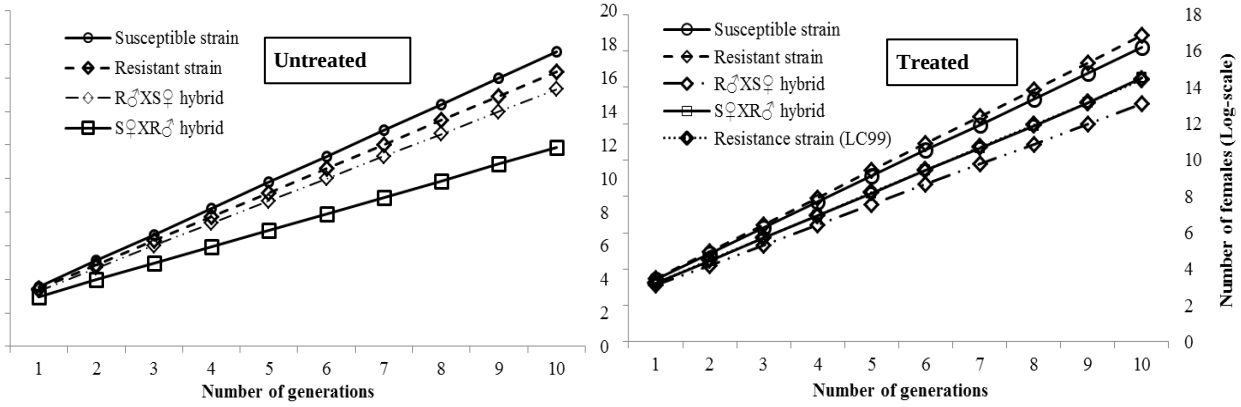

Figure 3. Projected number of untreated and treated *Tetranychus urticae* females of the susceptible and propargite-resistant strains and reciprocal *F1* hybrids after 10 generations.

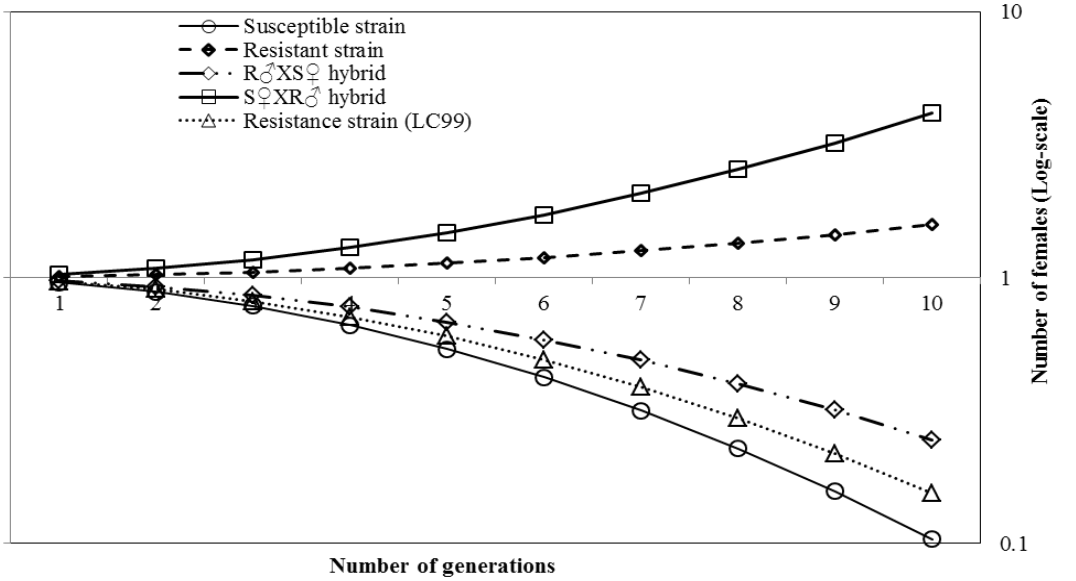

Figure 4. Rate of increase/decrease of number of *Tetranychus urticae* females of the susceptible, propargite-resistant strains and reciprocal *F1* hybrids at each generation treated with LC_{50} of the susceptible strain.

Discussion

Andres (1957) reported a mean developmental time (from egg to adult) of 10.5 and 7.0 d at 24 and 35ºC, respectively, for *T. urticae.* Whereas, Laing (1969) recorded a mean developmental time of 16.9 d for females of *T. urticae* reared on strawberry at an average temperature of 20.3ºC. Carey & Bradley (1982) recorded mean developmental time of 10.5 and 6.2 d at 23.8ºC and 29.4ºC, respectively, for the same species reared on cotton seedlings. Given these data, and assuming a linear developmental rate versus temperature relationship, developmental times of around 11 d would be expected at 22.4ºC as used in this study. Developmental times close to 11 d were recorded for all the strains and hybrids in the control groups and those treated with the LC_{50} of the susceptible strain, and no significant differences were found between these. Several authors have reported no significant difference in the developmental times of the susceptible and resistant strains of different insects, for example, Kasamatsu & Ogawa (1992), Saito et al. (1992) and Omer et al. (1992). Sabelis (1985) suggested that tetranychid mites have been intensively selected for reduced developmental times, and possibly have reached their physiological limit. This may explain why no significant differences were detected in the developmental time among the different strains and hybrids of the control groups.

While the treatment of eggs of both the strains and hybrids with the LC_{50} of the susceptible strain did not significantly affect the developmental time from egg to adult, the treatment of eggs of the propargite-resistant strain with LC_{99} of the susceptible strain did increase the developmental time by about 2 d. No physiological reason for this is evident. All the other strains/hybrids died as a result of this treatment. The higher concentrations (e.g., LC_{99} of susceptible strain) intended to kill spider mites would kill not only the susceptible strain but the heterozygous hybrids as well while the propargite-resistant strain would survive with prolonged developmental time showing pleiotropic effects. This longer developmental time, combined with several other factors shown in Table 2 could possibly decrease the rate of resistance development at any location.

Reported intrinsic rates of increase (r_m) for *T. urticae* differ widely from study to study. Watson (1964) determined r_m to be between 0.202 and 0.256 depending on the age of the host plant. Shih et al. (1976) determined r_m to be 0.336 at 27ºC. Whereas, Laing (1969) reported an r_m of 0.143 at 20.3ºC on cotton seedlings, although Carey & Bradley (1982) reported r_m to be 0.219 at 23.8ºC reared on the same host. Herron & Rophail (1993) reported an r_m values of 0.285 and 0.292 at 28.4ºC and finite rates of increase (λ) of 1.33 and 1.34 for susceptible and clofentezine-hexythiazox-resistant strains of *T. urticae* reared on *P. vulgaris*, respectively. Other researchers have reported the *r*-values of spider mites from 0.212 to 0.480 per d (Razmjou et al., 2008; Sedaratian et al., 2011). In this study the values of r_m was found to be 0.214 and 0.188 per d for untreated susceptible and propargite-resistant strain of *T. urticae.* The finite rates of increase (λ) for the susceptible and propargite-resistant strains of *T. urticae* was 1.243 and 1.204, respectively. The values of both r_m and λ were smaller than those given by Herron & Rophail (1993), which was probably are due to the lower developmental temperature of 22.4ºC.

To estimate the variability of life-table parameters, jackknife and bootstrap techniques are usually used. However, Huang & Chi (2013) reported that the jackknife technique may overestimate the variability. They found that the bootstrap method generated normally distributed estimates and smaller variances. In this study bootstrap technique was used to estimate variability of life-table parameters in two-sex life-table program.

The R_0 value describes the physiological capability of an individual relative to its reproductive capacity. The R_o values of TSSM decreased significantly for the susceptible strain and $R^{\male} \times S^{\female}$ hybrid treated with LC_{50} of susceptible strain, however its value increased for the $S^{\female} \times R^{\male}$ hybrid (from 7.09 to 18.38). The R_o value for TSSM was reported as 11.25 on bean, 29.13 on cowpea and 53.84 on soybean (Razmjou et al., 2009).

The value of r_m for the susceptible strain and the $R^{\male} \times S^{\female}$ hybrid treated with LC_{50} of susceptible strain and the propargite-resistant strain treated with LC_{99} of susceptible strain decreased compared with that of the untreated controls, whereas, that of the propargite-resistant strain and $S^{\male} \times R^{\female}$ treated with LC_{50} of the susceptible strain increased. Wrensch (1985) suggests that such differences, although small, are sufficient for differential success within a species. Roush & McKenzie (1987) were also of the opinion that although the difference in fitness may be small, it is important to determine whether such selective disadvantages are sufficiently large to be useful in practical situations. Figure 4 clearly shows that small (possibly statistically nonsignificant) differences as found in this study have biological significance. If each generation of *T. urticae* receives sublethal concentrations of propargite (e.g., LC_{50} of the susceptible strain), the number of females of the propargite-resistant strain and the hybrid $S^{\male} \times R^{\female}$ would continue to increase with each generation while those of the susceptible strain and $R^{\male} \times S^{\female}$ hybrids would decline increasing the resistance frequency each generation. Also, Smirnova (1987) found that the progeny of resistant females and sensitive males ($S^{\male} \times R^{\female}$) of carbaryl-resistant tick, *Hyalomma plumbeum* (Panzer, 1795), were resistant and resistance increased from generation to generation. Inadequate spray coverage of propargite, possibly producing sublethal effects, could also increase resistance frequency. If each generation of *T. urticae* is treated with higher concentrations intended to kill most of the mites (e.g., LC_{99} of susceptible strain or higher) then all the susceptible and hybrid mites will most likely die. However, the numbers of the propargite-resistant strain would still increase, but at slower rate compared with the untreated resistant strain. Under field conditions, areas poorly sprayed (inadequate coverage) and/or inadequate dosage can be common (Hoy et al., 1998). As a result, in crops where propargite-resistant mites occur, this would increase the chances of an increase in resistance frequency after each application of propargite compared with a field properly sprayed with the right dose where resistance build up would be relatively slow.

The changing frequency/percentage of adult females of the susceptible and propargite-resistant strains and hybrids, based on their differential success (difference in the values of r_m), and propargite application at each generation will continue to change the resistance frequency throughout a growing season at a particular location. For example, Dennehy & Granett (1984) reported an increase in the proportion of locations with detectable levels of dicofol-resistant spider mites late in the cotton growing season. Any decrease in resistance frequency at a location throughout a growing season may be the result of the resistance management strategy in place but any increase in resistance frequency, with continuous selection pressure, could possibly be the result of the differential success of different strains within a species. Application of any selective force, together with a lower number of adults exhibiting dispersal behavior (Shah & Worner, 2018) would then help create areas of intense infestation. Similarly, as Wang et al. (2010) stated, an appropriate resistance management strategy could promote reversion of the resistant populations back to susceptibility.

This study demonstrated that the duration of different life stages changes according to the propargite dosage applied to the field. In case of inadequate dosage (e.g., 0.006% ai) there is higher probability that the propargite-resistant strain and $S^{\male} \times R^{\female}$ hybrid will produce more progeny and increase in population will occur at a higher rate. Depending upon the prevalent resistance frequency, an adequate dosage (e.g., 0.032% ai) may kill the susceptible individuals and hybrids present in the field but the propargite-resistant individuals may still continue to reproduce (although at a slower rate). These finding could be incorporated into any new or existing integrated resistant management program.

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Original article (Orijinal araştırma)

Cannibalistic behavior of aphidophagous coccinellid, *Hippodamia variegata* (Goeze, 1777) (Coleoptera: Coccinellidae)[1]

Afidofag coccinellid, *Hippodamia variegata* (Goeze, 1777) (Coleoptera: Coccinellidae) 'nın kannibalistik davranışları

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Abstract

The variegated lady beetle, *Hippodamia variegata* (Goeze, 1777) is widely distributed in different agroecosystems of Turkey. Cannibalism, intraspecific predation, where individuals of the same species feed upon each other, is a common phenomenon in most aphidophagous coccinellids including *H. variegata.* We investigated the cannibalistic behavior of various growth stages of *H. variegata* in the presence and absence of *Acyrthosiphon pisum* Harris, 1776 under laboratory conditions. The results for cannibalism of eggs and larvae by adults revealed that eggs and younger larvae were more vulnerable to cannibalism. Notably, egg cannibalism by adults was found to be higher even at high prey abundance. Whereas, larval cannibalism was found significantly lower. Cannibalism of eggs by larvae and within the larvae showed that older larvae consumed significantly higher number of eggs and younger larvae in the absence of *A. pisum* indicating that cannibalism was mainly influenced by scarcity of prey. However, all the larval instars, especially 4th and 3rd instars, also consumed a substantial number of eggs even in the presence of prey. Cannibalism within the same stage/age larvae showed a successive increase with the successive larval stage showing minimum cannibalism by 1st instar larvae and maximum by 4th instar larvae. The study found that scarcity of prey leads to cannibalism in *H. variegata* and that egg cannibalism occurs even at high prey densities.

Keywords: *Acyrthosiphon pisum*, cannibalism, eggs, *Hippodamia variegata,* larval instars

Öz

Afidofag coccinellid, *Hippodamia variegata* (Goeze, 1777), Türkiye'nin farklı agroekosistemlerinde yaygın olarak bulunmaktadır. Aynı türün bireylerinin birbirlerine besledikleri kannibalizm, intraspesifik avcılık, *H. variegata* da dahil olmak üzere birçok afidofag coccinellidlerde yaygın olan bir davranıştır. Bu çalışmada laboratuvar koşullarında *Acyrthosiphon pisum* Harris, 1776 varlığında ve yokluğunda *H. variegata*'nın ergin ve ergin öncesi dönemlerine ait kannibalizm davranışları araştırılmıştır. Erginler tarafından yumurta ve larva kannibalizmi üzerine yapılan denemelerde, yumurta ve genç larvanın kannibalizme karşı daha savunmasız olduğunu ortaya koymuştur. İlginç şekilde, erginler tarafından yumurta kannibalizmi, avın bolluğunda dahi belirgin şekilde daha yüksek bulunurken, larva kannibalizmi önemli ölçüde azalmıştır. Larvaların yumurta kannibalizmi ve larva içerisindeki kannibalizm sonuçları, olgun larvaların yaprak biti yokluğunda daha fazla sayıda yumurta ve daha genç yaşta larva tükettiklerini ve avın olmamasının kannibalizme neden olan başlıca etken olduğunu göstermiştir. Bununla birlikte, tüm larva dönemlerinde, özellikle 3. ve 4. larva dönemlerinde, yaprak biti varlığında bile önemli miktarda yumurta tükettikleri saptanmıştır. Aynı yaştaki larvalar arasındaki kannibalizmde, larva dönemleri ile kannibalizm arasında gençlerden yaşlılara doğru olmak üzere doğru orantılı bir korelasyon olduğu belirlenmiştir. Araştırmalarımız, avın azlığının *H. variegata*' da kannibalizme neden olduğunu ancak yumurta kannibalizminin avın yüksek yoğunluklarında bile gerçekleştiğini ortaya koymuştur.

Anahtar sözcükler: *Acyrthosiphon pisum*, kannibalizm, yumurta, *Hippodamia variegata,* larva dönemleri

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Introduction

The predacious coccinellids (Coleoptera: Coccinellidae) are well-known beneficial insects which are important voracious feeders of many pests of economic importance, like aphids, leaf hoppers, jassids, thrips, whiteflies, scale insects, mealybugs, mites, lepidopteran insects and other soft bodied insects in natural and agricultural habitats (Dixon, 2000; William, 2002; Silva et al., 2009; Khan et al., 2009; Shah & Khan, 2014).

Palearctic coccinellid species, *Hippodamia (Adonia) variegata* (Goeze, 1777) is a widespread aphidophagous predator in many parts of the world including different agroecosystems of Turkey. It feeds on many insect pests including aphids, whiteflies, jassids, psyllids, mealybugs and lepidopteran insects (Franzman, 2002; Kontodimas & Stathas, 2005). It has been efficiently used in biological control program against a number of insect pests especially aphids. Cannibalism, intraspecific predation, is a well-known behavior of predaceous coccinellids including *H. variegata* and is one of the important mortality factors of coccinellids (Osawa, 1989; Khan et al., 2003; Rondoni et al., 2012; Jafari, 2013).

In aphidophagous coccinellids, cannibalism is an important evolutionary behavior during times of food scarcity that enable them to survive and complete their development. Aphidophagous coccinellids are usually more prone to cannibalism compared to coccidophagous coccinellids as the latter encounter each other less frequently and more likely complete their development before the population collapse of their prey (Agarwala & Dixon, 1992; Dixon, 2000). The eggs and younger larvae appeared to be more helpless against older larvae and adults and are easily devoured (Dimetry, 1974; Hodek, 1996; Nakamura et al., 2006). Cannibalistic behavior of coccinellid adults and older larvae like feeding on eggs, younger or same stage larvae, prepupae/pupae is common in the field. Cannibalism of unhatched eggs in the same egg cluster by the newly hatched first instar larvae is very common and is regarded as sibling cannibalism. This sibling egg cannibalism in ladybirds before dispersion avoid starvation risk and may have adaptive significance in term of increased survival and development by providing essential nutrients and critical energy (Kawai, 1976; Wratten, 1976; Roy et al., 2007).

The adults and larvae of aphidophagous coccinellids have been reported to eat conspecifics in the field (Agarwala & Dixon, 1992; Hindayana et al., 2001; Nakamura et al., 2006; Omkar et al., 2007). The scarcity or presence of extraguild prey greatly influences the intensity of cannibalism (Rosenheim et al., 1995; Lucas et al., 1998; Rondoni et al., 2012). This means that the rate of cannibalism is somehow inversely proportion to the availability of natural food. The abundance of prey also lowers the relative frequency of encounter of adults and older larvae with eggs and smaller larvae leading to minimize the rate of cannibalism (Agarwala & Dixon, 1992; Dixon, 2000; Aleosfoor et al., 2014). However, some research has also revealed cannibalism even in the presence of prey (Osawa, 1989; Agarwala, 1991). Aleosfoor et al. (2014) reported that the rate of cannibalism under starvation was enormously higher in the variegated lady beetle, *H. variegata* compared to satiation conditions and that the rate of cannibalism in *Coccinella septempunctata* (L., 1758) was greater and more frequent than that of *H. variegata*.

A variety of research has been conducted on various aspects of cannibalism in *H. variegata*. However, the present study was designed to conduct detailed investigations on cannibalism in controlled conditions in order to provide a complete set of information on the cannibalistic behavior of *H. variegata* under laboratory conditions.

Material and Methods

Rearing of pea aphid, *Acyrthosiphon pisum*

The pea aphids, *Acyrthosiphon pisum* Harris, 1776 were collected from lucerne and clover and brought to the laboratory. Rearing was maintained on broad bean, *Vicia faba* L., plants grown in sterilized soil in 500 ml pots under laboratory conditions (23±1ºC and 65±5% RH).

Rearing of *Hippodamia variegata*

Adults of *Hippodamia variegata* were collected from lucerne, clover, plum, wheat and weeds (grown at the experimental farm of the Faculty of Agriculture, Ege University, Izmir, Turkey) and brought to the laboratory. They were kept in plexiglass cages (20 cm high by 15 cm in diameter) with ventilation holes, two on the side and one in the lid screened with fine mesh. The jars were kept in wooden framed

cage (100 x 70 x 75 cm) in a 16:8 h L:D photoperiod. The aphids were regularly provided to these beetles on their host leaves. The eggs laid by females were collected and transferred to other cages for hatching. Similarly, the larvae emerged from eggs were transferred to plexiglass cages with abundant supply of aphids on leaves. The rearing was maintained at 24±1ºC, 65±5% RH and 16:8 h L:D photoperiod.

Cannibalism of eggs and larvae by adults

Cannibalism of eggs and larvae by adults was accomplished by keeping 24-h starved male and female (Agarwala & Dixon, 1992) adult beetles individually in Petri dishes (9 cm for eggs and 15 cm for larvae). Each adult (male or female) was provided 100 conspecific eggs with and without 80 aphids, 60 1st instar larvae newly emerged from eggs with and without 350 aphids, or 35 2nd instar larvae with and without 350 aphids for 24 h. The experiments included 10 replicate adult males and females for each treatment. Petri dishes were examined after 24 h for evidence of cannibalism.

Cannibalism of eggs by larvae

In this experiment all the four larval instars were kept individually in Petri dishes (9 cm) and starved for 12 h to standardize their appetite (Yasuda et al., 2001; Khan et al., 2003). Known numbers of the conspecific eggs were transferred with the help of soft camel hair brush to the Petri dishes. The different numbers of conspecific eggs presented to each larval instar were: 1st instar 20 eggs, 2nd instar 30, 3rd instar 40 and 4th instar 100. The number of eggs consumed by larvae was recorded after 24 h. In another experiment the same number of conspecific eggs were provided to all the four larval instars along with different densities of aphids (20, 30, 40 and 80 for 1st, 2nd, 3rd and 4th instars, respectively) in order to record cannibalism in the presence of natural food. All treatments in the experiments consisted of 10 replicates.

Cannibalism within the larval stages

To determine cannibalism within larvae, two experiments were conducted. In first experiment cannibalism of younger larvae by older was investigated in the presence and absence of aphids. The 4th, 3rd and 2nd larval instars were kept singly in 15-cm Petri dishes and starved for 12 h to standardize their appetite. Well-fed 1st and 2nd larval instars (60 and 35, respectively) were provided with 4th instar larvae in separate Petri dishes in the presence (350 aphids for each of 1st and 2nd instars) and absence of aphids. Similarly, 1st and 2nd instar larvae (35 and 20, respectively) were provided with 3rd instar larvae in the presence (250 aphids for each of 1st and 2nd instars) and absence of aphids, and 20 1st instar larvae were given to 2nd instar larvae (n = 10) with and without 100 aphids. Examination for evidence of cannibalism was made after 24 h. In the second experiment, same age larvae of each stage (1st, 2nd, 3rd and 4th) were starved for 12 h as above and then kept together for 24 h in 12-cm Petri dishes in the presence and absence of prey. The aphid densities provided were 70, 100, 250 and 500 aphids for each of the Petri dishes with 10 1st, 2nd, 3rd and 4th larval instars, respectively.

The cannibalistic behavior of *H. variegata* was studied at 24±1ºC and 16:8 h L:D photoperiod. Similar aged adult beetles (male and female), different larval instars and eggs used in the experiments were taken from stock culture maintained on *A. pisum*.

Statistical analysis

Data on the cannibalistic behavior of *H. variegata* were subjected to one-way analysis of variance using statistical software SPSS, 2008. Multiple comparison among the means was made using Tukey's HSD test ($P < 0.05$). Independent sample t-test was used where two variables were involved.

Results and Discussion

Cannibalism of eggs by adults

The data on cannibalism of eggs by adult male and female of *H. variegata* revealed that females consumed significantly higher mean number of eggs (95.3±1.53) than males (52.7±1.39) in the absence of aphids (Figure 1) The availability of high density of *A. pisum* resulted in a significant reduction in the conspecific egg consumption, however, it was still substantially higher especially for females, which consumed significantly more eggs (32.8±1.86) than males (19.4±1.59) ($F = 427.23$, $df = 3$, $P < 0.001$).

Cannibalism of 1st and 2nd instar larvae by adults

The data on cannibalism of 1st and 2nd instar larvae by adult males and females of *H. variegata* revealed that both the sexes were voracious towards 1st instar larvae. However, adult females showed a more vigorous response towards 1st and 2nd instar larvae in absence of aphids, consuming on average 52.5±1.5 and 31.5±0.9 larvae, respectively, which was significantly greater than that of adult males (46.2±1.59 and 26.3±0.76, respectively) ($F = 455$, $df = 7$, $P < 0.001$). The presence of aphids caused a drastic reduction in consumption of larvae and negligible amounts of 1st and 2nd instar larvae were consumed by both adult females and males (Figure 2).

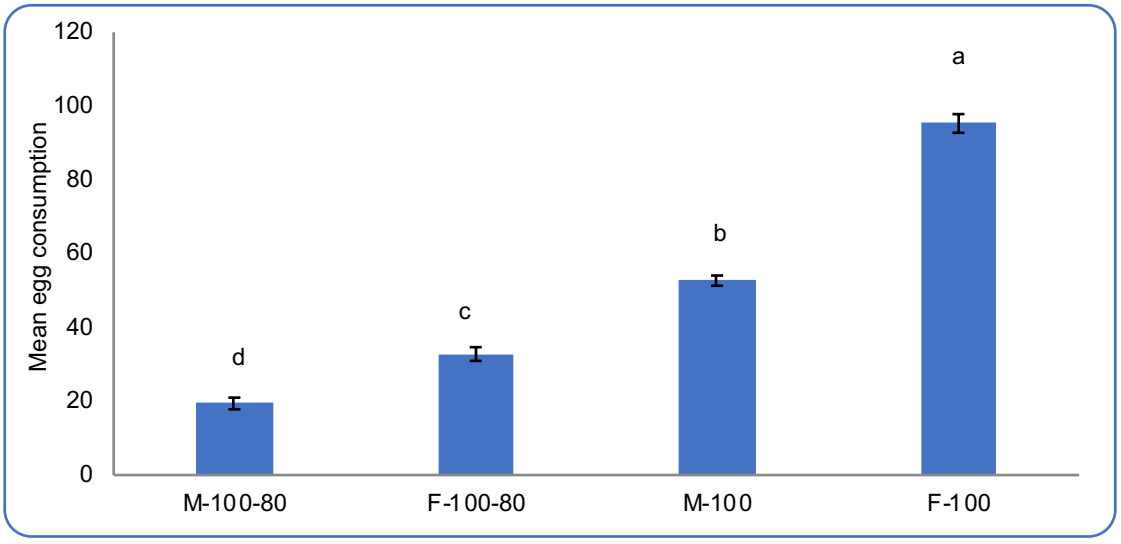

Figure 1. Cannibalism of conspecific eggs by adult male and female of *Hippodamia variegata*. Each bar represents mean egg consumption (±SEM). M-100 = 100 eggs provided to adult males, F-100 = 100 eggs provided to adult females, M-100-80 = 100 eggs along with 80 *Acyrthosiphon pisum* provided to males, and F-100-80= 100 eggs along with 80 *A. pisum* provided to females. Different letters above the bars indicate significant differences ($P < 0.05$).

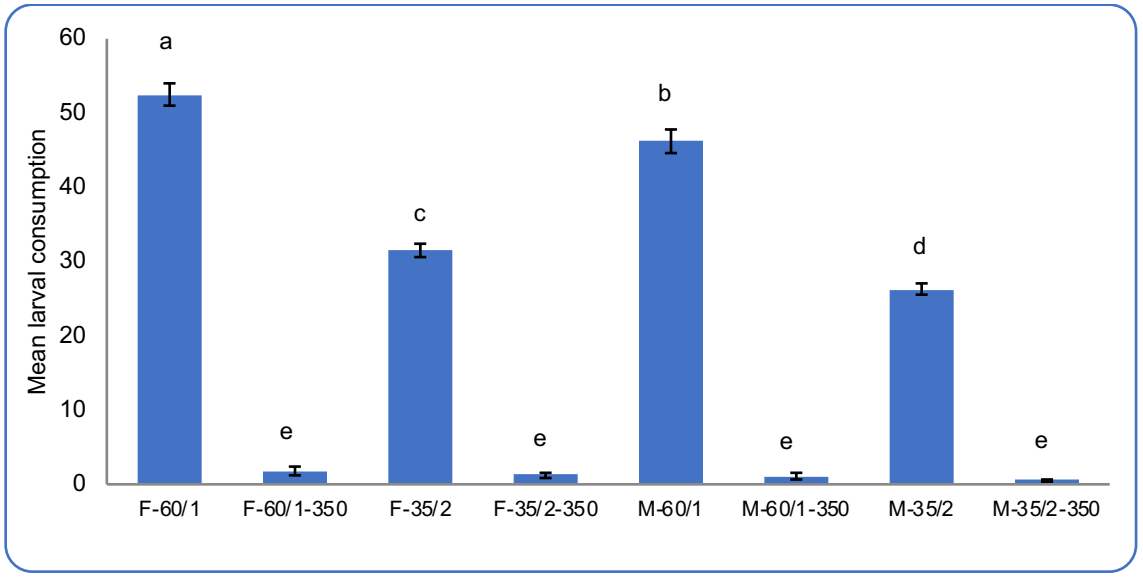

Figure 2. Cannibalism of 1st instar and 2nd instar larvae by male and female of *Hippodamia variegata*. F-60/1 = 60 1st instar larvae provided to adult females, F-60/1-350 = 60 1st instar larvae provided to adult females along with 350 *Acyrthosiphon pisum*, F-35/2 = 35 2nd instars provided to females, F-35/2-350 = 35 2nd instar larvae provided to females along with 350 *A. pisum*, M-60/1 = 60 1st instars provided to adult males, M-60/1-350 = 60 1st instar larvae provided to male with 350 *A. pisum*, M-35/2 = 35 2nd instars provided to males, and M-35/2-350 = 35 2nd instar larvae provided to male along with 350 *A. pisum*. Different letters above the bars indicate significant differences ($P < 0.05$).

Cannibalism of eggs by larvae

The rate of cannibalism by the four larval instars of *H. variegata* on the conspecific eggs varied greatly with significant differences among the different growth stages (Figure 3). In the absence of prey, 4th instar larvae proved to be the most voracious consuming significantly higher number of eggs (86.1±1.89), followed by 3rd instar larvae (29.4±1.22), during the 24-h exposure period ($F = 451$, $df = 7$, $P < 0.001$). The presence of aphids significantly reduced egg consumption by all the larval instars, however, the 4th instar larvae showed some degree of preference for the eggs even in presence of aphids consuming 25.8±2.4 eggs. The 3rd instar larvae consumed 9.37±0.84 eggs in the presence of aphids, this was not significantly different from the number of eggs consumed by 2nd instar larvae in the absence of aphids (11.6±0.86). The lowest number of eggs was consumed by the 1st instar larvae (5.37±0.46), but this was insignificantly different from its consumption of eggs in presence of aphids (2.37±0.56) (Figure 3).

Cannibalism within larvae

The data on cannibalism of younger larvae by older larvae of *H. variegata* revealed that all the starved older larval stages voraciously fed on younger larvae (Figure 4). The 4th instar larvae consumed a significantly higher number of 1st and 2nd instar larvae (51.7±1.55 and 29.7±1.10, respectively) in absence of prey followed by 3rd instar larvae with average consumption of 26.8±1 and 16.7±0.66, respectively ($P < 0.05$). The 2nd instar larvae consumed significantly less 1st instar larvae (5.1±0.31) in absence of *A. pisum* ($F = 548$, $df = 9$, $P < 0.001$). The presence of a high density of aphids caused a marked reduction in larval consumption by the 2nd, 3rd and 4th instars with only a negligible number of larvae consumed (Figure 4).

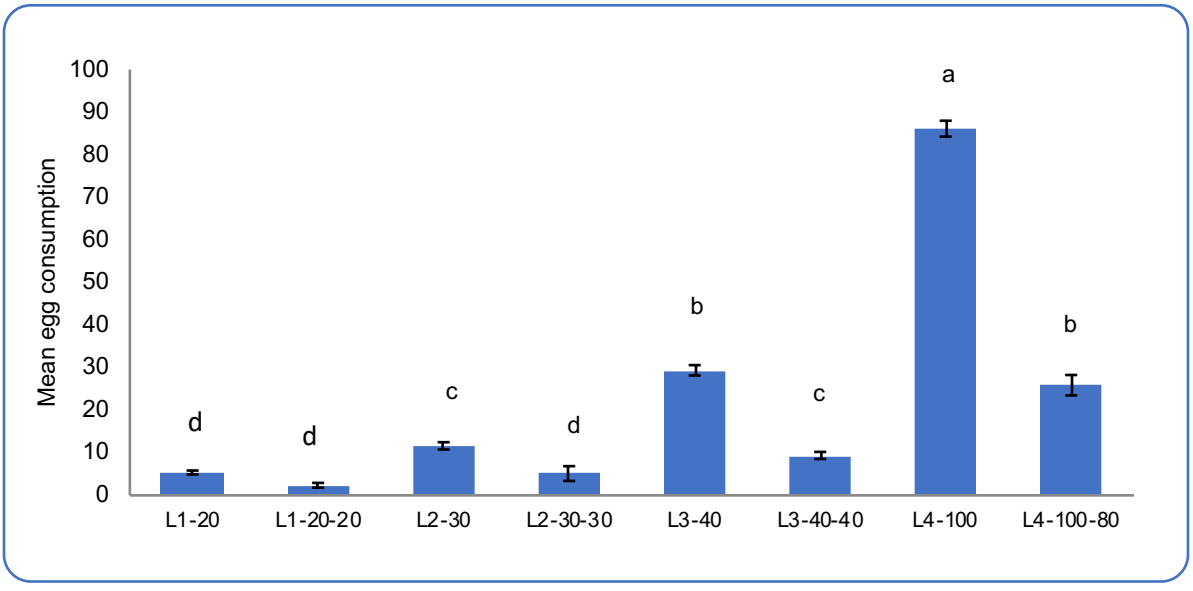

Figure 3. Cannibalism of eggs by different larval instars of *Hippodamia variegata* in the presence and absence of aphids. L1-20 = 20 eggs provided to 1st instars, L1-20-20 = 20 eggs along with 20 *Acyrthosiphon pisum* provided to 1st instars, L2-30 = 30 eggs provided to 2nd instars, L2-30-30 = 30 eggs along with 30 *A. pisum* provided to 2nd instars, L3-40 = 50 eggs provided to 3rd instars, L3-40-40 = 50 eggs along with 60 *A. pisum* provided to 3rd instars, L4-100 = 100 eggs provided to 4th instars, L4-100-80 = 100 eggs along with 80 *A. pisum* provided to 4th instars. Different letters above the bars indicate significant differences ($P < 0.05$).

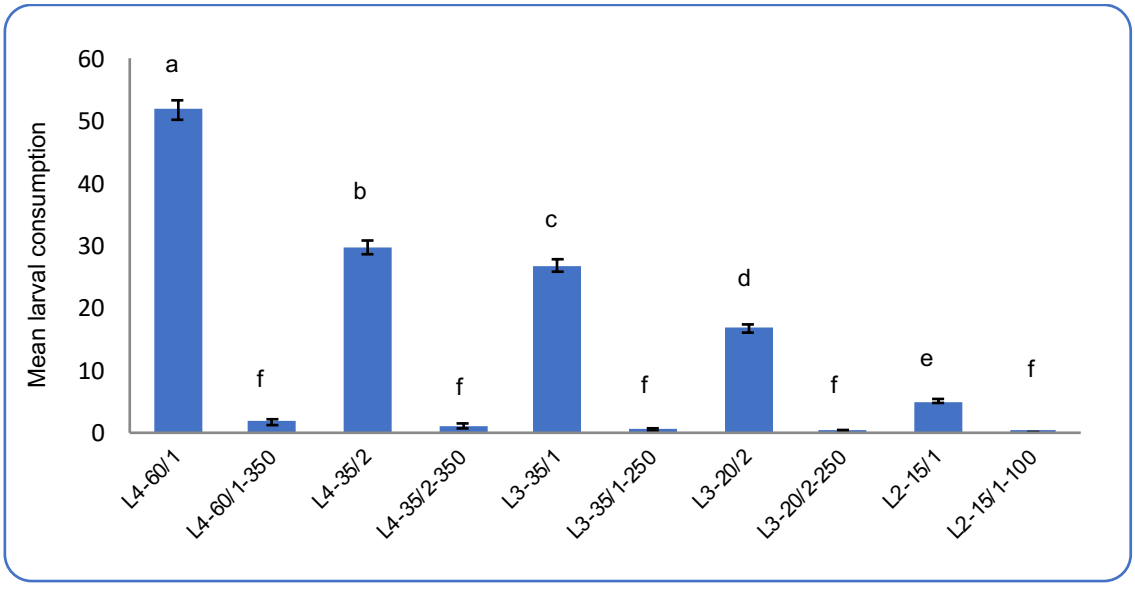

Figure 4. Cannibalism of younger larvae by older ones. L4-60/1 = 60 1st instars provided to 4th instars, L4-60/1-350 = 60 1st instars provided to 4th instars with 350 *Acyrthosiphon pisum*, L4-35/2 = 35 2nd instars provided to 4th instars, L4-35/2-350 = 35 2nd instars provided to 4th instars along with 350 *A. pisum*, L3-35/1 = 35 1st instars given to 3rd instars, L3-35/1-250 = 35 1st instars provided to 3rd instars with 250 *A. pisum,* L3-20/2 = 20 2nd instars provided to 3rd instars, L3-20/2-250 = 20 2nd instars given to 3rd instars with 250 *A. pisum*, L2-15/1 = 15 1st instars provided to 2nd instars, and L2-15/1-100 = 15 1st instars provided to 2nd instars along with 100 *A. pisum*. Different letters above the bars indicate significant differences ($P < 0.05$).

Cannibalistic behavior of the same age larvae of *H. variegata* in the absence of *A. pisum* increased successively with larval stage. The highest ($P < 0.05$) cannibalism rate of 3.7±0.30 was recorded for 4th instar larvae. The rate of cannibalism within 3rd (2.8±0.29) and 2nd instar larvae (2.6±0.33) was not significantly different. The same was the lowest ($P < 0.05$) for the 1st instar larvae (1.7±0.42). In the presence of aphids, cannibalism was negligible and not statistically different between the same age larvae of the 4 instars (Figure 5).

These results revealed that cannibalism in *H. variegata* took place mainly when aphid prey was scarce and the predators were starving. Eggs and younger larvae appeared to be the most vulnerable stages which were consumed in large numbers by both the adults and older larvae. Many researchers report that egg and younger larval cannibalism is a widespread phenomenon in predacious ladybirds (Takahashi, 1987; Osawa, 1989, 1992; Agarwala & Dixon, 1992; Kajita et al., 2010). The sibling cannibalism where newly hatched larvae in the same egg cluster cannibalize the sibling unhatched eggs is also well known in the ladybirds (Hodek, 1996; Agarwala, 1991). A cannibalizing predator has an advantage by increasing its chances of survival in times of food scarcity, and also by killing or eradication of a potential competitor sharing similar resources.

Our results confirmed that all the growth stages fed on the conspecifics in the absence of aphids indicating that the presence or absence of alternative food source influences the frequency of cannibalism (Osawa, 1989; Burgio et al., 2002). Whereas, the provision of high density of natural prey led to a marked reduction in the rate of cannibalism by all the growth stages of *H. variegata*. This is consistent with the findings of Agarwala (1991) and Aleosfoor et al. (2014), who reported that collapse of the aphid population in an ecosystem exerts great pressure on larvae and adults of coccinellids to survive, consequently unhatched eggs or minor larvae become the readily available food source.

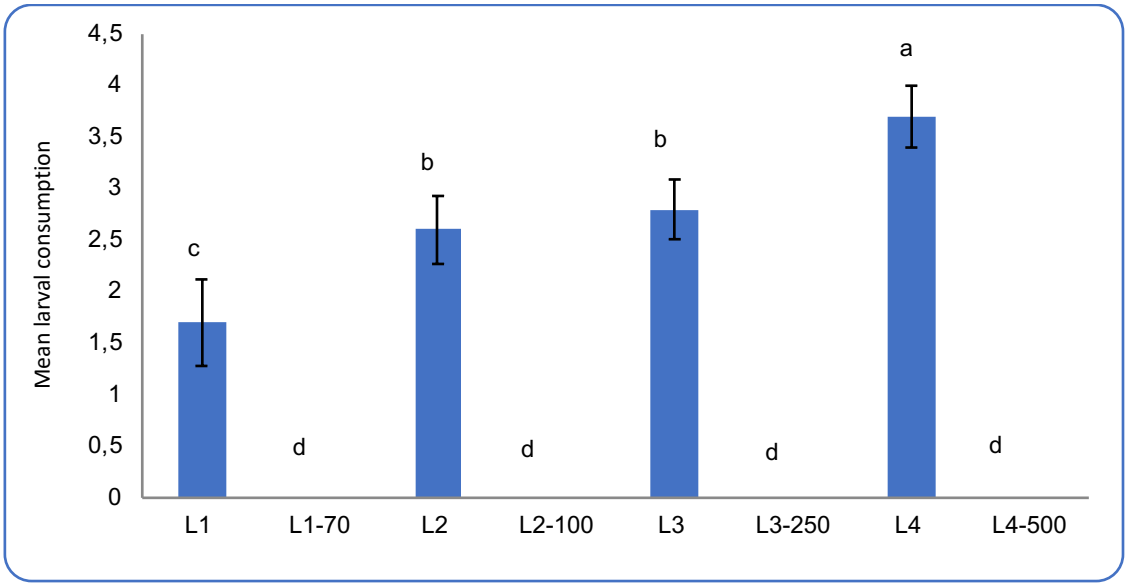

Figure 5. Cannibalism within the same age larvae. L1 = 10 1st instars alone, L1-70 = 10 1st instars along with 70 aphids, L2 = 10 2nd instars alone, L2-100 = 10 2nd instars along with 100 aphids, L3 = 10 3rd instars alone, L3-250 = 10 3rd instars along with 300 aphids, L4 = 10 4th instars alone, and L4-500= 10 4th instars along with 500 aphids. Different letters above the bars indicate significant differences ($P < 0.05$).

Adult females appeared to be more voracious towards eggs and younger larvae in the absence of prey followed by 4th instar larvae and adult males. The egg consumption of males was, however, far lower than that of adult females and 4th instar larvae (Figure 1). The greater voracity of these growth stages for conspecific eggs and larvae can be attributed to their larger size (Agarwala & Dixon, 1992; Cottrell, 2005; Sato et al., 2011; Lucas, 2012; Jafari, 2013) and higher nutritional requirements, especially in case of reproductive females and 4th instar larvae completing their development (Fox, 1975; Polis, 1981; Takahashi, 1987; Hodek & Honek, 1996).

It is noteworthy that all the growth stages of *H. variegata* showed some degree of preference for the conspecific eggs even when there was an abundance of prey. The number of eggs eaten by 1st instar larvae in the absence of aphids was not significantly different than when aphids were present. Adult females and males, and 4th instar larvae, in particular, consumed substantial amount of the conspecific eggs even in the presence of aphids, with the most voracious response exhibited by adult females. This could be attributed to eggs being immobile and unable to escape (Takahashi, 1987; Agarwala, 1991; Hodek & Honek, 1996). Agarwala (1991) reported higher rates of egg cannibalism in ladybirds at lower prey densities. Also, non-sibling cannibalism of eggs in ladybirds mostly happens without the influence of prey availability (Fox, 1975; Polis, 1981; Mills, 1982; Osawa, 1989).

The phenomenon of egg cannibalism in ladybirds, including *H. variegata,* has also been regarded as a mean of nutritional and energetic gains in term of larger body size, faster development, earlier maturity, competitive advantage in mating and higher rate of reproduction (Osawa, 1992; Felix & Soares, 2004; Michaud & Grant, 2004; Omkar et al., 2004; Pell et al., 2008; Rondoni et al., 2014). Poor diet quality also exerts pressure on predators to feed on their conspecifics. However, there appears to be no published information on the nutritional quality of *A. pisum* for *H. variegata* or other coccinellids.

The higher egg consumption by females in the presence of prey also indicates that the females are more sensitive than males to resource limitation, and hence get maximum benefit from egg cannibalism. Another reason could be the higher amount of energy required by female for reproduction (Michaud & Grant, 2004). There is also some evidence that the coccinellids do not tend to lay their eggs close to the

aphid colony (Banks, 1954; Dixon, 1959; Osawa, 1989) as near the aphid colony the risk of being cannibalized is higher (Osawa, 1989). However, Dixon (1959) reported that ladybirds will oviposit in the vicinity of an aphid colony to enable their progeny to easily access the food. Cannibalism of eggs by the adults and larvae has also been widely recorded in the field in several species of ladybirds (Mills, 1982; Takahashi, 1989).

It is also worth noting that coccinellid eggs are protected from predation by the presence of defensive alkaloids and other chemicals. Hemptinne et al. (2000) reported many compounds found on the egg surface of *Adalia bipunctata* (L. 1758) and *C. septempunctata*, which serve to reduce predation by other coccinellid predators. However, in most coccinellids egg cannibalism is widespread as there are no disadvantages linked with the consumption of conspecific eggs with a positive impact on development and survival (Felix & Soares, 2004; Ware & Majerus, 2008; Kajita et al., 2010). Similarly, Osawa (1989), Agarwala (1991), Agarwala & Dixon (1992), Agarwala et al. (1998), Michaud (2002), Omkar et al. (2006), Ware & Majerus (2008) and Kajita et al. (2010) reported that the coccinellid predators tend to prey on conspecific eggs instead of heterospecific eggs. This may probably be due to the occurrence of alkanes and alkaloids on the surface of conspecific eggs. These surface chemicals seemingly act as feeding attractants for individuals of same species and hence increase cannibalism to some extent (Omkar et al., 2004).

Base on the data collected, it is concluded that cannibalism in *H. variegata* is inversely proportional to the availability of prey. Adult females and males, and 4th instar larvae are voracious feeders of eggs and younger larvae, and that egg cannibalism occurs irrespective of the aphid presence.

Acknowledgments

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Original article (Orijinal araştırma)

Determination of the changes in the process of degradation of some pesticides applied in mixtures with plant growth regulators, foliar fertilizers and spreader-stricker in a vineyard[1]

Bağda bitki gelişim düzenleyicisi, yaprak gübresi ve yayıcı-yapıştırıcılarla karıştırılarak uygulanan bazı pestisitlerin parçalanma sürecindeki değişimlerin belirlenmesi

Gülçin Pınar ÖRNEK[2*] **Enver DURMUŞOĞLU[3]**

Abstract

The study conducted in 2015-2016 examined effects of plant growth regulators (PGR), foliar fertilizers (FF) and spreader-sticker (SS) on the process of degradation of pesticides. First physical then chromatographic analyses were conducted in laboratory to determine whether degradation occurred even in spray tanks. In order to determine if changes occurred during the process of decomposition of pesticides in mixtures under field conditions, two experiments were set up in Izmir during the shooting and fruiting of grapevines in 2016. In shooting period, a mixture of azoxystrobin, imidacloprid and metalaxyl was blended with PGR, FF and SS in double and triple combinations to create eight treatments applied to three replicates to determine the process of degradation of active ingredients on leaf samples collected at given intervals. In general, it was observed that degradation of azoxystrobin, imidacloprid and metalaxyl on leaves applied with PGR, FF and SS was slower and the residues did not drop below maximum residue limits even after the preharvest interval. During the fruiting period, a mixture of boscalid, chlorpyrifos ethyl and hexythiazox was applied in the same way as for the shooting period. All mixtures with PGR, FF and SS tended to increase degradation of boscalid, but had no effect on the degradation on chlorpyrifos ethyl and hexythiazox.

Keywords: Foliar fertilizer, pesticide, plant growth regulator, preharvest interval, spreader-sticker, tank mix

Öz

2015-2016 yıllarında yürütülen bu çalışmada, asma yapraklarını toplama ve üzüm hasadı öncesi dönemlerde kullanılan bazı pestisitler ile bitki gelişim düzenleyicisi (BGD), yaprak gübresi (YG) ve yayıcı-yapıştırıcıların (YY) pestisitlerin parçalanma sürecine etkisi araştırılmıştır. Bağda karışım halinde kullanılan pestisitlerin, ilaçlama alet deposunda parçalanma olup olmadığının belirlenmesi amacıyla laboratuvarda gerçekleştirilen önce fiziksel sonra da kromatografik analizlerde gerek pestisitlerin birbirleriyle gerekse diğer preparatlar ile karışımlarında herhangi bir önemli etkileşim görülmemiştir. Karışım halinde kullanılan pestisitlerin arazi koşullarında parçalanma sürecinde değişim olup olmadığını belirlemek üzere 2016 yılında İzmir'in Kemalpaşa ilçesindeki bir bağda iki farklı dönemde iki deneme kurulmuştur. Asmanın yaprak toplama döneminde azoxystrobin, imidacloprid ve metalaxyl içeren preparatların üçlü karışımı BGD, YG ve YY ile tekli, ikili ve üçlü olarak karıştırılarak 8 karakterli bir deneme üç tekerrürlü olarak uygulanmış, amaca uygun aralıklarla alınan yaprak örneklerinde aktif maddelerin degredasyon süreci belirlenmiştir. Genel olarak çoğu karışımlarda degredasyonunun yavaşladığı, bekleme süresi sonunda bile kalıntı miktarının MRL'nin altına düşürmediği görülmüştür. Üzüm döneminde ise boscalid, chlorpyrifos ethyl ve hexythiazox içeren preparatların üçlü karışımı, aynı asma yaprak döneminde olduğu gibi uygulanmıştır. Bu dönemde kullanılan tüm karışımlar, boscalidin degredasyon sürecini uzatmış, chlorpyrifos ethyl ve hexythiazoxun degredasyon sürecinde ise önemli bir etki görülmemiştir.

Anahtar sözcükler: Yaprak gübresi, pestisit, bitki gelişim düzenleyicisi, bekleme süresi, yayıcı-yapıştırıcı, tank karışımı

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Introduction

A total of 65 Mt of grapes from 7.5 Mha are produced in the world every year, of which Turkey produces 3.7 Mt from 0.46 Mha. Viticulture has a long history in Turkey dating back to antiquity as well as being a centre of genetic diversity of grapevines (Anonymous, 2014; TUIK, 2015). Given that there are numerous pests affecting grapevines, control of vineyard pests is vital. Although a great variety of control methods exist, it is chemical control that is most widely and effectively used as it achieves rapid, high level control (Durmuşoğlu et al., 2010). In the Aegean Region of Turkey, there are numerous sprays applied to grapevines to control diseases and pests during different phenological periods. Copçu et al. (2002) reported that about 27 different chemical sprays are applied annually in and around Manisa Province, which is the main area for viticulture in Turkey.

It is known that tank mixtures of chemicals are extensively used even though this is considered to be undesirable for vineyard production. When mixtures are made that are not recommended, phytotoxicity can occur or the intended desirable effects might not be obtained because the active ingredients and/or other substances react with each.

Pesticides are used separately in all registration experiments. Values such as maximum residue limits (MRL) and preharvest interval (PHI) are determined using data from these experiments. However, chemical can be applied in mixtures to save time and reduce depreciation of spray equipment. There are few studies of the problems caused by pesticides used in mixtures with fertilizers and adjuvants anywhere in the world, including changes in effects on pesticide residues. Ryckaert et al. (2007) measured effects of tolyfluanid mixed with four different adjuvants in lettuce and propiconazole mixed with six different adjuvants in wheat on pesticide degradation using chromatographic methods to determine effects of adjuvants on fungicides residues in leaves. The results showed that the adjuvants used in mixtures with fungicides slowed the process of degradation. Kucharski (2007) studied the effect of three different adjuvants (mineral oil, plant oil and surfactant) on phenmedipham, desmedipham and ethofumesate finding that they increased residues of the active ingredients by 52 and 33% in soil and plant, respectively. Kucharski & Sadowski (2009a) found that mixtures of phenmedipham with oil adjuvant applied to soil slowed degradation of the pesticide. Similarly, Kucharski et al. (2011, 2012) and Kucharski & Sadowski (2009b) showed that applications of separate ethofumesate, lenacil, chloridazon and their mixtures with oil adjuvant and surfactant applied to soil extended the PHI. Swarcewicz & Gregorczyk (2012) applied pendimethalin to soil then single, double and triple combinations of metribuzin, mancozeb and thiamethoxam. Compared to application of pendimethalin alone, the mixtures were found to extend the PHI. Swarcewicz et al. (2013) tested the application of linuron alone and mixtures with mancozeb and thiamethoxam in soil under laboratory conditions and found that the PHI for linuron was extended. Another study conducted in Turkey found that humic materials mixed with pesticides impacted on the degradation process. Yılmaz & Durmuşoğlu (2012) applied separate mixtures of imidacloprid to tomato leaves with humic acid and fulvic acid, and found that degradation of imidacloprid was accelerated and found the the same effect in the soil with humic acid, but with fulvic acid degradation was slower.

None of these studies examined degradation of pesticides mixed with plant growth regulators (PGR), foliar fertilizers (FF) and spreader-sticker (SS), as currently practiced. Viticulture is important globally and in Turkey, both for domestic consumption and export, however, these different additives have not been fully tested with the numerous pesticides used either in Turkey or elsewhere. Therefore, the main aim of this study was to determine whether degradation of pesticides mixed with PGR, FF and SS was changed.

Material and Methods

A range of experiments were performed under laboratory and field conditions to determine changes in the process of degradation of pesticides in samples from spray tanks, and of grapevine leaves and fruit where PGR, FF and SS had been mixed with pesticides alone or various combinations. The study was undertaken during two grapevine development periods, shooting and fruiting, when pesticide residue problems particularly emerge. The shooting period is of concern because foliage is harvested for making a popular dish (sarma) in which meat or rice are wrapped in grapevine leaves. During both periods, pesticides and adjuvants of choice are used for controlling many significant pests and diseases, especially in Izmir and Manisa which are major production areas in Turkey. The details of the pesticides and adjuvants used in the experiments are presented in Tables 1 to 3.

Table 1. Pesticides used for the shooting period

Commercial name	Active ingredient and rate	Target organism	Application dose (g or ml/100 l water)	PHI (d)
QuadrisMaxx SC	Azoxystrobin 250 g/l	Powdery mildew	75	21
Confidor SC	Imidacloprid 350 g/l	Thrips	50	14
Ridomil Gold MZ WG	Metalaxyl 4%	Downy mildew	250	14

Table 2. Pesticides used for the fruiting period

Commercial name	Active ingredient and rate	Target organism	Application dose (g or ml/100 l water)	PHI (d)
Cantus WG	Boscalid 50%	Gray mold	120	7
Dursban 4 EC	Chlorpyrifos-ethyl 480g/l	Grapevine moth	100	14
Nissorun 5 EC	Hexythiazox 50 g/l	Spider mite	50	7

Table 3. Adjuvants used for the shooting and fruiting periods

Commercial name	Content and rate	Purpose	Application dose (g or ml/100 l water)
Vulcana Gold	Gibberellic acid 20 g/l	Plant Growth Regulators	120
Carnival - calcium nitrate	9% nitrate nitrogen, 15% water-soluble calcium oxide, 0.05% water-soluble boron, 0.02% water-soluble zinc	Foliar Fertilizers	150
Slygard 309 - organic silicon	80% 3-(3-hydroxypropyl)- hepta-methyltrisiloxane, ethoxlated, acetate	Spreader-Sticker	30

Laboratory studies

Physical and chromatographic analyses were performed in laboratory to establish whether the rate of degradation of pesticides in mixtures with PGR, FF and SS changed in spray tanks or on the grapevine. Physical and chromatographic analyses were performed at the Izmir Food Control Laboratory and Izmir Radix Analysis Laboratory (both accredited for pesticide residue analysis), respectively.

Physical analyses

Physical analyses of pesticides and their mixtures with PGR, FF and SS were conducted using the criteria of pesticide formulations by FAO, WHO, EPA and CIPAC (WHO, 1984; FAO, 1985; EPA, 1996; CIPAC, 2006). The criteria evaluated included reactions in the solution (sudden cooling or warming, unexpected odor release or permanent foaming), changes in appearance (precipitation, agglomeration, sedimentation, decomposition or unexpected turbidity) and changes in pH.

Liquid formulations were pipetted and solid formulations were weighed. These were placed in a 1-L beaker half filled with water and mixed with a magnetic stirrer. If a pesticide was analyzed alone, hard water is carefully added to the beaker to a final volume of 1 L. If a pesticide was analyzed in a mixture, all ingredients were added to the beaker before the water and then mixed with a magnetic stirrer before dilution to the final volume.

Physical analyses of mixtures of pesticides

All pesticides were prepared according to the combinations shown in Table 4, such that the doses were twice the recommended dose.

Table 4. Doses prepared for physical analyses of mixtures of pesticides

Commercial names and active ingredients of pesticides used for the shooting period	Used dose of pesticides (g or ml/l water)	Commercial names and active ingredients of pesticides used for the fruiting period	Used dose of pesticides (g or ml/l water)
QuadrisMaxx - Azoxystrobin (A)	1.5	Cantus - Boscalid (B)	2.4
Confidor - Imidacloprid (I)	1.0	Dursban - Chlorpyrifos-ethyl (C)	2.0
Ridomil Gold - Metalaxyl (M)	5.0	Nissorun - Hexythiazox (H)	1.0
A + I	1.5 + 1.0	B + C	2.4 + 2.0
A + M	1.5 + 5.0	B + H	2.4 + 1.0
I + M	1.0 + 5.0	C + H	2.0 + 1.0
A+ I + M	1.5 + 1.0 + 5.0	B + C + H	2.4 + 2.0 + 1.0

An experiment with three replicates and 14 treatments (two mixtures in seven combination) was conducted to determine if mixtures of pesticides lead to degradation in a spray tank. Mixtures were kept at room temperature for 10 min, before preparation and assessment under the Regulations of Plant Protection Products by Ministry of Food Agriculture and Livestock, according to criteria given above.

Physical analyses of mixtures of pesticides and adjuvants

The mixture of pesticides including azoxystrobin, metalaxyl and imidacloprid is named Mixture 1, which was to represent combinations used during the shooting period when leaves can be harvested. The mixture was prepared using hard water. The mixture of pesticides including boscalid, chlorpyrifos ethyl and hexythiazox were called the Mixture 2, which was to represent combinations used during the fruiting periods when grapes are harvested, and was also prepared with hard water. Table 5 details the combination of these two mixtures with adjuvants.

Table 5. Doses prepared for physical analyses of mixtures of pesticides with adjuvants

Mixtures of pesticides with adjuvants used for the shooting period*	Used dose of adjuvants (g or ml/l water)	Mixtures of pesticides with adjuvants used for the fruiting period	Used dose of adjuvants (g or ml/l water)
Mixture 1 + PGR	2.4	Mixture 2 + PGR	2.4
Mixture 1 + FF	3.0	Mixture 2 + FF	3.0
Mixture 1 + SS	0.6	Mixture 2 + SS	0.6
Mixture 1 + PGR + FF	2.4 + 3.0	Mixture 2 + PGR + FF	2.4 + 3.0
Mixture 1 + PGR + SS	2.4 + 0.6	Mixture 2 + PGR + SS	2.4 + 0.6
Mixture 1 + FF + SS	3.0 + 0.6	Mixture 2 + FF + SS	3.0 + 0.6
Mixture 1 + PGR + FF + SS	2.4 + 3.0 + 0.6	Mixture 2 + PGR + FF + SS	2.4 + 3.0 + 0.6

* PGR, plant growth regulators; FF, foliar fertilizers; SS, spreader-sticker.

The combinations including PGR, FF and SS in single, double and triple combinations in three pesticides, which made it possible to reduce number of samples and analytical costs by avoiding unnecessary tests.

The second experiment was conducted with three replicates of these 14 treatments (Table 5) to determine if mixture of pesticides with PGR, FF and SS caused degradation in a spray tank. Assessments were the same as in the first experiment.

Chromatographic analyses

Chromatographic analyses were performed with Agilent 6460 Triple Quad LC-MS/MS (Liquid chromatography-tandem mass spectrometry) equipment. Calibration and recovery studies were conducted to determine recovery performance of calibration and extraction methods of the equipment used to perform residue analyses before assessing experimental samples. According to SANTE/11945/2015 document, at least three different levels are required for calibration. So, calibration was done with six concentrations (5, 10, 25, 50, 100 and 200 µg/kg) considering the measurement range for each active ingredient used in the field. In the analyses, matrix matched calibration was used to compensate the matrix effect. Untreated leaf samples were collected from the vineyard and recovery studies conducted at three concentrations (10, 50 and 200 µg/kg) for each active ingredient. Details of the operating conditions of LC-MS/MS equipment used in chromatographic analyses are presented in Table 6.

Table 6. Operating parameters used for chromatographic analyses with the Agilent 6460 Triple Quard LC-MS/MS

Equipment Model	Agilent 6460 LC-MS/MS
Detector	Triple Quard MS
Column	Poroshell C_{18}, 2.7µm, 3.0 x 75 mm
Mobile Phase A	Ultra-pure water with 5mM ammonium formatted
Mobile Phase B	100% acetonitrile
Flow Rate	0.6 ml/min
Injection Volume	5 µl
Run Time	12 min

Chromatographic analyses of mixtures of pesticides and adjuvants

Pesticide and adjuvant mixtures (Table 5) for chromatographic analyses were prepared again as for the physical analyses.

Aliquots of 50 µl were taken from each mixture and transferred to Teflon tubes with 50 ml of ultrapure water to give a concentration of 0.1% to avoid any damage to the chromatographic equipment. These solutions were filtered through PTFE-Polytetrafluoro ethylene-0.20 µl to remove particulate matter. Three replicates of each solution were analyzed with the LC-MS/MS system. Samples were taken and analyzed at three different times (10, 60 and 120 min) after preparation to simulate a situation in which pesticide mixtures are prepared in spray tank and used within 2 h. Therefore, the experiment of 28 treatments (Tables 4 and 5) was analyzed in triplicate and at three times.

Field experiments

Field experiments were conducted in a vineyard located in Kemalpaşa County, Izmir Province**.** Two experiments were conducted, one during the shooting period (3 May 2016) and the other during the fruiting period (20 July 2016). The experiments were conducted using standard methods (Anonymous, 2011) for residue trials for plant protection products in plant products issued by Ministry of Food, Agriculture and Livestock. They were performed using back pulverizes in doses recommended for all pesticides and adjuvants. Experiments had a complete block trial design with in three replicates plots of four vines each.

Assessment of degradation of pesticide residues on leave

Degradation of the pesticides on grapevine leaves was determined following their application of Mixture 1 alone and in single, double and triple combinations with the adjuvants. Pesticide mixtures were applied soon after unsprayed control samples had been taken to determine if there had been any previous pesticide application. The sampling procedure was made considering days since the last standardized spraying and collect following the relevant ministry standards for such sampling. Leaf samples were taken for residue analyses at nine times; immediately before treatment, and 2 h and 1, 3, 5, 7, 10, 14 and 21 d after treatment. All samples were taken to laboratory in cold chain and stored in a deep freezer at -80ºC until analysis. The buffered solution of QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used for the extraction (Lehotay et al., 2005; Lehotay, 2007).

The QuEChERS method uses a single-step buffered acetonitrile (MeCN) extraction and salting out liquid-liquid partitioning of the water in the sample with MgSO4. Dispersive-solid-phase extraction (dispersive-SPE) cleanup was done to remove organic acids, excess water, and other components with a combination of primary secondary amine (PSA) sorbent and MgSO4; then the extracts are analyzed by mass spectrometry (MS) after a chromatographic analytical separation (Lehotay, 2007). The basic steps of the QuEChERS method used were as follows. The sample was homogenized by blender, then 15 g transferred to a 50-ml teflon tube and shaken vigorously for 1 min. Then 1.5 g of sodium acetate + 6 g magnesium sulfate was added and vortexed for 1 min. After centrifuging for 4 min at 5000 rpm, an aliquot of 8 ml of the acetonitrile phase was transferred to a 15-ml dispersive SPE tube containing 0.2 g PSA (primary secondary amine) and 0.6 g MgSO4, and vortexed again for 1 min, before centrifuging for 4 min at 5000 rpm. The upper phase was filtered and transferred to a vial for LC-MS/MS analysis.

Analysis of variance was used to statistically examine treatment effects. If significant differences ($P < 0.05$) were found, multiple comparison tests were applied to compare the means and LSMeans Student's t-test used for groupings. Data was analyzed with JMP 7.0 statistical program (JMP, 2007).

Assessment of degradation of pesticide residues on fruit

Degradation of the pesticide residues on grapes was determined after application of Mixture 2 alone and in single, double and triple combinations with the adjuvants using the methods described above for the leaf samples.

Results and Discussion

Physical analyses of mixtures of pesticides

Table 7 gives the pH of solutions prepared with Mixtures 1 and 2. There were no significant changes in pH values and no other reactions were observed.

Table 7. pH of pesticide mixtures

Treatment (shooting period)	pH (mean±SD)	Treatment (fruiting period)	pH (mean±SD)
Azoxystrobin (A)	7.64±0.01	Boscalid (B)	7.48±0.10
Imidacloprid (I)	7.09±0.03	Chlorpyrifos-ethyl (C)	7.97±0.41
Metalaxyl (M)	7.59±0.02	Hexythiazox (H)	7.45±0.11
A + I	7.21±0.02	B + C	7.93±0.05
A + M	7.24±0.07	B + H	7.22±0.04
I + M	7.11±0.04	C + H	7.84±0.04
A+ I + M	7.12±0.03	B + C + H	7.83±0.05

Physical analyses of mixtures of pesticides and adjuvants

Table 8 gives the pH of solutions prepared with Mixtures 1 and 2 and adjuvants. There were no significant changes in pH values and no other reactions were observed. The pH of the PGR, FF and SS alone were 7.25, 6.72 and 7.59, respectively.

Table 8. pH of pesticide and adjuvant mixtures

Treatment* (shooting period)	pH (mean±SD)	Treatment (fruiting period)	pH (mean±SD)
Mixture 1 + FF	7.04±0.06	Mixture 2 + FF	7.27±0.05
Mixture 1 + SS	7.43±0.05	Mixture 2 + SS	7.62±0.04
Mixture 1 + PGR	7.19±0.05	Mixture 2 + PGR	7.43±0.06
Mixture 1 + FF + SS	7.03±0.05	Mixture 2 + FF + SS	7.55±0.20
Mixture 1 + FF + PGR	6.99±0.07	Mixture 2 + FF + PGR	7.49±0.07
Mixture 1 + SS + PGR	7.19±0.06	Mixture 2 + SS + PGR	7.34±0.02
Mixture 1 + FF + SS + PGR	7.13±0.05	Mixture 2 + FF + SS + PGR	7.53±0.06

* PGR, plant growth regulators; FF, foliar fertilizers; SS, spreader-sticker.

Chromatographic analyses

Calibration and recovery

Correlation coefficients calculated for the calibration curves were 0.996 for azoxystrobin, 0.995 for metalaxyl, 0.992 for imidacloprid, 0.990 for boscalid, 0.997 for chlorpyrifos ethyl, 0.993 for hexythiazox. Miller & Ambrus (2005) reported that the coefficients for acceptance of linear calibration should be ≥0.99.

Triplicate recovery studies conducted for three different concentrations (0.01, 0.05 and 0.20 mg/kg) of each active ingredient gave average recovery of 0.0105, 0.0524, 0.2098 mg/kg for azoxystrobin; 0.079, 0.0546, 0.1991 mg/kg for imidacloprid; 0.0804, 0.0530, 0.1992 mg/kg for metalaxyl; 0.0924, 0.0576, 0.1983 mg/kg for boscalid; 11.24, 48.24, 200.31 mg/kg for chlorpyrifos; and 0.0106, 0.0492 0.2002 mg/kg for hexythiazox, respectively. According to SANTE (2015) 'Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Foods and Feeds', recovery values must be within the range of 70-120%, which was achieved in the current study.

Mixtures of pesticides and adjuvants in a spray tank

Tables 9 to 12 summarizes the results the degradation of pesticides in mixtures with and without adjuvants in a spray tank. Given the dilutions used in the analysis, the expect concentrations for Mixture 1 were 0.5, 11.5 and 1.5 mg/kg for azoxystrobin, imidacloprid and metalaxyl, respectively. In Mixture 2 to the expected concentrations were 11.5, 10 and 2 mg/kg for boscalid, chlorpyrifos ethyl and hexythiazox, respectively.

It is clear from Tables 9 to 12 that values quite very close to the expected values when measured 2-h period after pesticides mixtures were prepared. The variation between the values is consistent with normal experimental error, and variation in laboratory measurement variation and the of sensitivity in equipment. Therefore, mixtures of pesticides did not cause and statistically significant changes in in the first 2 h, which is the normally recommended maximum time between mixing and application of pesticides.

Considering various factors, such as pH and hardness and mineral content of water in spray tank as well as chemical properties of pesticides, numerous studies (Okdemir et al., 1965; Ağar et al., 1991; Fishel, 2002; Whitford, 2009; Lo & Lee, 2010; Park & Chong, 2010) have examined the effects of such factors on degradation of pesticides. For example, one study examining the effects of humic matter mixed with pesticides (Yılmaz & Durmuşoğlu, 2012) showed no significant effects of humic matter on acetamiprid, imidacloprid and pymetrozine.

Table 9. Residues (mg/kg) in pesticides mixtures (Mixture 1) at different sampling times

Active Ingredient	Treatment	Sampling times		
		10 min Mean±SD	60 min Mean±SD	120 min Mean±SD
Azoxystrobin	Azoxystrobin	0.48±0.01	0.48±0.00	0.47±0.01
	Azoxystrobin + Metalaxyl	0.46±0.00	0.48±0.01	0.45±0.02
	Azoxystrobin + Imidacloprid	0.47±0.00	0.46±0.00	0.46±0.00
	Azoxystrobin + Metalaxyl + Imidacloprid	0.49±0.01	0.48±0.01	0.48±0.01
Imidacloprid	Imidacloprid	11.57±0.05	11.15±0.08	11.52±0.07
	Imidacloprid + Azoxystrobin	11.02±0.15	11.13±0.07	11.40±0.08
	Imidacloprid + Metalaxyl	11.11±0.09	11.06±0.10	11.22±0.32
	Imidacloprid + Azoxystrobin + Metalaxyl	11.47±0.31	11.70±0.15	11.43±0.14
Metalaxyl	Metalaxyl	1.40±0.01	1.45±0.01	1.45±0.01
	Metalaxyl + Azoxystrobin	1.50±0.01	1.46±0.00	1.48±0.02
	Metalaxyl + Imidacloprid	1.60±0.01	1.60±0.01	1.58±0.01
	Metalaxyl + Azoxystrobin + Imidacloprid	1.54±0.00	1.54±0.01	1.52±0.02

According to the LSMeans Student's t-test ($P > 0.05$) there was no difference between the values in the groups.

Table 10. Residues (mg/kg) in pesticides mixtures (Mixture 2) at different sampling times

Active Ingredient	Treatment	Sampling times		
		10 min Mean±SD	60 min Mean±SD	120 min Mean±SD
Boscalid	Boscalid	11.85±0.09	11.60±0.07	11.17±0.07
	Boscalid + Chlorpyrifos ethyl	11.72±0.06	11.42±0.10	11.01±0.20
	Boscalid + Hexythiazox	11.39±0.43	11.27±0.33	10.81±0.75
	Boscalid + Chlorpyrifos ethyl + Heyxthiazox	10.82±1.20	10.96±0.41	11.52±0.41
Chlorpyrifos-ethyl	Chlorpyrifos ethyl	9.79±0.42	9.42±0.68	10.05±0.73
	Chlorpyrifos ethyl + Boscalid	10.17±0.03	10.59±0.36	11.11±0.17
	Chlorpyrifos ethyl + Hexythiazox	9.70±1.45	9.68±0.54	10.12±0.44
	Chlorpyrifos ethyl + Boscalid + Hexythiazox	10.28±0.38	10.33±0.36	9.88±0.41
Hexythiazox	Hexythiazox	2.27±0.10	2.22±0.10	2.36±0.12
	Hexythiazox + Boscalid	2.05±0.10	1.98±0.12	2.10±0.18
	Hexythiazox + Chlorpyrifos ethyl	1.93±0.23	1.90±0.23	2.02±0.16
	Hexythiazox + Boscalid + Chlorpyrifos ethyl	2.15±0.15	2.06±0.10	2.08±0.23

According to the LSMeans Student's t-test (P > 0.05) there was no difference between the values in the groups.

Table 11. Residues (mg/kg) in pesticide (Mixture 1) and adjuvant mixtures at different sampling times

Active Ingredient	Treatment	Sampling times		
		10 min (mean±SD)	60 min (mean±SD)	120 min (mean±SD)
Azoxystrobib	Mixture 1 (A + I + M)*	0.49±0.01	0.48±0.01	0.48±0.02
	Mixture 1 + FF	0.54±0.01	0.49±0.02	0.50±0.02
	Mixture 1 + PGR	0.51±0.01	0.49±0.01	0.51±0.01
	Mixture 1 + SS	0.49±0.02	0.48±0.03	0.51±0.02
	Mixture 1 + FF + PGR	0.50±0.01	0.50±0.04	0.53±0.03
	Mixture 1 + FF + SS	0.50±0.01	0.55±0.04	0.54±0.05
	Mixture 1 + PGR + SS	0.52±0.02	0.49±0.01	0.49±0.03
	Mixture 1 + FF + PGR + SS	0.51±0.02	0.50±0.04	0.51±0.03
Imidacloprid	Mixture 1 (A + I + M)	11.47±0.31	11.70±0.15	11.43±0.14
	Mixture 1 + FF	11.60±0.31	11.23±0.18	11.25±0.52
	Mixture 1 + PGR	11.49±0.42	11.87±0.55	11.57±0.30
	Mixture 1 + SS	11.74±0.34	11.48±0.39	11.22±0.20
	Mixture 1 + FF + PGR	11.98±0.67	11.80±0.59	11.48±0.27
	Mixture 1 + FF + SS	11.22±0.61	11.59±0.38	11.55±0.16
	Mixture 1 + PGR + SS	11.80±0.98	11.44±0.21	11.67±0.54
	Mixture 1 + FF + PGR + SS	11.82±1.11	11.93±0.34	11.92±0.21
Metalaxyl	Mixture 1 (A + I + M)	1.54±0.00	1.54±0.01	1.52±0.02
	Mixture 1 + FF	1.63±0.03	1.57±0.04	1.55±0.07
	Mixture 1 + PGR	1.55±0.06	1.58±0.02	1.54±0.08
	Mixture 1 + SS	1.55±0.08	1.56±0.10	1.53±0.05
	Mixture 1 + FF + PGR	1.58±0.05	1.49±0.07	1.58±0.05
	Mixture 1 + FF + SS	1.57±0.04	1.65±0.10	1.56±0.06
	Mixture 1 + PGR + SS	1.58±0.05	1.50±0.04	1.53±0.04
	Mixture 1 + FF + PGR + SS	1.61±0.05	1.59±0.08	1.63±0.10

*A + I + M, Azoxystrobin + Imidacloprid + Metalaxyl; PGR, plant growth regulators; FF, foliar fertilizers; SS, spreader-sticker
According to the LSMeans Student's t-test (P > 0.05) there was no difference between the values in the groups.

Table 12. Residues (mg/kg) in pesticide (Mixture 2) and adjuvant mixtures at different sampling times

Active Ingredient	Treatment	Sampling times		
		10 min (mean±SD)	60 min (mean±SD)	120 min (mean±SD)
Boscalid	Mixture 2 (B + C + H)*	10.82±1.20	10.96±0.41	11.52±.041
	Mixture 2 + FF	11.34±0.37	11.30±0.32	10.22±0.58
	Mixture 2 + PGR	11.30±0.08	11.25±0.35	11.17±0.21
	Mixture 2 + SS	11.76±0.74	11.53±0.63	11.86±0.55
	Mixture 2 + FF + PGR	11.58±0.38	11.33±0.34	11.41±0.35
	Mixture 2+ FF + SS	11.80±0.42	11.55±0.44	11.41±0.52
	Mixture 2 + PGR + SS	11.86±0.09	11.94±0.52	11.71±0.54
	Mixture 2 + FF + PGR + SS	11.44±0.25	11.32±0.17	11.41±0.12
Chlorpyrifos ethyl	Mixture 2 (B + C + H)	10.28±0.38	10.33±0.36	9.88±0.41
	Mixture 2 + FF	9.93±0.63	9.87±0.32	10.16±0.27
	Mixture 2 + PGR	9.97±0.64	10.32±0.49	9.85±0.20
	Mixture 2 + SS	10.24±0.43	10.10±0.20	10.36±0.04
	Mixture 2 + FF + PGR	9.57±0.59	9.90±0.23	10.11±0.18
	Mixture 2 + FF + SS	10.06±0.73	9.93±0.35	9.77±0.44
	Mixture 2 + PGR + SS	10.10±0.55	9.66±0.88	10.00±0.39
	Mixture 2 + FF + PGR + SS	10.39±0.52	9.98±0.51	9.78±0.42
Hexythiazox	Mixture 2 (B + C + H)	2.15±0.15	2.06±0.10	2.08±0.23
	Mixture 2 + FF	2.13±0.12	2.03±0.06	2.09±0.11
	Mixture 2 + PGR	1.84±0.04	1.85±0.14	1.89±0.11
	Mixture 2 + SS	1.98±0.25	1.94±0.07	2.08±0.22
	Mixture 2 + FF + PGR	2.01±0.18	2.04±0.12	2.02±0.17
	Mixture 2 + FF + SS	1.92±0.11	2.16±0.17	2.18±0.11
	Mixture 2 + PGR + SS	1.85±0.11	1.94±0.09	1.90±0.04
	Mixture 2 + FF + PGR + SS	2.09±0.20	2.10±0.24	1.96±0.02

*B + C + H, Boscalid + Chlorpyrifos ethyl + Hexythiazox; PGR, plant growth regulators; FF, foliar fertilizers; SS, spreader-sticker
According to the LSMeans Student's t-test (P > 0.05) there was no difference between the values in the groups.

Pesticide residues on leaves

No residues were found on the unsprayed control leaf samples collected immediately before treatment. Tables 13 to 15 summarizes the results of the degradation of active ingredients in mixtures containing azoxystrobin, imidacloprid and metalaxyl applied during the shooting period.

Table 13 shows the residues of azoxystrobin on leaves in different sampling times. The residues on leaves at the end of PHI (21 d) for azoxystrobin were over MRL in mixtures with PGR + SS, PGR + FF and PGR + FF + SS.

Table 13. Residues of azoxystrobin (mg/kg) on leaves at different sampling times in pesticide (Mixture 1) and adjuvant mixtures

Active Ingredient	Treatment	Sampling times							
		2h (mean±SD)	1d (mean±SD)	3d (mean±SD)	5d (mean±SD)	7d (mean±SD)	10d (mean±SD)	14d* (mean±SD)	21d (mean±SD)
Azoxystrobin	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mixture 1	10.39±1.72 c**	5.65±1.21 c	1.63±0.81 c	0.85±0.20 bc	0.22±0.04 d	0.02±0.01 c	0.01±0.00 d	0.00±0.00 b
	Mixture 1 +PGR	7.22±0.21 d	3.32±0.03 d	1.15±0.09 cd	0.23±0.05 d	0.20±0.03 d	0.12±0.03 c	0.08±0.07 c	0.00±0.00 b
	Mixture 1 +SS	4.68±1.23 e	0.97±0.60 e	0.24±0.04 e	0.20±0.07 d	0.05±0.03 e	0.02±0.01 c	0.01±0.00 d	0.00±0.00 b
	Mixture 1 +FF	4.42±0.92 e	1.37±0.58 de	0.80±0.06 cde	0.09±0.01 d	0.02±0.00 e	0.01±0.00 c	0.01±0.00 cd	0.00±0.00 b
	Mixture 1 + SS + PGR	28.60±0.91 a	10.78±0.59 b	6.19±1.18 a	2.18±0.90 a	1.58±0.11 a	0.76±0.21 a	0.25±0.06 a	**0.08±0.01 a**
	Mixture 1 + SS + FF	10.18±1.11 c	2.63±0.43 de	0.76±0.07 de	0.33±0.07 cd	0.20±0.05 d	0.05±0.03 c	0.01±0.01 cd	0.00±0.00 b
	Mixture 1 + PGR + FF	17.68±1.20 b	7.36±2.75 c	2.59±0.65 b	0.95±0.04 b	0.46±0.02 c	0.32±0.04 b	0.29±0.07 a	**0.07±0.04 a**
	Mixture 1 + SS+ FF + PGR	19.34±0.89 b	13.43±2.09 a	5.51±0.64 a	2.28±0.20 a	1.25±0.06 b	0.69±0.01 a	0.17±0.02 b	**0.07±0.01 a**

* PHI 21 d, values in bold are above the MRL of 0.05 mg/kg.
** Means in a column followed by the same letter are not statistical significantly different by LSMeans Student's t-test (P > 0.05).

Table 14. Residues of imidacloprid (mg/kg) on leaves at different sampling times in pesticide (Mixture 1) and adjuvant mixtures

Active Ingredient	Treatment	Sampling time							
		2h (mean±SD)	1d (mean±SD)	3d (mean±SD)	5d (mean±SD)	7d (mean±SD)	10d (mean±SD)	14d* (mean±SD)	21d (mean±SD)
Imidacloprid	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mixture 1	78.81±17.65 de**	43.16±2.91 c	37.04±3.86 b	22.73±3.10 b	9.24±1.39 c	5.04±0.33 b	0.82±0.05 c	0.77±0.05 c
	Mixture 1 +PGR	94.15±4.55 bc	73.36±1.29 a	62.96±2.32 a	47.45±1.00 a	12.44±0.26 b	10.36±0.43 a	**4.63±0.70 a**	**2.82±0.66 b**
	Mixture 1 +SS	29.48±1.02 f	27.34±1.00 d	17.10±0.58 e	13.07±0.37 d	6.65±0.31 d	2.32±0.05 c	0.89±0.06 c	0.32±0.03 c
	Mixture 1 +FF	39.09±5.42 f	24.61±7.63 d	13.64±1.70 f	7.42±0.97 e	6.01±1.48 d	3.46±1.57 c	**2.23±1.43 c**	0.66±0.29 c
	Mixture 1 + SS + PGR	89.85±0.86 cd	47.77±0.69 bc	27.16±0.62 c	17.68±0.29 c	6.39±0.06 d	5.23±0.20 b	**2.62±0.73 bc**	0.89±0.23 c
	Mixture 1 + SS + FF	65.79±1.85 e	43.36±2.59 bc	28.30±0.83 c	11. 36±0.93 d	6.44±0.13 d	3.18±0.06 c	1.12±0.47 c	0.55±0.07 c
	Mixture 1 + PGR + FF	129.34±13.69 a	52.95±10.40 b	23.97±0.76 d	19.89±3.03 bc	16.98±1.38 a	11.10±0.72 a	**4.37±2.13 ab**	**2.11±1.32 b**
	Mixture 1 + SS+ FF + PGR	105.17±8.7 b	47.19±1.93 bc	25.98±0.63 cd	21.64±3.54 b	12.94±1.50 b	6.23±1.24 b	**5.06±1.39 a**	**4.01±0.48 a**

* PHI 14 d, values in bold are above the MRL of 2 mg /kg.
** Means in a column followed by the same letter are not statistical significantly different by LSMeans Student's t-test (P > 0.05).

Table 15. Residues of metalaxyl (mg/kg) on leaves at different sampling times in pesticide (Mixture 1) and adjuvant mixtures

Active Ingredient	Treatment	Sampling times							
		2h (mean±SD)	1d (mean±SD)	3d (mean±SD)	5d (mean±SD)	7d (mean±SD)	10d (mean±SD)	14d* (mean±SD)	21d (mean±SD)
Metalaxyl	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mixture 1	18.26±0.85 d**	13.81±0.88 b	4.28±0.34 b	2.71±0.17 b	2.52±0.33 a	1.15±0.08 c	0.05±0.00 e	0.01±0.00 e
	Mixture 1 +PGR	30.31±0.92 a	18.73±1.11 a	6.71±0.45 a	3.51±0.39 a	2.60±0.21 a	1.49±0.23 b	**1.40±0.20 a**	**0.67±0.15 a**
	Mixture 1 +SS	24.25±1.01 c	10.24±2.12 d	2.75±0.80 c	1.68±0.81 d	0.77±0.10 d	0.59±0.06 e	**0.43±0.05 d**	**0.14±0.01 d**
	Mixture 1 +FF	16.24±2.73 d	7.60±0.91 e	4.44±0.75 b	2.68±0.31 bc	1.70±0.34 bc	0.75±0.21 de	0.04±0.01 e	0.00±0.00 e
	Mixture 1 + SS + PGR	26.92±1.56 bc	11.00±0.19 cd	4.23±0.09 b	2.34±0.15 bcd	0.89±0.09 d	0.71±0.02 de	**0.59±0.06 cd**	**0.27±0.02 c**
	Mixture 1 + SS + FF	26.11±2.17 c	11.94±0.27 c	4.46±0.23 b	2.01±0.57 cd	1.06±0.17 d	0.74±0.08 de	**0.54±0.03 d**	**0.26±0.03 c**
	Mixture 1 + PGR + FF	29.63±2.50 ab	18.10±0.13 a	6.63±0.77 a	3.97±0.22 a	2.09±0.59 ab	1.84±0.12 a	**0.77±0.11 b**	**0.44±0.03 b**
	Mixture 1 + SS+ FF + PGR	29.88±1.16 a	11.04±0.15 cd	3.99±0.35 b	2.00±0.53 cd	1.25±0.30 cd	0.92±0.12 cd	**0.75±0.10 bc**	**0.48±0.09 b**

* PHI 14 d, values in bold are above the MRL of 0.05 mg /kg.
** Means in a column followed by the same letter are not statistical significantly different by LSMeans Student's t-test (P > 0.05).

Table 14 shows the residue of imidacloprid on leaf samples after 14 d dropped below the expected level following its application without adjuvants, but in mixtures with SS and with SS + FF the residues of remained above the MRL despite PHI (14 d). Therefore, regardless of PHI of 14 d, mixtures were found with residues over the MRL even in the samples collected after 21 d. For example, leaf samples from plots treated with a mixture including PGR had 2.82 mg/kg imidacloprid even after 21 d. Also, when it was applied with PGR + FF, the same problem occurred and its residue approached the MRL at 2.11 mg/kg. The treatment with the three adjuvants had a residue of 5.06 mg/kg in leaf samples collected after 14 d, remaining much higher than MRL and even leaf samples following 21 d also exhibited a residue as much twice the MRL. Mixing with PGR + FF + SS slowed degradation of imidacloprid leading to the highest residue.

For metalaxyl only two treatments were below of MRL at 14 and 21 d; i.e., metalaxyl with adjuvants and with FF (Table 15). All other treatments have residues above MRL even after 21 d, so they had slowed the degradation of metalaxyl.

Pesticide residues on fruit

Residues of boscalid, chlorpyrifos ethyl and hexythiazox for treatments applied during the fruiting period are given in Tables 16 to 18. No residues were detected in the unsprayed controls collected just before the application of the treatments.

Degradation of boscalid (Table 16) on the grape samples when applied without adjuvants was faster than in mixtures with adjuvants, and after 7 d its residue was under the MRL. Wheres, with all combinations with adjuvants after 7 d residue exceeded the MRL. Chlorpyrifos ethyl residues at PHI of 14 d were below the MRL in all treatments (Table 17). However, considering the reports published by EPA and EFSA concerning plant protection products including chlorpyrifos ethyl emphasized its danger for human health and the directive of EU in August 2016 to set the MRL for chlorpyrifos ethyl to 0.01 mg/kg, the residues in all the samples after 14 d were above this EU MRL. The resides of hexythiazox, were below the MRL in all treatments after 7 d, in other words at the end of PHI (Table 18).

Although there are no similar studies published for grapevines or other contexts, there are some studies on effects of a number of adjuvants on the degradation of pesticides. Kucharski (2007) reported that three different adjuvants (mineral oil, plant oil and surfactant) applied to sugar beet and soil increased the residues of phenmedipham, desmedipham and ethofumesate. Consistent with the findings reported hare, Kucharski & Sadowski (2009b) reported that mixture of an oil adjuvant with ethofumesate tended to decrease degradation of ethofumesate applied alone (PHI increased at 8 to10 d) and the residue in soil was higher. Kucharski et al. (2011; 2012) repeated the same study using lenacil and chloridazon with similar results. Another study supportive of the present one was reported by Ryckaert et al. (2007). It was reported that some adjuvants mixed with tolyfluanid increased residues compared to controls in pepper, but mixture of tolyfluanid with another adjuvant, magic sticker, showed the opposite effect. The sticker increased the rate of degradation of tolyfluanid and thus decreased its residue. From the present study, it follows that mixture of FF with pesticides reduces residues of some active ingredients but decelerated the degradation of others with a risk that the residues could exceed the MRL. In fact, another study similar to the present one conducted by Yılmaz & Durmuşoğlu (2012) showed that combination of humic matter with imidacloprid applied on leaves, (humic and fulvic acid) accelerated up the degradation of that active ingredient. However, application of imidacloprid with fulvic acid to soil was observed to accelerate uptake of imidacloprid by the plant, as well as decelerate and lengthen the process of degradation. Application of imidacloprid with humic acid to soil was found to retard transport of imidacloprid to leaves, as well as speed up the process of degradation. Consequently, application of pesticides with humic matter was reported to be likely to cause changes in the process of degradation residues in plants, which could cause variable results depending the humic matter content and chemical properties of the pesticides.

It is clear from the results for grapevine leaves that most mixtures gave residues above MRL s for most of active ingredients, with a few exceptions. The MRL for grapevine leaves are the lowest, based on LOD (limit of detection), under Turkish Food Codex which adopts the EU analytically lowest limit.

Table 16. Residues of boscalid (mg/kg) on grapes on leaves at different sampling times in pesticide (Mixture 2) and adjuvant mixtures

Active Ingredient	Treatment	Sampling times						
		2h (mean±SD)	1d (mean±SD)	3d (mean±SD)	5d (mean±SD)	7d* (mean±SD)	10d (mean±SD)	14d (mean±SD)
Boscalid	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mixture 2	155.46±2.52 b**	105.92±2.65 e	70.03±2.97 bc	21.84±3.22 f	4.88±1.46 c	1.98±0.54 b	0.52±0.16 c
	Mixture 2 +PGR	126.28±2.53 d	96.35±3.32 f	64.67±3.32 cd	18.88±2.14 f	**8.74±1.35 ab**	3.88±0.66 a	0.31±0.06 c
	Mixture 2 +SS	152.10±3.27 b	116.29±4.37 d	73.91±2.54 b	26.31±0.69 e	**8.31±1.01 b**	2.24±0.32 b	0.43±0.20 c
	Mixture 2 +FF	120.78±2.35 d	100.28±0.97 f	60.58±2.62 d	27.39±0.69 de	**10.48±0.47 ab**	4.31±0.05 a	0.90±0.29 b
	Mixture 2 + SS + PGR	142.17±3.35 c	129.10±1.51 c	74.73±2.21 b	48.45±1.15 b	**10.97±2.37 a**	4.48±0.86 a	0.63±0.07 bc
	Mixture 2 + SS + FF	173.89±3.94 a	158.30±4.21 a	86.31±7.38 a	53.67±3.08 a	**10.19±0.88 ab**	3.83±0.50 a	0.58±0.25 bc
	Mixture 2 + PGR + FF	153.43±4.05 b	145.70±2.46 b	68.38±4.08 bc	39.55±0.42 c	**8.93±1.65 ab**	4.15±1.34 a	0.49±0.04 c
	Mixture 2 + SS+ FF + PGR	126.49±5.82 d	61.64±2.53 g	46.74±2.10 e	30.65±1.99 d	**9.26±1.80 ab**	3.90±0.56 a	1.27±0.25 a

* PHI 7 d, values in bold are above the MRL of 5 mg /kg.
** Means in a column followed by the same letter are not statistical significantly different by LSMeans Student's t-test (P > 0.05).

Table 17. Residues of chlorpyrifos ethyl (mg/kg) on grapes on leaves at different sampling times in pesticide (Mixture 2) and adjuvant mixtures

Active Ingredient	Treatment	Sampling times						
		2h (mean±SD)	1d (mean±SD)	3d (mean±SD)	5d (mean±SD)	7d (mean±SD)	10d (mean±SD)	14d* (mean±SD)
Chlorpyrifos ethyl	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Mixture 2	28.11±2.54 b**	20.53±0.46 b	10.04±0.70 bc	4.70±0.57 bc	2.27±0.35 bcd	0.81±0.17 cd	0.15±0.09 bc
	Mixture 2 +PGR	36.63±4.14 a	29.63±1.41 a	18.76±1.42 a	8.51±0.91 a	3.56±0.41 a	1.10±0.11 ab	0.23±0.02 ab
	Mixture 2 +SS	22.48±3.12 c	18.49±2.52 bc	11.24±2.75 bc	5.33±1.02 bc	2.91±.23 ab	0.76±0.11 de	0.10±0.02 c
	Mixture 2 +FF	22.06±1.12 c	16.40±1.22 cd	10.71±0.73 bc	4.15±0.17 cd	1.97±0.12 bcd	0.66±0.09 de	0.07±0.00 c
	Mixture 2 + SS + PGR	24.50±1.06 bc	19.08±1.70 bc	11.07±0.05 bc	4.92±0.23 d	2.83±0.51 abc	1.22±0.27 a	0.07±0.02 c
	Mixture 2 + SS + FF	23.51±2.07 c	16.28±2.38 cd	6.04±1.62 d	9.80±0.42 a	1.39±0.46 d	0.28±0.20 f	0.09±0.01 c
	Mixture 2 + PGR + FF	24.08±2.02 bc	18.57±1.57 bc	11.96±0.31 b	6.15±0.17 b	2.53±0.48 bc	0.96±0.08 bc	0.33±0.09 a
	Mixture 2 + SS+ FF + PGR	37.68±1.36 a	14.38±1.45 d	9.10±0.92 c	4.37±0.55 c	1.88±0.29 cd	0.57±0.19 e	0.16±0.08 bc

* PHI 14 d, values in bold are above the MRL of 0.5 mg /kg.
** Means in a column followed by the same letter are not statistical significantly different by LSMeans Student's t-test (P > 0.05).

Table 18. Residues of hexythiazox (mg/kg) on grapes on leaves at different sampling times in pesticide (Mixture 2) and adjuvant mixtures

Active Ingredient	Treatment	Sampling time						
		2h (mean±SD)	1d (mean±SD)	3d (mean±SD)	5d (mean±SD)	7d* (mean±SD)	10d (mean±SD)	14d (mean±SD)
Hexythiazox	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00 a
	Mixture 2	3.41±0.58 bc*	3.13±0.70 b	2.14±0.13 b	1.21±0.18 b	0.40±0.08 a	0.16±0.03 a	0.00±0.00 a
	Mixture 2 +PGR	4.57±1.43 ab	2.37±0.14 c	1.91±0.24 bc	0.80±0.08 c	0.28±0.05 abc	0.11±0.02 bc	0.00±0.00 a
	Mixture 2 +SS	4.19±1.23 bc	2.10±0.11 cd	1.86±0.03 bc	0.76±0.11 cd	0.35±0.04 abc	0.12±0.04 abc	0.00±0.00 a
	Mixture 2 +FF	3.27±0.66 c	1.89±0.12 cd	1.74±0.40 bc	0.60±0.02 d	0.27±0.05 bc	0.09±0.01 cd	0.00±0.00 a
	Mixture 2 + SS + PGR	3.69±0.34 bc	1.69±0.23 d	1.42±0.27 c	0.87±0.12 c	0.38±0.08 ab	0.13±0.01 ab	0.00±0.00 a
	Mixture 2 + SS + FF	4.31±0.67 bc	2.30±0.15 c	1.96±0.48 bc	1.11±0.11 b	0.24±0.09 cd	0.07±0.03 de	0.00±0.00 a
	Mixture 2 + PGR + FF	5.56±0.33 a	4.59±0.19 a	3.35±1.00 a	1.69±0.06 a	0.35±0.11 abc	0.06±0.01 de	0.00±0.00 a
	Mixture 2 + SS+ FF + PGR	4.16±0.02 bc	1.89±0.21 cd	1.56±0.20 bc	0.76±0.12 cd	0.12±0.04 d	0.03±0.01 e	0.00±0.00 a

* PHI 7 d, values in bold are above the MRL of 1 mg /kg.
** Means in a column followed by the same letter are not statistical significantly different by LSMeans Student's t-test (P > 0.05).

Considering the results from the fruiting period with application of a mixture of boscalid, chlorpyrifos ethyl and hexythiazox (Mixture 2), single, double and triple mixtures with PGR, FF and SS tended to lengthen the process of degradation of boscalid but did not affect the degradation of chlorpyrifos ethyl and hexythiazox. In summary, PGR, FF and SS affected the process of degradation of some active ingredients with some failing to drop below the MRL even after the PHI.

Despite the fact that producers comply with the PHI on the pesticide labels, the unexpected residue problem caused by the additives in mixtures could negatively affects the health of consumers. The practical benefits of mixtures in conjunction with the lack of information on the effects of these mixtures motivated for this work, which attempted address some unanswered questions on this topic. Nevertheless, the pesticides used in this study should be further investigated by examining the effects of other preparations using PGR, FF and SS in different concentrations. Likewise, other active ingredients and mixtures, and other cultivated plants need to be studied. It is also recommended that manufacturers be informed about tank mixes and their potential risks. Similarly, it should be emphasized that, while pesticides approved for cultivated plants are recommended to producers, producers should not mix with unapproved adjuvants.

In conclusion, it was observed that using pesticides in mixtures with PGR, FF and SS could lead to slower degradation and higher residues, and this could vary with chemical properties of the pesticide, and nature and concentration of the adjuvants.

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Original article (Orijinal araştırma)

House dust mite population in the bedrooms of people with dust mite allergy in the city of Ordu, Turkey[1]

Ordu (Türkiye)'da toz akar alerjisi olan kişilerin yatak odalarında toz akarı popülasyonu

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Abstract

House dust mites are a major source of allergens that are responsible for allergic symptoms such as asthma and atopic diseases. The warm and humid climate of Ordu, which is situated on the Black Sea coast of Turkey is highly suitable for the growth of house dust mites. Seasonal changes in the populations of the house dust mites were studied in five houses of dust-mite sensitive people with asthma between 2013 and 2015. Dust samples were obtained once a month to assess the monthly distribution of dust mites. House dust mites were isolated from samples by a wet-sieving method. Permanent slides were prepared for morphologically based species determination. The most abundant species were *Dermatophagoides pteronyssinus* (Trouessart, 1897) and *Dermatophagoides farinae* (Hughes, 1961) (Astigmata: Pyroglyphidae), 65.4 and 31.0% of the total count of mites, respectively. Dust mites can survive all year round and are widely distributed in different types of houses in the city of Ordu. The mite abundance reached its peak in all houses during August (26-28ºC and 64-78% RH). The discomfort for people who have a dust mite allergy may reach a peak specifically during this time and they need to take extra precautions to reduce exposure to dust mites as much as possible during this period.

Keywords: Allergy, *Dermatophagoides*, house dust mites, seasonal population fluctuation, Turkey

Öz

Ev tozu akarları, astım ve atopik dermatit gibi birçok alerjik simptoma neden olabilen ana alerjen kaynaklarından biridir. Türkiye'nin Karadeniz kıyısında yer alan Ordu şehrinin ılık ve nemli iklimi, ev tozu akarları için oldukça uygundur. Bu nedenle Ordu ilinde 2013-2015 yılları arasında, toz akarına duyarlı astımlı hastaların yaşadığı beş evde ev tozu akarı popülasyonundaki mevsimsel değişiklikler incelenmiştir. Toz akarı popülasyonundaki aylık değişimleri tespit etmek için, her evde ayda bir toz örneklemesi yapılmıştır. Akarlar ıslak- elek analiz yöntemiyle elde edilmiştir. Elde edilen türlerin morfolojik tür teşhisleri için preparatları yapılmıştır. Sonuçlar, en baskın türlerin *Dermatophagoides pteronyssinus* (Trouessart, 1897) ve *Dermatophagoides farinae* (Hughes, 1961) (Astigmata: Pyroglyphidae) olduğunu göstermiştir. Bu türler, toplam yoğunluğun sırası ile %65.4 ve %31.0 kadarını oluşturmuştur. Toz akarları, Ordu ilinde yıl boyunca tüm evlerde yaygın olarak bulunmuştur. Evlerdeki toz akar popülasyonunun ağustos ayında (26-28ºC ve %64-78 RH) en yüksek seviyelere ulaştığı da belirlenmiştir. Toz akarı alerjisi olanların özellikle bu süre zarfında şikayetleri artabilir ve ekstra önlemlere ihtiyaç duyabilirler.

Anahtar sözcükler: Alerji, *Dermatophagoides*, ev tozu akarları, mevsimlik popülasyon değişimi, Türkiye

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Introduction

House dust is often heavily contaminated with many allergens. One of the important allergenic components of house dust is dust mites (Colloff, 2009). The house dust mite was first suspected as a source of allergen in 1928 and has been recognized as an important cause of allergic disorders since 1964 (Voorhorst et al., 1964). In particular, the mite species, *Dermatophagoides pteronyssinus* (Trouessart, 1897) and *Dermatophagoides farinae* (Hughes, 1961) (Astigmata: Pyroglyphidae), have been shown to be important sources of inhalant and contact allergens associated with asthma and atopic diseases (Chew et al., 1999). They primarily live on dander (skin scales) shed from humans and household animal pets. Temperature and humidity are the major factors that influence the distribution and abundance of dust mites. Additional factors such as ventilation, floor level, orientation, age of home, or living habits of the occupants may contribute to the differences in indoor humidity that can influence the growth conditions of mites (Terra et al., 2004). A large number of studies on seasonal variation in house dust mite population have been carried out in many countries. Some of these studies were conducted in North America by Murray & Zuk (1979), Santiago De Compostela (Galicia, Spain) by Agratorres et al. (1999), Lithuania (Vilnius region) by Duatartiene (2001), northwestern Poland by Kosik-Bogacka et al. (2012) and India (South Assam) by Sharma et al. (2011). Population changes in dust mites have also determined in the different parts of Turkey by various researcher [Ozman-Sullivan & Celik, 2010 (Samsun); Aykut et al., 2013 (Bitlis and Muş Provinces)]. However, the seasonal dynamics of house dust mites in Ordu city have not been studied. Ordu is a seaside city and has a very humid climate. It is known that there is a direct relationship between relative humidity (RH) and house dust mite growth (Arlian et al., 1992). The aim of this work was to study the seasonal changes and abundance of the house dust mites in five houses of dust-mite sensitive people with asthma over two years between in the city of Ordu.

Material and Methods

Study area and houses

Experiments were conducted in the bedrooms of five houses (A, B, C, D and E) of dust mite sensitive people in Ordu (Turkey) during 2013 to 2015. Brief characteristics of each house are as follows:

A: The apartment is on the fourth floor of a block house and about 20 years old. The bed is about 5 years old, while the carpet is 10 years old. The bedroom is heated with gas. The inhabitant of this apartment is highly sensitive to house dust and 25 years old.

B: The apartment is on the third floor of a block house and about 45 years old. The bed is about 15 years old. Carpet is about 5 years old. The bedroom is heated with stove. The inhabitant with asthma of this apartment is 70 years old.

C: The apartment is on the fourth floor of a block house and about 15 years old. The bed is about 20 years old. Carpet is about 10 years old. The room is heated with gas. The inhabitant of this apartment is highly sensitive to house dust and 25 years old. The home is shared with a dog.

D: The apartment is on the fourth floor of a block house and about 20 years old. The bed is about 20 years old and used for sleeping except during August. Carpet is about 5 years old. The room is heated with gas. The inhabitant with asthma of this apartment is 21 years old.

E: The apartment is on the second floor of a block house and about 15 years old. The bed is about 1 year old. There is no carpet in the room. The room is heated with gas. The inhabitant with asthma of this apartment is 13 years old.

The temperature and RH in the bedroom of each house were measured and recorded daily using a data logger (CEM DT-172). These data were used to calculate the mean monthly temperature and RH.

Dust sample collection

Dust samples were obtained once a month from August 2013 until August 2015 in one house (A) and from August 2013 till July 2014 in four houses (B, C, D, E). House dust samples were taken from the entire surface of the mattress (about 3 m^2) (van Strien et al., 2002) and, carpet or floor near the bed (about 2 m^2). The samples were collected with a portable vacuum cleaner for 2 min/m^2 (Ozman-Sullivan & Celik, 2010; Zeytun et al., 2015).

A new bag for each vacuuming was used. After each vacuuming, the dust bag was taken out, placed in a plastic bag and brought to the laboratory for analysis (Wassenaar, 1988). The samples were usually analyzed within 24 h. Dust samples that were examined later were stored in a refrigerator at 4ºC.

Mite isolation and enumeration

The large particles were removed from each dust sample. House dust mites were isolated from 1 g fine dust by a wet-sieving method adapted from Natuhara (1989). The mites within the samples were stored in 70% alcohol. All mites were counted and mounted whether they were alive, dead or physically damaged. The percentage of each species detected during the sampling period was calculated as follows:

Percentage of each species (%) = (Number of each mite species / Total mite number) × 100.

Mite density was reported as number of mites/g dust.

Mite preparation and identification

Specimens were cleared using lactophenol and then mounted using Hoyer's medium on microscope slides and dried for 5 to 7 d in an oven at 50ºC according to the method of Krantz & Walter (2009). The mites were identified to species level using a Leica DM 2500 phase contrast microscope. The identification of mites was made according to the appropriate keys (Fain et al., 1990; Solarz, 2010; Solarz et al., 2016).

Confirmation of species identification was made in University of Michigan, Department of Ecology and Evolutionary Biology, USA by Dr. Pavel B. Klimov.

Results and Discussion

The monthly distribution of dust mite numbers (mites/g dust) in house A in 2013-2015 and in houses B, C, D, E in 2013-2014 in Ordu city, and weekly temperature and RH during the study are given in Figure 1-5.

A total of 72 dust samples were examined and all houses sampled were found to be infested with house dust mites. A total of 1,143 mite specimens in various developmental stages were collected during the study.

A total of 1105 mites were identified to the species level. This group included *D. pteronyssinus, D. farinae, Lepidoglyphus destructor* (Schrank, 1781), *Chortoglyphus arcuatus* (Troupeau, 1879). The 38 damaged mites were identified only to the genus level as *Cheyletus* sp*., Dermatophagoides* sp*., Tyrophagus* sp*.* and *Rhizoglyphus* sp*.*

The most abundant species were *D. pteronyssinus* and *D. farinae*. They constituted 65.4% (748) and 31.0% (354) of the total count of mites collected from the bedrooms, respectively. The next six rare mite species and their abundance were *L. destructor* at 0.2% (2), *C. arcuatus* 0.1% (1), *Cheyletus* sp.1.8% (21), *Dermatophagoides* sp. 0.9% (10), *Tyrophagus* sp. 0.4% (5) and *Rhizoglyphus* sp. 0.2% (2).

In house A, the mite population reached the highest with 33 mites/g dust in the first year and 35 mites/g dust in the second year on August (26.2ºC and 65.4% RH, and 26.9ºC and 73.0% RH, respectively). During the study, the lowest mite population density was recorded between March-June. The mite population ranged between 12 and 16 mites/g dust during this period (21.9-23.8ºC; 53.8-74.8% RH) (Figure 1). A total of 465 mite specimens in various developmental stages were determined. They were; *D. pteronyssinus* (251 ♀♀, 20 ♂♂, 12 tritonymphs, 8 protonymphs), *D. farinae* (95 ♀♀, 31 ♂♂, 5 tritonymphs, 14 protonymphs), *L. destructor* (1 ♂), *Cheyletus* sp. (14 ♀♀), *Tyrophagus* sp. (2 ♀♀, 1 tritonymphs), *Rhizoglyphus* sp. (1 ♂), and *Dermatophagoides* sp. (5 ♀♀, 2 ♂♂, 3 larvae).

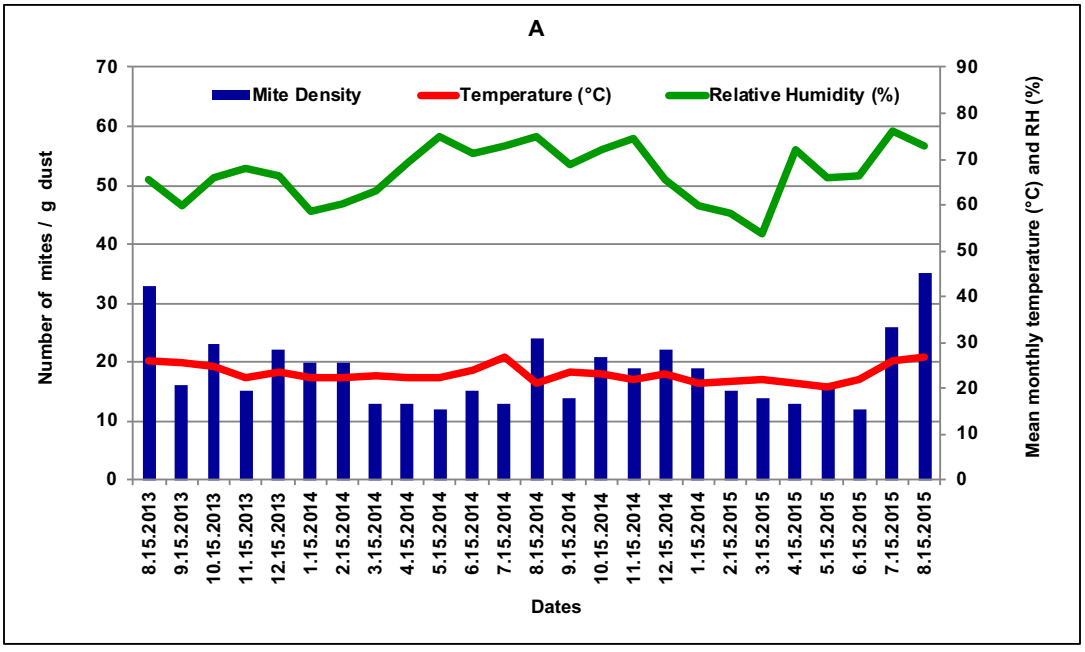

Figure 1. Number of mites/g dust in the house A in the city of Ordu between 2013 and 2015.

In house B, the highest (48 mites/g dust) and lowest (11 mites/g dust) mite numbers were observed in August (27.9ºC and 77.2% RH) and January (22.1ºC and 51.6% RH), respectively. The total number of mites (247) obtained during the sampling period was the highest of all the houses (Figure 2). A total of 247 mite specimens in various developmental stages were determined in this house during the study. They were *D. pteronyssinus* (136 ♀♀, 52 ♂♂, 12 tritonymphs, 5 protonymphs), *D. farinae* (25 ♀♀, 10 ♂♂, 3 tritonymphs, 1 protonymph), *C. arcuatus* (2 ♀♀) and *L. destructor* (1 ♂).

In house C, the highest population density was observed in August (67 mites/g dust) (26.9ºC and 65.3% RH) and the lowest in January (9 mites/g dust) (21.8ºC and 58.3% RH) (Figure 3). A total of 238 mite specimens in various developmental stages were determined in this house during the study. They were *D. pteronyssinus* (80 ♀♀, 35 ♂♂, 1 tritonymphs, 2 protonymphs), *D. farinae* (83 ♀♀, 23 ♂♂, 6 tritonymphs, 7 protonymphs) and *Cheyletus* sp. (1 ♀).

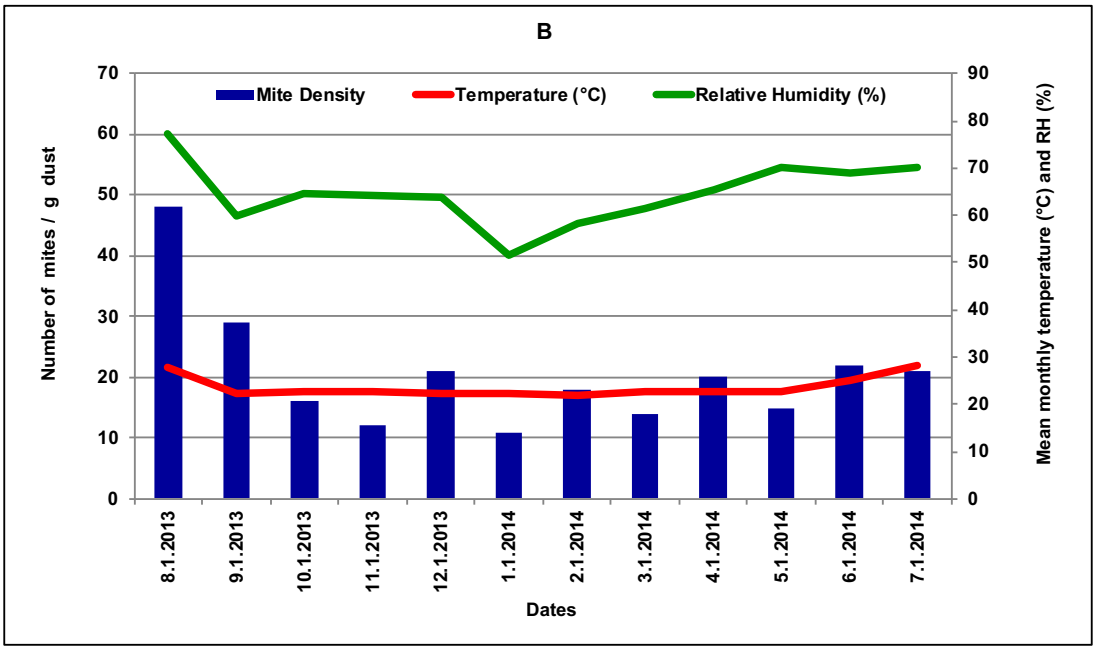

Figure 2. Number of mites/g dust in the house B in the city of Ordu between 2013 and 2014.

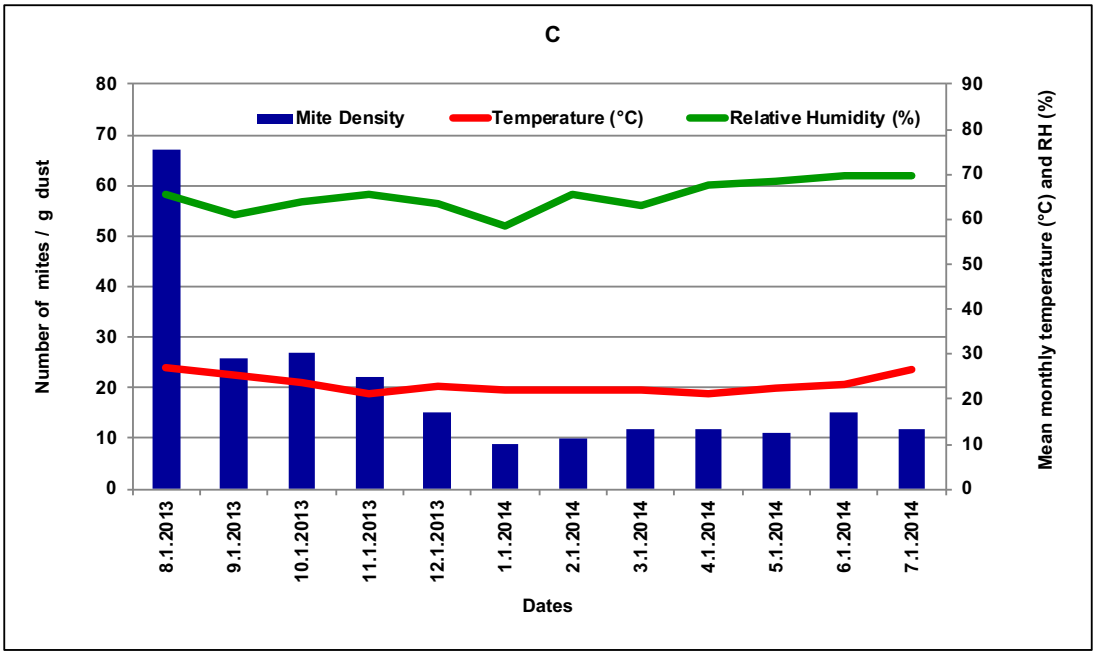

Figure 3. Number of mites/g dust in the house C in the city of Ordu between 2013 and 2014.

In house D, the dust mite density was highest (27 mites/g dust) in August (26.9ºC and 59.33% RH) and May (22.6ºC and 59.1% RH). The lowest mite population was 3 mites/g dust in January (24.3ºC and 59.4% RH) (Figure 4). A total of 171 mite specimens in various developmental stages were determined in this house during the study. They were *D. pteronyssinus* (48 ♀♀, 55 ♂♂, 8 tritonymphs, 4 protonymphs), *D. farinae* (37 ♀♀, 10 ♂♂, 3 tritonymphs, 1 protonymphs), *C. arcuatus* (1 ♂), *Cheyletus* sp. (1 ♀), *Tyrophagus* sp. (2 ♀♀) and *Rhizoglyphus* sp. (1 ♀).

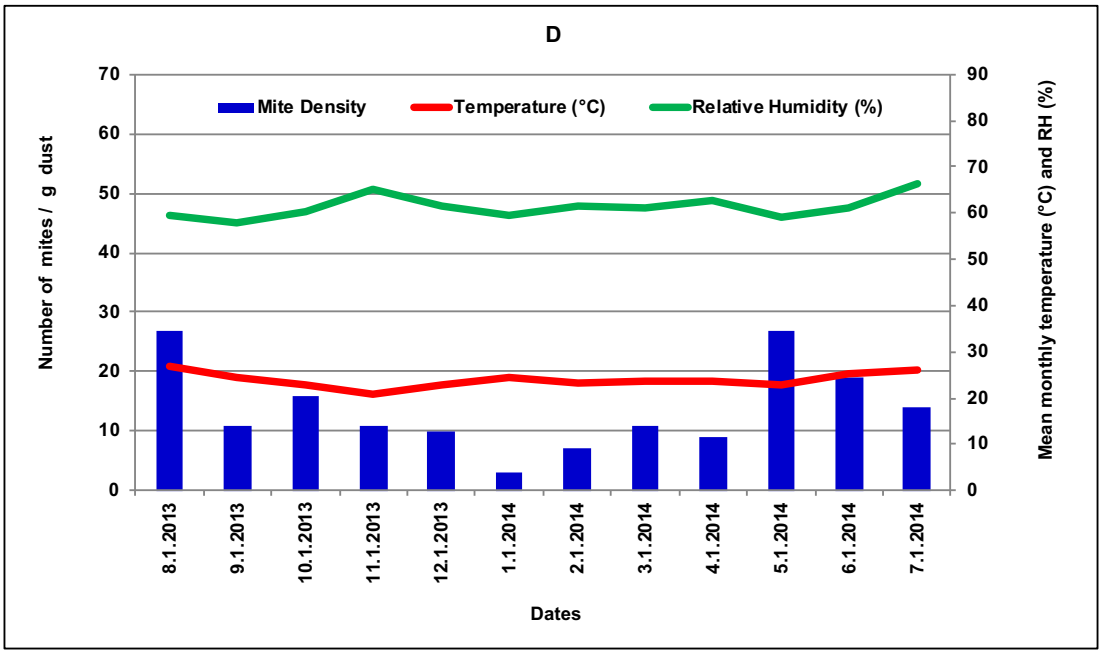

Figure 4. Number of mites/g dust in the house D in the city of Ordu between 2013 and 2014.

In house E, the mite density peaked in August (9 mites/g dust) (28.4ºC and 77.4% RH). The total mite number (22) obtained during the sampling period was the lowest of all the houses (Figure 5). A total of 22 mite specimens in various development stages were determined in this house during the study. They were *D. pteronyssinus* (9 ♀♀, 9 ♂♂, 1 tritonymphs) and *Cheyletus* sp. (3 ♀♀).

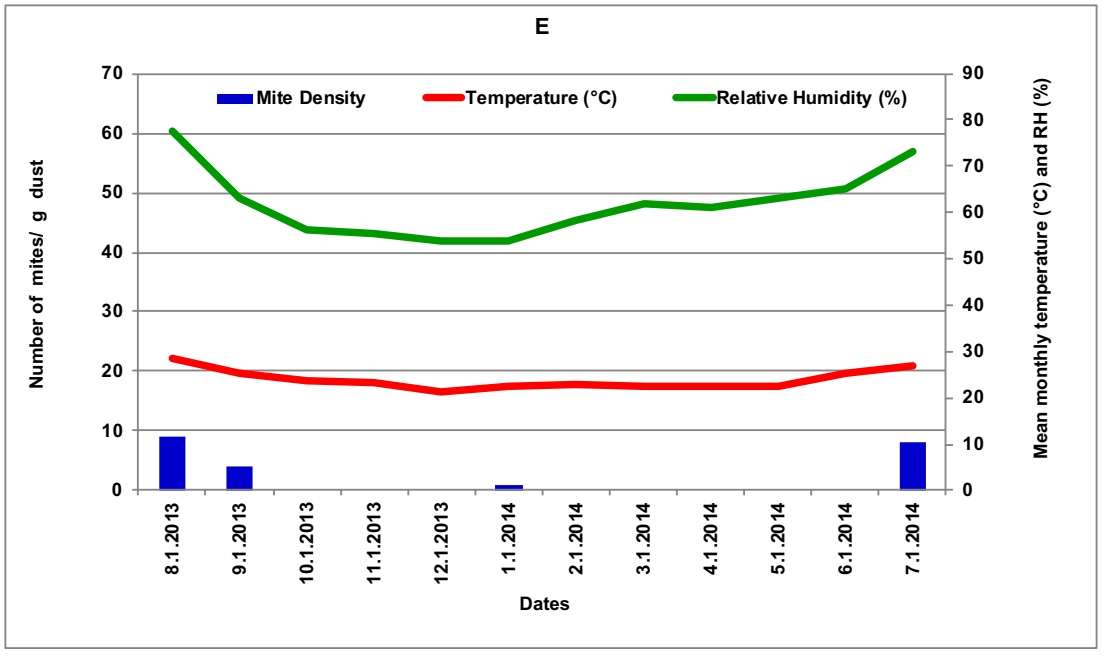

Figure 5. Number of mites/g dust in the house E in the city of Ordu between 2013 and 2014.

The total number of mites obtained from house A, B, C, D and E during the sampling period was 465 (215 in first year and 250 in second year), 247, 238, 171 and 22, respectively.

The highest density of mites (67 mites/g dust) was observed in house C and the lowest (1 mite/g dust) in house E. The person with asthma who lived in house E was highly sensitive to the house dust. It was observed that he took many precautions such as cleaning air vents and ducts, routine cleaning, using of synthetic, hypoallergenic, washable bedding and pillows and special vacuum cleaners to reduce his exposure to indoor dust. It is hypothesized that because of this, the number of mites/g dust in this house was lower than the others. Although completely eliminating dust mites from a given apartment is virtually impossible, their numbers can be reduced by adopting some of the precautionary measures as those mentioned above.

The people who lived in the other houses (A, B, C and D) were also sensitive to house dust. They also took many precautions to reduce exposure to indoor dust. It is also hypothesized that because of this reason, the number of mites in these houses was lower than expected.

Living mites were found all year around in all houses. This is consistent with the results from northern Poland (Racewicz, 2001), Vilnius Region (Duatartiene, 2001) and Turkey (Tatvan) (Aykut et al., 2013).

The mean monthly number of mites changed depending on the room temperature and RH. When we compared the results, the dust mite population was generally higher in August (26-28ºC and 64-78% RH) in all houses. Alternatively, the lowest density was observed in January (21-22ºC and 51-58% RH) in all houses except in house A. These results are consistent with the results obtained by various researchers from other parts of Turkey including Budak (1984) and Kalpaklıoğlu et al. (1997). An increase in the number of mites was observed between June and September in Hasköy (Muş) and November in Dağdibi (Muş) by Aykut et al. (2013) when the outside mean monthly temperature and RH were generally above 20ºC and 50%, respectively. However, the same researchers found that mite numbers were high during the whole year in one house and lowest in August-October in the second house in Tatvan (Bitlis). The mite number was highest from June to September (temperature and RH unspecified) in Kütahya (Akdemir & Gürdal, 2005). In contrast, Ozman-Sullivan & Celik (2010) found that *D. pteronyssinus* had the highest population density in September (temperature and RH unspecified) in Samsun which is another city along the Black Sea coast in Turkey.

Mumcuoglu et al. (1999) found that the highest prevalence of mites occurred in April-November and May-November with a maximum temperature ranging between 30 and 32ºC and RH 45% or above. Sharma et al. (2011) detected higher mite population during the summer and early autumn in South Assam, India. The mean atmospheric temperature and RH of this part of India ranges from 29 to 32ºC and 90 to 94%, respectively. Kosik-Bogacka et al. (2012) examined the seasonal dynamics of mite populations in dust samples collected from sleeping places in northwestern Poland. The mite numbers were high in October to December and low in April to June corresponding to the highest and lowest temperature (24.5 and 19.0ºC) and RH (90 and 68%).

In the current study, *Cheyletus* sp. which is known as one of the major predators of house dust mites were also detected in the dust samples. In accordance with other studies conducted in Turkey, Poland, Chile, Lithuania (Franjola & Malonnek, 1995, Duatartiene, 2001; Racewicz, 2001; Solarz, 2001; Akdemir & Gürdal, 2005; Ozman- Sullivan & Celik 2010), the total number of cheyletid mites obtained during the sampling period was quite low (21 mites, 1.8%) in Ordu. The frequency of *Cheyletus* spp. in house dust samples was higher (20.0-48.5%) in other parts of the world (Montealegre et al., 1997; Baqueiro et al., 2006; Sharma et al., 2011; Kosik-Bogacka et al., 2012). According to Ree et al. (1997) and Henszel et al. (2010), these mites can only develop with particularly high RH and therefore are more common in the tropics than in Europe.

Our results show that the mite abundance reached its peak in each house during August in Ordu. The average temperature and RH during this period in the Ordu homes varied from 26 to 28ºC and 64 to 78% RH, respectively. It is known that dust mites develop optimally at temperatures between 25 to 30ºC and RH between 75 to 95% (Arlian et al., 1990; Collof, 2009).

It was also shown that house dust mites can survive all year round in the city because the mean room temperature and RH during the study in all houses were generally within the optimum range for growth and development of dust mites.

The inhabitants of the sampled houses were sensitive to house dust and accordingly took many precautions to reduce exposure to indoor dust. Given this, it is considered that the number of mites in these houses were lower than expected.

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Original article (Orijinal araştırma)

Ichneumonidae (Hymenoptera) from Northeastern Anatolia Region (Erzurum, Aşkale)[1]

Kuzeydoğu Anadolu Bölgesi (Erzurum, Aşkale)'nden Ichneumonidae (Hymenoptera) türleri

Ümit SARI[2] **Saliha ÇORUH[3*]**

Abstract

This study examined material from the family Ichneumonidae collected from Turkey (Aşkale, Erzurum Province) between 2015-2017. Nineteen species belonging to Acaenitinae, Anomaloninae, Banchinae, Campopleginae, Collyriinae, Cremastinae, Cryptinae, Diplazontinae, Ichneumoninae, Tryphoninae and Pimplinae were recorded. Additional collecting locations and distribution of Turkey with maps are given for each species. Also, the areas of expansion in the world are given. Four species are new records for the Turkish fauna; *Campoletis holmgreni* Tschek, 1871, *Hellwigia elegans* Gravenhorst, 1823 (Campopleginae), *Hemichneumon subdolus* Wesmael, 1857 and *Bathyplectes rufigaster* Horstmann, 1977 (Ichneumoninae).

Keywords: Aşkale, Erzurum, Hymenoptera, Ichneumonidae, new records, Turkey

Öz

Bu çalışmada, 2015-2017 yılları arasında Aşkale merkez ilçeden toplanan Ichneumonidae türleri incelenmiştir. Çalışma sonucunda, Acaenitinae, Anomaloninae, Banchinae, Campopleginae, Collyriinae, Cremastinae, Cryptinae, Diplazontinae, Ichneumoninae, Tryphoninae ve Pimplinae altfamilyalarına ait ondokuz tür tespit edilmiştir. Her bir tür için, ilave yayılım alanları ve haritaları ile birlikte Türkiye'deki dağılımları, dünyadaki dağılım alanları da verilmiştir. Dört tür Türkiye faunası için yeni kayıttır; *Campoletis holmgreni* Tschek, 1871, *Hellwigia elegans* Gravenhorst, 1823 (Campopleginae), *Hemichneumon subdolus* Wesmael, 1857 ve *Bathyplectes rufigaster* Horstmann, 1977 (Ichneumoninae).

Anahtar sözcükler: Aşkale, Erzurum, Hymenoptera, Ichneumonidae, yeni kayıtlar, Türkiye

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Introduction

Hymenoptera (sawflies, wasps, ants and bees) are one of four megadiverse insect orders, comprising more than 153,000 described and possibly up to one million undescribed extant species (Grimaldi & Engel, 2005; Aguiar et al., 2013). As parasitoids, predators and pollinators, Hymenoptera are fundamental in all terrestrial ecosystems and are of substantial economic importance (Quicke, 1997; Grimaldi & Engel, 2005).

The Hymenopteran family, Ichneumonidae, is particularly species-rich, and one of the largest and most diverse insect families in the world (Riedel & Magnusson, 2014). This family includes 1579 genera and 24,281 described species (Yu et al., 2012). Townes (1969) estimated that there could be about 60,000 ichneumonid species in the world, and subsequently Gauld (1997) revised this figure upwards to potentially more than 100,000 species.

The Ichneumon wasps include some of the most important parasitic species so that they are important in the suppression of harmful species especially caterpillars (Lepidoptera) (Kasparyan, 1981).

In Turkey, there was no detailed information available on Ichneumonidae until 1995. Kolarov (1995) listed 383 ichneumonid species in a catalog of the Ichneumonidae of Turkey. Over the past 22 years, many studies have been conducted on this group. Each study contributed to the enrichment of the fauna. Relatively recently, the number of recorded Ichneumonidae species was 1228 (Kolarov et al., 2017). However, with more recent contributions (Çoruh et al., 2018; Riedel et al., 2018) and this paper, the number of Ichneumonidae fauna of Turkey has reached to 1257 species and 287 genera.

The aim of this study is to determine Ichneumonidae species found in various habitats on Erzurum, Aşkale.

Material and Methods

Study area

Aşkale district (39º92'02" N, 40º69'47" E) in Erzurum Province, situated in the Northeastern Anatolia of Turkey (Figure 1), was selected as the study area (Figure 2). Aşkale is west of Erzurum and ranges from sea level up to 1625 m. There are Dumanlı, Güllü, Merşem and Kop Mountains on the borders of Aşkale.

Sampling method and collection

Materials used in this study were collected by sweep net from flowering plants in the summers of 2015 to 2017. Specimens were transferred by aspirator and killed with ethyl acetate. Standard methods were used for preparation of the samples, after which they were deposited in the Entomological Museum, Erzurum, Turkey. The pictures of the specimens were taken by a Leica CLS 50x microscope connected to a computer.

All ichneumonid specimens were collected by first author. Specimens were identified by Dr. Janko Kolarov and Dr. Saliha Çoruh. The terminology used for morphological features mostly follows that of Townes (1969). Distributional data mainly follows that of Yu et al. (2012).

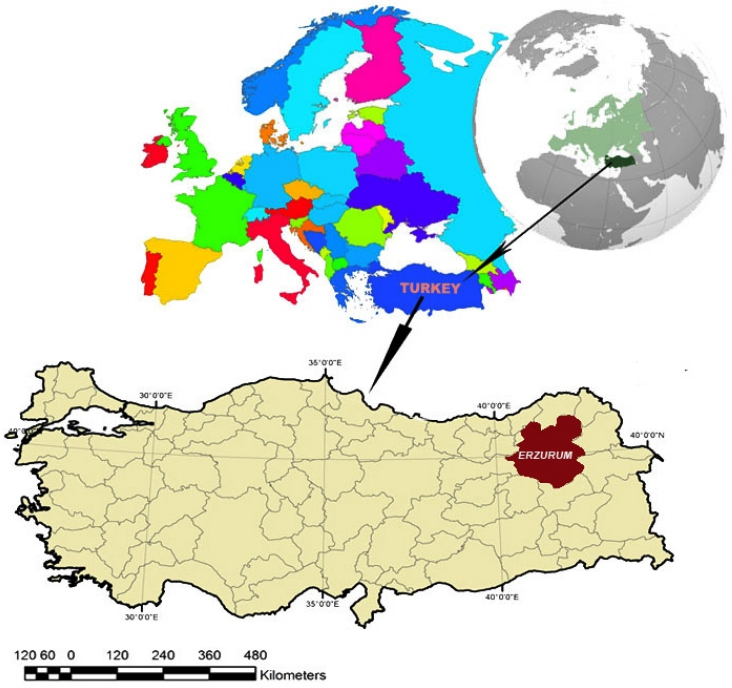

Figure 1. Map of the study area.

Figure 2. Study area.

Results

A total of 185 specimens were collected in Aşkale village during the three summers by Ümit Sarı, with 19 species in 11 subfamilies were recorded. Four species, marked in the text with an asterisk, are new records for the Turkish fauna.

Subfamily Acaenitinae Förster, 1869

Phaenolobus fulvicornis (Gravenhorst, 1829) (Figure 3a)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 25.VI.2015, 1681 m, 11 ♂♂; 39°56'580" N, 40°46'163" E, 1688 m, 21.VII.2016, 2 ♂♂.

Distribution in Turkey: Asia Minor (Kolarov, 1995); Erzurum (Kolarov et al., 2002a); Isparta (Gürbüz, 2005; Gürbüz et al., 2009b); Artvin, Bayburt, Erzurum, Kars (Çoruh & Özbek, 2013); Erzurum (Çoruh et al., 2014b); Bayburt (Çoruh & Çalmaşur, 2016); Rize (Kolarov et al., 2016) (Figure 4a).

General distribution: Palearctic Region.

Subfamily Anomaloninae Viereck, 1918

Anomalon cruentatum (Geoffroy, 1785) (Figure 3b)

Material examined: Aşkale: 39°56'598' N, 40°46'172" E, 12.VI.2015, 1691 m, 5 ♀♀; 39°56'580" N, 40°46'163" E, 08.VIII.2016, 1688 m, 3 ♀♀.

Distribution in Turkey: Afyonkarahisar, Ankara, Çanakkale, Istanbul, Kayseri, Muğla, Tekirdağ, Yozgat (Yurtcan et al., 1994; Kolarov, 1995; Kolarov et al., 1997a; Beyarslan et al., 2006); Afyonkarahisar, Isparta, Muğla (Kolarov et al., 2002b; Gürbüz, 2004); Antalya, Bayburt, Bingöl, Diyarbakır, Erzincan, Erzurum, Iğdır, Kahramanmaraş, Kars (Çoruh et al., 2004); Adıyaman, Batman, Diyarbakır, Elazığ, Malatya, Mardin (Akkaya, 2005); Bolu, Isparta, Kastamonu, Zonguldak (Okyar & Yurtcan, 2007; Buncukçu, 2008; Kırtay, 2008; Birol, 2010); Erzurum, Tunceli (Kolarov et al., 2014b); Erzurum (Kolarov et al., 2016); Bayburt, Erzurum, Kars (Çoruh & Kolarov, 2016); Erzincan, Erzurum, Gümüşhane (Kolarov et al., 2017) (Figure 4b).

General distribution: Palearctic Region.

Subfamily Banchinae Wesmael, 1845

Lissonata (*Loxonota*) *flavovariegata* (Lucas, 1849) (Figure 3c)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 05.VII.2015, 1681 m, 2 ♂♂; 39°56'580" N, 40°46'163" E, 07.VII.2015, 1688 m, 7 ♂♂, 3 ♀♀; 39°56'598' N, 40°46'172" E, 21.VI.2016, 1691 m, 2 ♂♂, 4 ♀♀.

Distribution in Turkey: Bolu, İzmir, Konya (Kolarov, 1995; Kolarov et al., 2002); Çankırı, Kırşehir, Nevşehir, Yozgat (Özdemir, 1996); Ankara, Bayburt, Erzincan, Erzurum, Kars, Trabzon (Pekel, 1999); Erzincan, Erzurum, Giresun, Gümüşhane, Ordu (Kolarov et al., 2017) (Figure 4c).

General distribution: Palearctic Region.

Subfamily Campopleginae Förster, 1869

**Campoletis holmgreni* (Tschek, 1871) (Figure 3d)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 09.VIII.2015, 1681 m, 1 ♂, Leg. Ümit Sarı.

General distribution: Palearctic Region.

Remarks: This species is new record from Turkey.

**Hellwigia elegans* Gravenhorst, 1823 (Figure 3e)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 08.VII.2015, 1681 m, 1 ♂; Leg. Ümit Sarı.

General distribution: Palearctic Region.

Remarks: This species is new record for Turkey.

Subfamily Cremastinae Förster, 1869

Cremastus pungens Gravenhorst, 1829 (Figure 3f)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 05.VII.2015, 1681 m, 2 ♀♀, Leg. Ümit Sarı.

Distribution in Turkey: Erzurum (Pekel, 1998; Pekel & Özbek, 2000); Tekirdağ (Beyarslan et al., 2006); Elazığ, Eskişehir, Kayseri, Malatya, Yozgat (Kolarov & Yurtcan, 2009); Batı Anadolu (Bozdağlar) (Anlaş et al., 2009); Hatay (Çoruh & Özbek, 2013) (Figure 4d).

General Distribution: Palearctic Region.

Pristomerus rivalis Narolsky, 1987 (Figure 3g)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 09.VIII.2015, 1681 m, 2 ♂♂; 39°56'598' N, 40°46'172" E, 21.VIII. 2016, 1691 m, 2 ♂♂, Leg. Ümit Sarı.

Distribution in Turkey: Erzurum (Pekel & Özbek, 2000) (Figure 4e).

General distribution: Palearctic Region.

Remarks: This species was collected for the first time in Palandöken Mountain in 1996, since then, it has not been found again in Turkey.

Temelucha pseudocaudata Kolarov, 1982 (Figure 3h)

Material examined: Aşkale: 39°56'566" N, 40°46' 156" E, 05.VII.2015, 1681 m, 2 ♀♀, Leg. Ümit Sarı.

Distribution in Turkey: Erzurum (Pekel, 1998; Pekel & Özbek, 2000; Çoruh et al., 2014b) (Figure 4f).

General Distribution: Palearctic Region.

Subfamily Collyriinae Cushman, 1924

Collyria coxator (Villers, 1789) (Figure 3i)

Material examined: Aşkale: 39°56'598' N, 40°46'172" E, 12.VI.2015, 1691 m, 15 ♀♀; 39°56'566" N, 40°46'156" E, 09.VIII.2015, 1681 m, 12 ♀♀; 39°56'598' N, 40°46'172" E, 21.VII.2016, 1691 m, 2 ♂♂, 3 ♀♀, Leg. Ümit Sarı.

Distribution in Turkey: Ankara, Istanbul (Kolarov, 1995); Erzurum, Kars (Çoruh et al., 2005a); Batman, Diyarbakır, Elazığ, Mardin (Akkaya, 2005); Isparta (Gürbüz, 2005; Gürbüz et al., 2009b); Afyonkarahisar, Konya (Özdemir & Güler, 2009); Sivas, Yozgat (Korkmaz et al., 2010); Erzurum (Çoruh et al., 2014b; Kolarov et al., 2014b); Afyonkarahisar, Aksaray, Amasya, Ankara, Ardahan, Çanakkale, Çorum, Düzce, Edirne, Elazığ, Erzurum, Eskişehir, Karaman, Kars, Kastamonu, Kayseri, Kırıkkale, Kırklareli, Kırşehir, Konya, Malatya, Nevşehir, Niğde, Sivas, Tekirdağ, Tokat, Yozgat, Zonguldak (Yurtcan & Kolarov, 2015) (Figure 4g).

General distribution: Holarctic Region.

Subfamily Cryptinae Kirby, 1837

Aritranis director (Thunberg, 1822) (Figure 3j)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 09.VIII.2015, 1681 m, 5 ♂♂; 39°56'598" N, 40°46'172" E, 12.VI.2015, 1691, 3 ♂♂; 39°56'566" N, 40°46'156" E, 30.VII.2016, 1681 m, 4 ♂♂, Leg. Ümit Sarı.

Distribution in Turkey: Antalya, Burdur, Isparta (Gürbüz & Kolarov, 2008); Isparta (Gürbüz et al., 2009b); Rize, Trabzon (Çoruh et al., 2014a); Isparta (Özdan, 2014; Özdan & Gürbüz, 2016) (Figure 4h).

General distribution: Palearctic Region.

Remarks: This species is new for the Eastern Anatolia and Erzurum.

Cryptus viduatorius Fabricius, 1804 (Figure 3k)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 25.VI.2015, 1681 m, 4 ♂♂, 3 ♀♀; 39°56'598' N, 40°46'172" E, 12.VI.2015, 1691 m, 4 ♂♂, 3 ♀♀; 39°56'580" N, 40°46' 163" E, 21.VII.2016, 1688 m, 6 ♂♂, 5 ♀♀; 39°56'566" N, 40°46'156" E, 30.VII.2016, 1681 m, 12 ♂♂, 3 ♀♀, Leg. Ümit Sarı.

Distribution in Turkey: Istanbul (Kolarov, 1995); Bilecik, Bursa (Kolarov et al., 1997b); Isparta (Gürbüz & Kolarov, 2008); Erzurum (Çoruh & Çoruh, 2008); Isparta (Gürbüz et al., 2009a; Özdan, 2014); Rize (Çoruh et al., 2014a); Erzurum (Çoruh & Kolarov, 2016; Kolarov et al., 2016) (Figure 4i).

General distribution: Palearctic Region.

Subfamily Diplazontinae Viereck, 1918

Diplazon laetatorius (Fabricius, 1781) (Figure 3l)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 03.VII.2015, 1681 m, 1 ♀; 39°56'598' N, 40°46'172" E, 21.VIII.2016, 1691 m, 2 ♀♀, Leg. Ümit Sarı.

Distribution in Turkey: Edirne, İstanbul, Kırklareli, Tekirdağ (Yurtcan et al., 1999); Afyonkarahisar, Ankara, Bolu, Burdur, Eskişehir, Isparta, Konya, Nevşehir (Özdemir, 2001); Artvin, Erzincan, Erzurum, Şanlıurfa, Trabzon (Çoruh, 2011); Adana, Hatay, Osmaniye (Gürbüz et al., 2011); Adana, Adıyaman, Afyonkarahisar, Aydın, Denizli, Erzincan, Erzurum, Isparta, İçel, İzmir, Kahramanmaraş, Kırklareli, Muğla, Sinop, Zonguldak (Kolarov, 2015) (Figure 4j).

General distribution: Afrotropical, Australasian, European, Holarctic, Nearctic, Neotropical, Oceanic, Oriental Regions.

Remarks: This species was obtained from pupae of *Episyrphus balteatus* (De Geer 1776) (Diptera: Syrphidae) in the University Campus, Erzurum in 2005.

Subfamily Ichneumoninae Latreille, 1802

Anisobas hostilis (Gravenhorst, 1820) (Figure 3m)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 03.VII.2015, 1681 m, 2 ♂♂; 39°56'598' N, 40°46'172" E, 21.VI.2016, 1691 m, 21.VIII.2016, 4 ♂♂, Leg. Ümit Sarı.

Distribution in Turkey: Tekirdağ (Yurtcan et al., 1999); Erzurum (Özbek et al., 2003); Kars (Riedel et al., 2010) (Figure 4k).

General distribution: Europea and West Palearctic Region.

**Bathyplectes rufigaster* Horstmann, 1977 (Figure 3n)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 9.VIII.2015, 1681 m, 5 ♀♀; 39°56'598' N, 40°46'172" E, 21.VI.2016, 21.VIII.2016, 1691 m, 5 ♂♂, Leg. Ümit Sarı.

General distribution: Europea and West Palearctic Region.

Remarks: This species is new record for Turkey.

Colpognathus divisus Thomson, 1891 (Figure 3o)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 05.VII.2015, 1681 m, 3 ♂♂; 39°56'598' N, 40°46'172" E, 12.VI.2015, 1691 m, 2 ♂♂; 39°56'566" N, 40°46'156" E, 30.VII.2016, 4 ♂♂; 39°56'598' N, 40°46'172" E, 21.VIII.2016, 3 ♂♂, Leg. Ümit Sarı.

Distribution in Turkey: Erzurum, Rize, Trabzon (Kolarov et al., 2014a; Çoruh, 2017) (Figure 4l).

General distribution: Palearctic Region.

**Hemichneumon subdolus* Wesmael, 1857

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 05.VII.2015, 1681 m, 1 ♂, Leg. Ümit Sarı.

General distribution: Palearctic Region.

Remarks: This species is new record from Turkey.

Subfamily Pimplinae Wesmael, 1848

Itoplectis maculator (Fabricius, 1775) (Figure 3p)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 03.VII.2015, 1681 m, 2 ♂♂; 39°56'598' N, 40°46'172" E, 21.VIII.2016, 1691 m, 2 ♀♀, Leg. Ümit Sarı.

Distribution in Turkey: Ankara, Eskişehir, Kırşehir, Konya, Nevşehir, Yozgat (Özdemir & Kılınçer, 1990); Adana, Balıkesir, İçel (Kolarov & Beyarslan, 1994); Çanakkale (Kolarov et al., 1997a); Erzurum (Kolarov et al., 1999); Afyonkarahisar, Balıkesir, Denizli, Muğla (Kolarov et al., 2002b); Çanakkale, Edirne, İstanbul, Kırklareli, Tekirdağ (Yurtcan, 2004); Artvin, Bayburt, Erzurum, Gümüşhane, Kars, Rize (Çoruh, 2005); Çanakkale, Edirne, İstanbul, Kırklareli, Tekirdağ (Yurtcan & Beyarslan, 2005); Kastamonu (Okyar & Yurtcan, 2007); Hatay (Gürbüz et al., 2008); Isparta (Kırtay, 2008; Birol, 2010; Eroğlu et al., 2011); Erzurum, Trabzon (Kolarov et al., 2016); Erzurum (Kolarov et al., 2017) (Figure 4m).

General distribution: Palearctic Region.

Remarks: This species was collected feeding on *Daucus carota*.

Pimpla spuria Gravenhorst, 1829 (Figure 3r)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 03.VII.2015, 1681 m, 4 ♂♂, 1 ♀; 39°56'598' N, 40°46'172" E, 21.VIII.2016, 1691 m, 2 ♂♂ 5 ♀♀, Leg. Ümit Sarı.

Distribution in Turkey: Ankara (Özdemir & Kılınçer, 1990); Adana, Adıyaman, Edirne, Gaziantep, Hatay, Kırklareli, Şanlıurfa, Tekirdağ (Kolarov & Beyarslan, 1994); Balıkesir, Bilecik, Bursa, Çanakkale (Kolarov at al., 1997b); Çanakkale (Kolarov et al., 1997a); Erzurum (Kolarov et al., 1999; Çoruh, 2016); Afyonkarahisar, Denizli, Manisa, Muğla, Uşak (Kolarov et al., 2002b); Çanakkale, Edirne, Istanbul, Kırklareli, Tekirdağ (Yurtcan, 2004); Çanakkale, Edirne, İstanbul, Kırklareli, Tekirdağ (Yurtcan & Beyarslan, 2005); Isparta (Gürbüz, 2005); Adana (Boncukcu, 2008); Eskişehir (Eroğlu et al., 2011); Erzurum, Tunceli (Kolarov et al., 2014b) (Figure 4n).

General Distribution: Oriental and Palearctic Region.

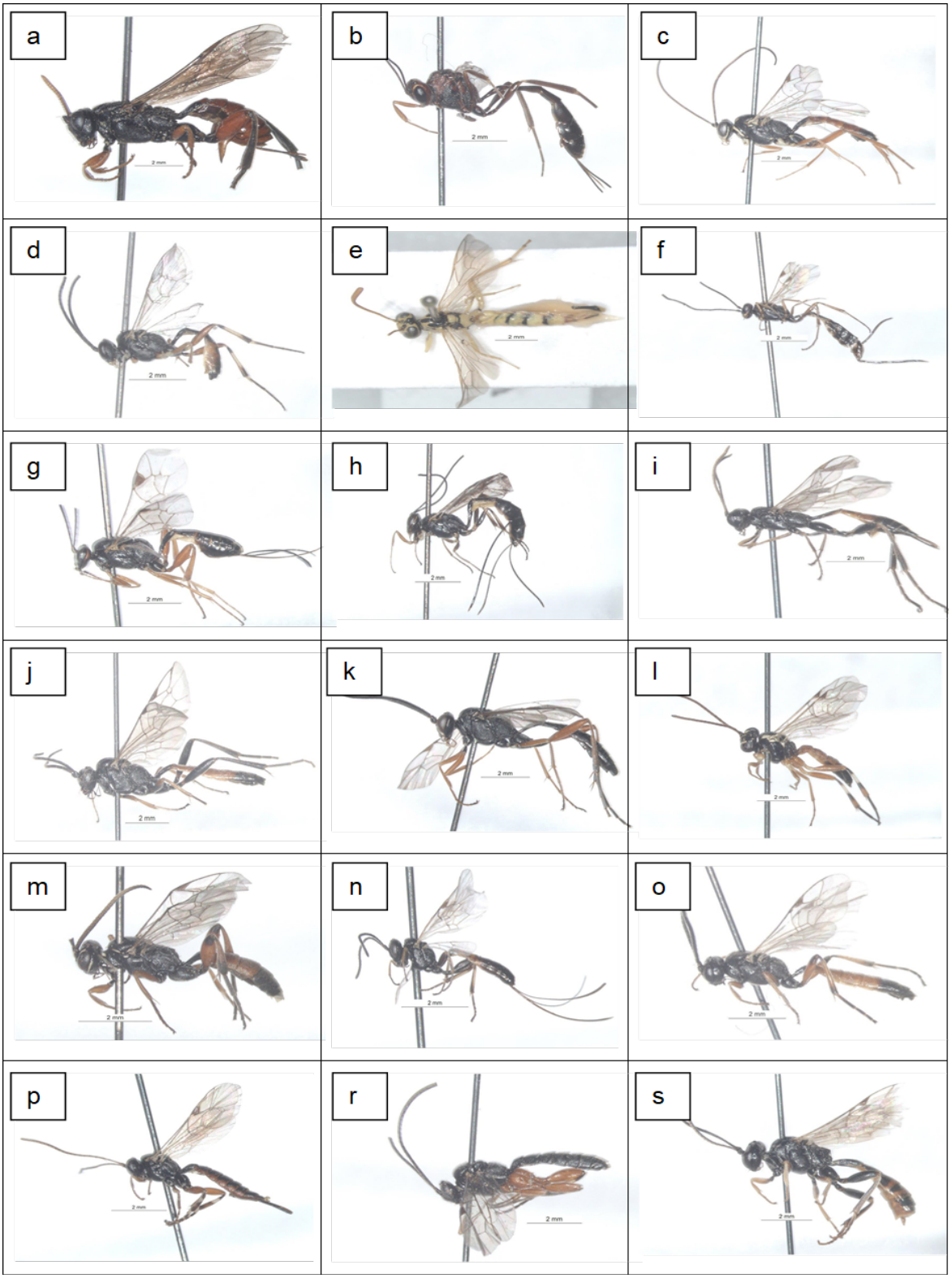

Figure 3. a) *Phaenolobus fulvicornis*; b) *Anomalon cruentatum*; c) *Lissonata (Loxonota) flavovariegata*; d) *Campoletis holmgreni*; e) *Hellwigia elegans*; f) *Cremastus pungens*; g) *Pristomerus rivalis*; h) *Temelucha pseudocaudata*; i) *Collyria coxator*; j) *Aritranis director*; k) *Cryptus viduatorius*; l) *Diplazon laetatorius*; m) *Anisobas hostilis*; n) *Bathyplectes rufigaster*; o) *Colpognathus divisus*; p) *Itoplectis maculator*; r) *Pimpla spuria*; and s) *Tryphon (Tryphon) atriceps*.

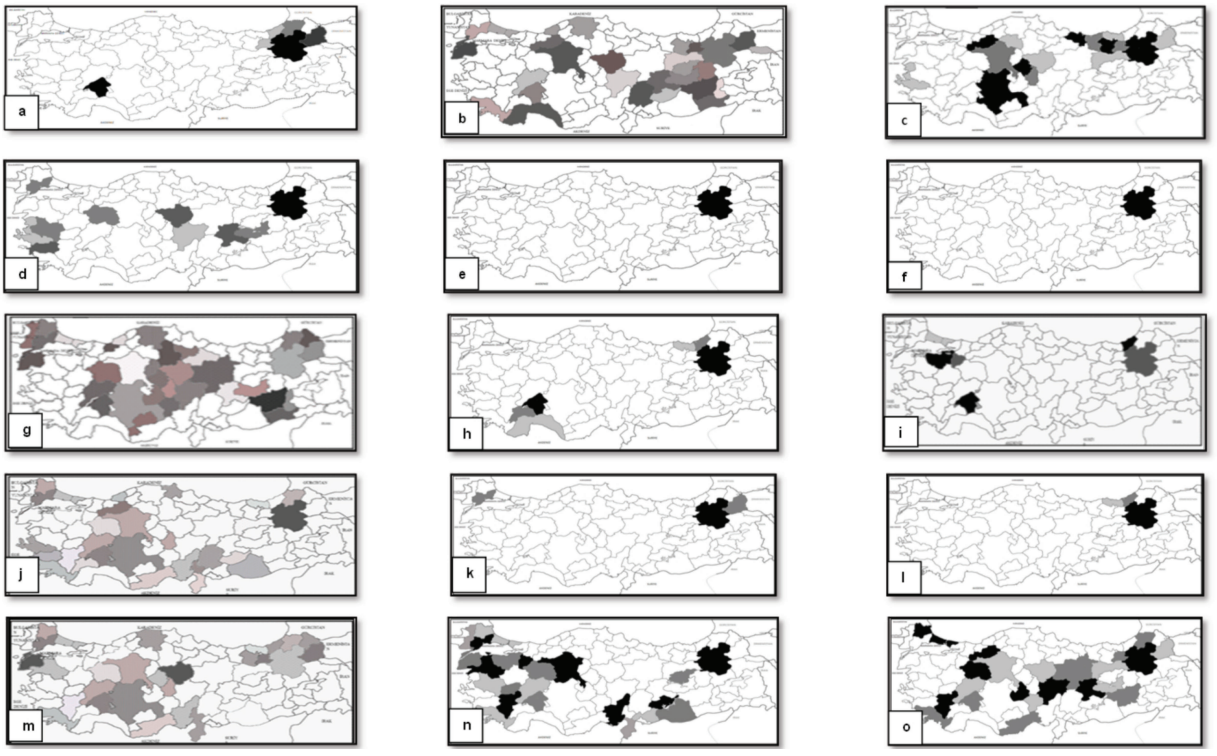

Figure 4. Distribution in Turkey of species: a) *Phaenolobus fulvicornis*; b) *Anomalon cruentatum*; c) *Lissonata (Loxonota) flavovariegata*; d) *Cremastus pungens*; e) *Pristomerus rivalis*; f) *Temelucha pseudocaudata*; g) *Collyria coxator*; h) *Aritranis director*; i) *Cryptus viduatorius*; j) *Diplazon laetatorius*; k) *Anisobas hostilis*; l) *Colpognathus divisus*; m) *Itoplectis maculator*; n) *Pimpla spuria*; and o) *Tryphon (Tryphon) atriceps.*

Subfamily Tryphoninae Shuckard, 1840

Tryphon (Tryphon) atriceps Stephens, 1835 (Figure 4o)

Material examined: Aşkale: 39°56'566" N, 40°46'156" E, 05.VII.2015, 1681 m, 2 ♂♂; 39°56'580" N, 40°46'163" E, 21.VII.2016, 3 ♀♀, Leg. Ümit Sarı.

Distribution in Turkey: Erzurum, İstanbul (Kolarov, 1994); Erzurum (Kolarov et al., 1999); Bolu, Eskişehir (Özdemir, 2001); Kırklareli (Yurtcan & Beyarslan, 2002); Sivas (Kasparyan & Shaw, 2005); Bayburt, Erzurum (Çoruh et al., 2005b); Isparta (Gürbüz, 2005; Beyarslan et al., 2006); Afyonkarahisar, Denizli, Muğla (Yurtcan & Beyarslan, 2006); Isparta (Gürbüz et al., 2009a; Eroğlu et al., 2011); Artvin, Bayburt, Diyarbakır, Erzincan, Erzurum, Isparta, Kars (Kolarov & Çoruh, 2012); Aksaray, Ankara, Edirne, Elazığ, İçel, Malatya, Niğde, Sivas, Yozgat (Yaman, 2014) (Figure 4o).

General Distribution: Palearctic Region.

Discussion

In “A catalogue of the Turkish Ichneumonidae” (Kolarov, 1995) 383 species were reported. After 1995, the first comprehensive studies conducted in the Thrace Region, and continued in the Eastern Anatolia Region and the Mediterranean Region, increased in the number of species to 1257 species.

When the total number is analyzed, three families, 34 genera and 274 species have been added Turkish Ichneumonidae fauna from Eastern Turkey since 1995 (Figure 5).

Figure 5. Eastern Turkey.

Among the 274 species which have been identified from Eastern Turkey, *Cymodusa (Cymodusa) yildirimi* Kolarov & Çoruh, 2008*, Exochus protuberans* Kolarov & Çoruh, 2009*, Coelichneumon nigritor* Riedel, Çoruh & Özbek 2010*, Coelichneumon problematicus* Riedel, Çoruh & Özbek, 2010*, Ichneumon sexcinctoides* Riedel, Çoruh & Özbek, 2010, *Phaenolobus trochanteralis* Çoruh & Kolarov, 2013 and *Collyria pronotalis* Yurtcan & Kolarov, 2015 were new species for the world. In addition, male of *Phaenolobus cornutus* (Victorov), *Ophion internigrans* Kokujev, *Eucremastus priebei* Kolarov, *Scallama triclistor* Aubert, and *Temelucha pseudocaudata* Kolarov were reported for the first time in Eastern Turkey. Also, *Alcima pictor* Aubert, *Colpotrochia triclistor* (Aubert), *Coelichneumon nigritor* Riedel, Çoruh & Özbek, *Exochus protuberans* Kolarov & Çoruh and *Heterischnus schachti* Diller, 1995 are endemic for the Turkey at now.

As shown in Figure 6a, of the 185 specimens collected, Cryptinae was found to be the most abundant at 28%, and Campopleginae has the least abundant at only 1%. The Ichneumoninae had the greatest species richness with 21% (Figure 6b).

Ichneumonid samples were collected between June and August, being more abundant in July. According to general distribution, 15 species have Palearctic distribution, two have West Palearctic distribution; one Holarctic and one species, *Diplazon laetatorius,* is cosmopolitan. According to these results, the species with Palearctic distribution are the better represented in this area.

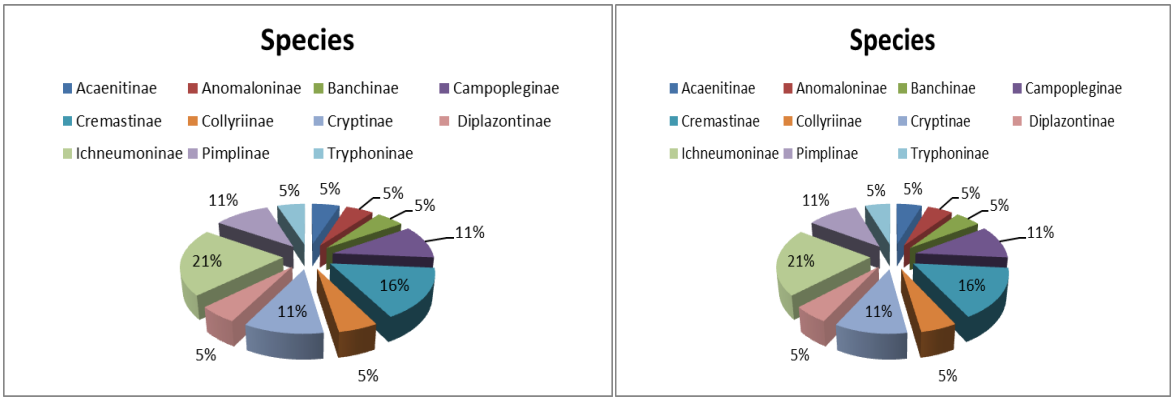

Figure 6. Distribution of subfamilies; a) abundance; and b) richness.

It is important to note that *Bathyplectes rufigaster* was recorded for the first time in Turkey during this study.

It should be noted that, except for *Aritranis director,* all other species have been collected from Erzurum before. *Aritranis director* was collected for the first time from both Erzurum and East Anatolia during this study.

Pristomerus rivalis and *Temelucha pseudocaudata* were found only from Erzurum being two species rare in Turkey.

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Original article (Orijinal araştırma)

Detection of the root-knot nematode *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae) in vegetable fields of Samsun Province, Turkey

Samsun İli sebze alanlarında kök-ur nematodu *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae)'nin belirlenmesi

Gökhan AYDINLI[1*]

Abstract

The root-knot nematode, *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae), is commonly found in greenhouses in Samsun Province in northern Turkey but has not been reported in open fields. However, the most recent study on the distribution of root-knot nematodes in open fields of this region was conducted more than 20 years ago and identification was based on perineal patterns. Therefore, the aim of the present study was to update the distribution of *Meloidogyne* spp. in the vegetable fields of Samsun Province. For that purpose, soil samples were collected from 50 vegetable fields during July 2017. Nematode isolates obtained from bioassay tests were identified based on their esterase enzyme phenotype and identification was confirmed with molecular techniques. Root-knot nematodes were detected in 20% of the fields sampled. *Meloidogyne luci* was the most prevalent species, as was reported from greenhouses in Samsun Province, followed by *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 and *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949. *Meloidogyne luci* is reported for the first time from open fields in Turkey with this study.

Keywords: Distribution, esterase phenotype, *Meloidogyne*, mitochondrial DNA, Turkey

Öz

Türkiye'nin kuzeyindeki Samsun İli'ndeki seralarda yaygın bulunan kök-ur nematodu *Meloidogyne luci* Carneiro et al., 2014 (Tylenchida: Meloidogynidae), bu çalışmadan önce açık alanlarda tespit edilmemiştir. Ayrıca, bu bölgedeki açık alanlarda kök-ur nematodlarının dağılımı ile ilgili en son çalışma, 20 yıldan daha uzun süre önce yürütülmüş ve teşhis perineal desenlere göre yapılmıştır. Bu yüzden, mevcut çalışmanın amacı, Samsun ilindeki sebze tarlalarında *Meloidogyne* türlerinin dağılımına ait sonuçların güncellenmesidir. Bu amaçla, Temmuz 2017'de 50 sebze tarlasından toprak örnekleri alınmıştır. Test bitkilerinden elde edilen nematod izolatları, esteraz enzim fenotiplerine göre teşhis edilmiş ve teşhis moleküler teknikler ile teyit edilmiştir. Örnekleme yapılan sebze tarlalarının %20'sinde kök-ur nematodları tespit edilmiştir. Samsun ilinde daha önce seralarda belirlenen *M. luci,* en yaygın tür olarak tespit edilirken, bu türü *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 ve *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 takip etmiştir. Türkiye'de açık alanlarda *M. luci*' nin varlığı ilk defa bu çalışma ile bildirilmiştir.

Anahtar sözcükler: Dağılım, esteraz fenotipi, *Meloidogyne,* mitokondrial DNA, Türkiye

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Introduction

Root-knot nematodes (*Meloidogyne* spp.) are among the most important threats to vegetable production. These parasitic nematodes infest the root system and typically cause abnormal swellings called galls which have adverse effects on the uptake of water and nutrients (Aydınlı & Mennan, 2016). This damage is seen above ground as the stunting and yellowing and results yield reduction. Wesemael et al. (2011) reported in a review that the degree of damage for important *Meloidogyne*-plant combinations in Europe ranges from 40% to 100%. Nearly 100 species have been described in this nematode genus and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949*, Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Meloidogynidae) are considered major pest species commonly detected in different vegetable production systems (Wesemael et al., 2011; Onkendi et al., 2014). Furthermore, *Meloidogyne chitwoodi* Golden et al., 1980 and *Meloidogyne fallax* Karssen, 1996, reported as emerging species by Moens et al. (2009), have become prevalent in vegetable production systems in European countries (Wesemael et al., 2011). Additionally, *Meloidogyne ethiopica* Whitehead, 1968 as a different species from those previously reported in Europe, was detected in tomatoes in Slovenia in 2003 (Sirca et al., 2004). After this report, *M. ethiopica* was recorded in Greece, Italy and Turkey (Conceição et al., 2012; Maleita et al., 2012; Aydınlı et al., 2013). In 2014, Carneiro et al. (2014) described *Meloidogyne luci* Carneiro et al., 2014 as a new root-knot nematode species from different crops in Brazil, Chile and Iran. This species has a very similar morphology to that of *M. ethiopica*. Moreover, a close relationship has been detected between *M. luci, M. ethiopica* and *Meloidogyne inornata* Lordello, 1956 based on phylogenetic analyses. Therefore, these three species are considered to be sister species (Carneiro et al., 2014; Gerič Stare et al., 2017). The esterase phenotypes of these species have three bands and are very similar but a slight difference in their band positions is sufficient to separate each of them (Carneiro et al., 2014), with only an esterase band position different between *M. ethiopica* and *M. luci*. However, before *M. luci* was described, populations in Europe were reported as *M. ethiopica*. Then, Janssen et al. (2016) reported that the Slovenian population accepted as *M. ethiopica* was actually *M. luci*. Later, Gerič Stare et al. (2017) reported that *M. ethiopica* populations in Europe, including Turkey, should be reclassified as *M. luci*. On the basis of that report, all *M. ethiopica* populations in Turkey were accepted as *M. luci*, including those found in vegetable greenhouses in Samsun Province (Aydınlı & Mennan, 2016).

Samsun Province, which is in the Middle Black Sea Region of northern Turkey, had total vegetable production of 1.16 Mt in 2017 (TUIK, 2017). The largest vegetable production areas are the Bafra and Çarşamba Plains, each with about 15,000 ha. Five *Meloidogyne* species, namely *M. arenaria, M. incognita, M. hapla, M. javanica* and *M. luci* (formerly *M. ethiopica*), have been reported from vegetable production areas of this province in different surveys (Bora, 1970; Yüksel, 1974; Mennan & Ecevit, 1996; Katı & Mennan, 2006; Aydınlı & Mennan, 2016). However, the most recent survey in open vegetable fields was conducted by Mennan & Ecevit (1996); they reported *M. incognita* as the most common root-knot nematode. Later surveys in the province were only conducted in greenhouses and the most recent showed that *M. luci* was the predominant species (Katı & Mennan, 2006; Aydınlı & Mennan, 2016). To date, there have been no reports of *M. luci* in open fields of Turkey. Hence, the aim of this study was to determine whether *M. luci* occurs in open vegetable fields. Additionally, the present study sought to update records of the distribution of *Meloidogyne* species in vegetable fields of Samsun Province.

Material and Methods

Surveys

Surveys were conducted in 2017 in 10 and 11 villages on the Bafra and Çarşamba Plains, respectively, in Samsun Province of northern Turkey (Figure 1). A total of 50 randomly selected vegetable fields in Bafra (25 fields; 8 pepper, 8 tomato, 6 melon and 3 watermelon) and Çarşamba (25 fields; 7 tomato, 7 bean, 6 pepper, 4 melon and 1 eggplant) were surveyed for the early presence of *Meloidogyne* infestation during July 2017. The roots of 20-30 plants from each field were checked for the presence of *Meloidogyne* galls and soil samples were collected from the rhizosphere of infected plants. The individual

soil samples collected in each field were combined into a composite sample, placed in a labeled, polyethylene bag and transported to the Nematology Laboratory of the Faculty of Agriculture at Ondokuz Mayıs University in Samsun for processing. Soil samples were used in bioassay tests within 2 days.

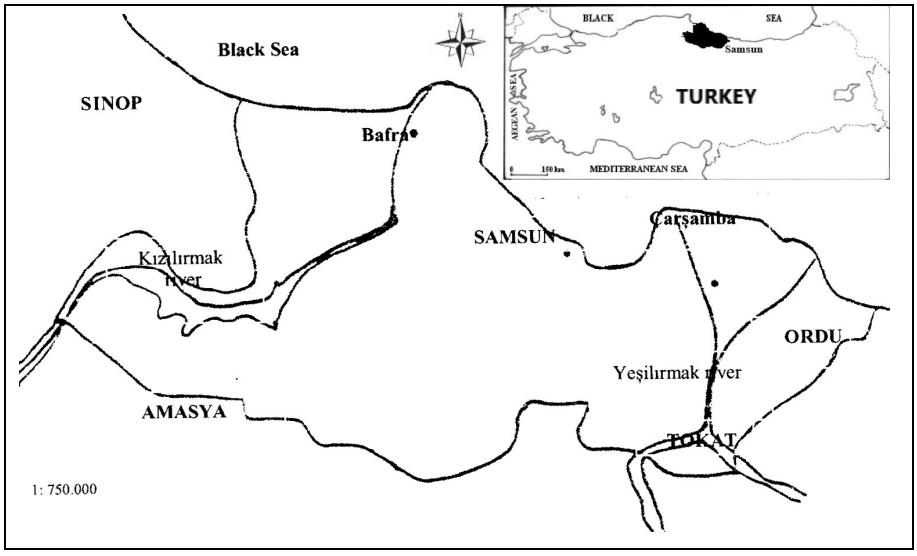

Figure 1. Locations of surveyed area in the map of Turkey.

Detection of *Meloidogyne* spp.

The presence of *Meloidogyne* spp. in soil samples was determined by bioassay test. For that purpose, 3-week-old seedlings of a nematode-susceptible tomato variety (*Solanum lycopersicum* L. cv. Falcon, May Seed, Turkey) were transplanted singly into pots containing 500 cm^3 of the homogenized soil sample from each field (Karuri et al., 2017). Plants were maintained at 25±2°C in the greenhouse, irrigated and fertilized as need. The plants were removed from the pots after 60 days and the roots were carefully washed with tap water. The roots were dissected under a stereomicroscope and females and egg masses were extracted from random positions of each root system. The females were immediately used and the egg masses were stored at -20ºC until analysis.

Identification of *Meloidogyne* spp. isolates

Meloidogyne spp. isolates were identified by the esterase phenotypes of females and identification was confirmed with molecular techniques based on DNA analysis. For the esterase studies, young females collected in 0.9% NaCl were transferred to an extraction buffer (20% sucrose and 1% Triton X-100) in a microhematocrit tube. Single or three females were crushed with a pestle. Samples were immediately stored at -20ºC. Twenty females from each plant root were analyzed. Females of *M. javanica* isolated from pot cultures in the laboratory were used as reference. The electrophoresis process was run at 6 mA per gel for the first 15 min and then at 20 mA per gel for 40-45 min in a Mini-Protean Tetra System (Bio-Rad Laboratories, Hercules, CA, USA). The gels were stained with the substrate α-naphthyl acetate for esterase activity (Esteves et al., 2015).

For the molecular studies, DNA was extracted from 10 egg masses for PCR with species-specific primers and from an individual male or second-stage juvenile for sequencing of the mitochondrial DNA (mtDNA) region. DNA was extracted with the DNeasy Tissue and Blood Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. PCR reactions were carried out with specific primers, namely Far/Rar (Zijlstra et al., 2000) for *M. arenaria*, Fjav/Rjav (Zijlstra et al., 2000) for *M. javanica*, inc-K14F/R (Randig et al., 2002) and SEC-1F/1R (Tesarova et al., 2003) for *M. incognita*, and JMV (Wishart

et al., 2002) for *M. hapla, M. chitwoodi* and *M. fallax*. Amplifications with specific primers were performed in a final volume of 25 µl that contained 2 µl of template DNA, 1 µl of 10 µM of each primer (0.4 µM of each primer) and 12.5 µl of BioMix Red (Bioline). PCR was conducted with a T-100 Thermal Cycler (Bio-Rad Laboratories) and amplification conditions were as follows: initial denaturation at 94ºC for 3 min; 35 cycles of 30 s at 94ºC, 30 s at 55ºC (SEC-1F/1R and JMV) or 60ºC (Far/ Rar, inc-K14F/R and Fjav/Rjav), and 1 min at 72ºC; and a final extension for 7 min at 72ºC. All samples were tested with all specific primer pairs in the study. The PCR products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide and then visualized.

The mtDNA COI region was amplified with JB3/COI2R5 primers (Kiewnick et al., 2014; Maleita et al., 2018) for the sequencing of the three phenotype L3 isolates. The PCR reaction mixtures contained 5 µl of DNA, 5 µl of 10X standard Taq buffer, 4 µl of 25 mM MgCl2, 2 µl of 5 mM dNTPs, 2 µl of 10 µM of each primer, and 0.5 µl of 5 U/µl Taq DNA polymerase (New England Biolabs, Ipswich, MA, United States) and nuclease-free water made up to 50 µl. Thermal cycling was programmed as reported by Kiewnick et al. (2014). Amplified DNA was sequenced in both directions with the respective primers used for amplification. Sequences were checked and aligned with the BioEdit software package (Hall, 1999). The edited sequences were then compared with the sequences of *Meloidogyne* species available in the GenBank database.

Results

Meloidogyne species was detected in 10 of the 50 fields sampled in Samsun Province (Table 1). Infested soil samples were recovered from vegetable fields in seven of 11 villages on the Carşamba Plain but no infestations were detected on the Bafra Plain. These infested samples were from four bean, four pepper and two tomato fields. The occurrence of *Meloidogyne* spp. varied among vegetable species. Overall, 57% of the bean fields surveyed were infested, followed by 29% of pepper fields and 13% of tomato fields. However, no *Meloidogyne* species were detected in watermelon, melon and eggplant fields.

Root-knot nematode species were identified on the basis of their esterase phenotypes. The three different esterase phenotypes detected, namely L3, A2 and I2, were indicative of *M. luci, M. arenaria* and *M. incognita*, respectively (Figure 2). L3, the most common esterase phenotype in this study, was detected in seven isolates (Figure 3), while A2 was in three isolates and I2 in one isolate. In addition, a sample obtained from a tomato field contained both L3 and A2 phenotypes indicating the presence of *M. luci* and *M. arenaria* isolates.

Identification based on esterase phenotype was confirmed by molecular assays, namely PCR with species-specific primers and sequencing of the mtDNA region. In addition, primers specific for *M. javanica* and *M. hapla*, which had been detected in previous studies in this survey region, were used to check the DNA of isolates identified with esterase assays. The DNA of isolates was not amplified with these primers (data not shown). The PCR conducted with the *M. arenaria* species-specific primer, Far/Rar, produced amplification fragments of 420 bp from the three isolates of the A2 esterase phenotype (Figure 4a). The inc-K14F/R from primer pairs used for the identification of *M. incognita* gave a positive band of 400 bp for an isolate with I2 esterase phenotype (Figure 4b), whereas PCR with SEC-1F/1R primers produced a single band of 500 bp for both an isolate with I2 and seven isolates with L3 (Figure 4c). The PCR with primer pair used for sequencing of the mtDNA COI region yielded single bands of about 800 bp (data not shown). The sequences from the mtDNA COI region were 100% identical with the *M. luci* sequences in GenBank (accession numbers MF280973, MF280974, MF280975, MF280976 and KY563093). The sequences obtained were deposited in GenBank under the accession numbers MG969509, MG969510 and MG969511.

Table 1. *Meloidogyne* isolates obtained from vegetable fields in Samsun Province, Turkey

Isolate No	Field coordinate	Sample field	Esterase phenotype	Primer pairs*			Species
				Far/Rar	inc-F/R	SEC-1F/1R	
103	41°16'39" N 36°33'13" E	Pepper	L3	-	-	+	*M. luci*
108	41°16'20" N 36°35'24" E	Bean	A2	+	-	-	*M. arenaria*
109	41°13'13" N 36°37'17" E	Pepper	I2	-	+	+	*M. incognita*
110	41°15'35" N 36°36'46" E	Bean	L3	-	-	+	*M. luci*
111	41°15'17" N 36°37'28" E	Pepper	L3	-	-	+	*M. luci*
112	41°15'37" N 36°36'52" E	Tomato	L3	-	-	+	*M. luci*
113	41°14'42" N 36°38'14" E	Bean	L3	-	-	+	*M. luci*
114	41°13'58" N 36°38'40" E	Bean	A2	+	-	-	*M. arenaria*
121	41°14'19" N 36°40'03" E	Tomato	A2+L3	+	-	+	*M. arenaria* + *M. luci*
125	41°17'39" N 36°34'40" E	Pepper	L3	-	-	+	*M. luci*

* DNA of isolates was not amplified with Fjav/Rjav, specific for *M. javanica,* and JMV, specific for *M. hapla, M. chitwoodi and M. fallax*.

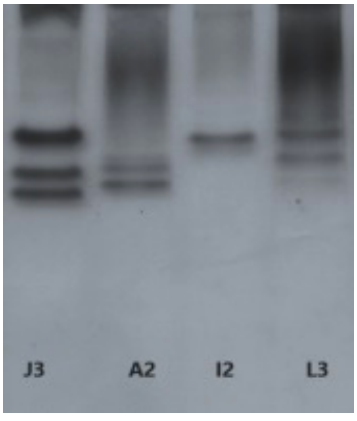

Figure 2. Esterase phenotypes detected from females of *Meloidogyne* isolates (J3, *M. javanica* as reference isolate; A2, *M. arenaria*; I2, *M. incognita*; L3, *M. luci*).

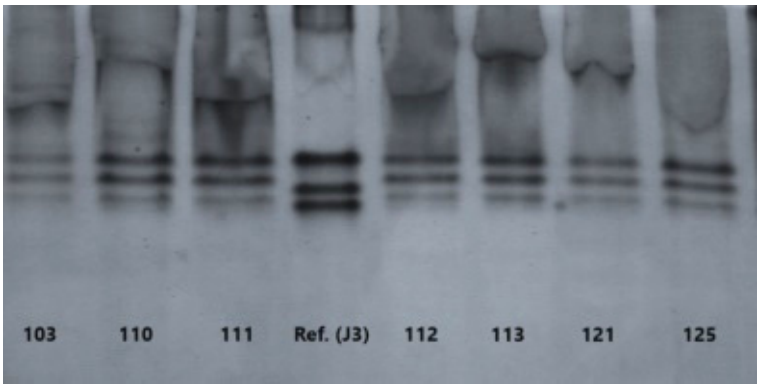

Figure 3. Esterase phenotypes (L3) of *Meloidogyne luci* isolates in this study (J3: reference isolate of *M. javanica*).

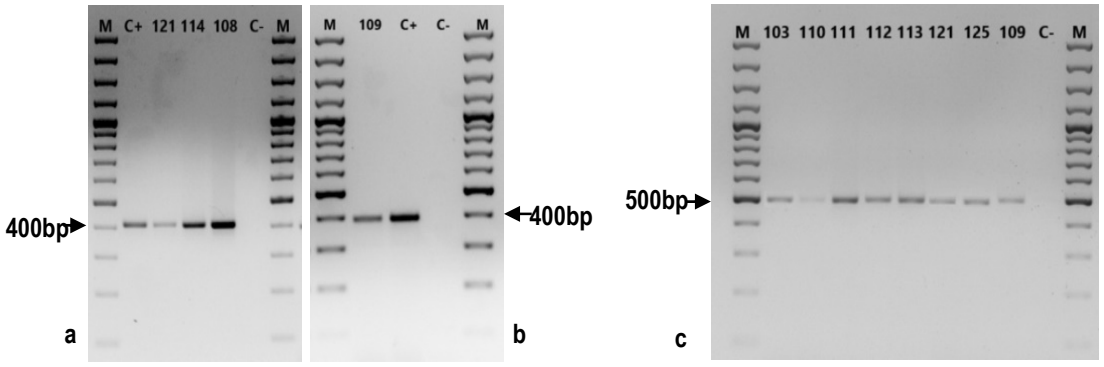

Figure 4. PCR products obtained with a) Far/Rar, primers specific for *Meloidogyne arenaria*; b) inc-K14F/R, primers specific for *Meloidogyne incognita*; and c) SEC-1F/1R, primers specific for *M. incognita* and *Meloidogyne luci* (Numbers indicated code of isolates in this study. C+: positive control DNA, C-: negative control (water), M: molecular marker with 100 bp.).

Discussion

This study reports the presence of *M. luci* (formerly *M. ethiopica*) in open fields in Turkey for the first time. The most recent open field survey for root-knot nematodes in Samsun was conducted over 20 years ago (Mennan & Ecevit, 1996). In that study, the identification of *Meloidogyne* species was based on the perineal patterns. Although the perineal patterns of females aid the identification of *Meloidogyne* species, this character alone is not sufficient anymore and can result in misidentification due to substantial similarity and overlap of perineal patterns between species (Brito et al., 2008; Garcia & Sanchez-Puerta, 2012). For example, the perineal patterns of *M. luci* are similar to those of *M. incognita* and are therefore not useful for identification purposes (Carneiro et al., 2014; Aydınlı & Mennan, 2016). In contrast, the present study reports the presence of *Meloidogyne* species in open fields through the use of reliable and sensitive methods, without relying on expertise in the interpretation of perineal morphology patterns.

Meloidogyne luci, the most prevalent root-knot nematode species in this survey, was collected from three pepper (isolates 103, 111 and 125), two bean (isolates 110 and 113) and two tomato fields (isolates 112 and 121), and *M. arenaria* was collected from two bean fields (isolates 108 and 114) and one tomato field (isolate 121). *Meloidogyne incognita* was detected in only one pepper field (isolate 109). In addition, a mixed population of *M. arenaria* and *M. luci* (isolate 121) was collected from a tomato field.

Meloidogyne luci was first detected in Turkey in 2009 in greenhouses in Samsun Province (Aydınlı et al., 2013). Later, an extensive survey of *Meloidogyne* species in greenhouses of the Middle Black Sea Region revealed that *M. luci* was the most common species, followed by *M. arenaria, M. javanica and*

M. incognita in Samsun (Aydınlı & Mennan, 2016). In the present survey, the occurrence of *Meloidogyne* species, except *M. javanica*, is consistent with the findings of Aydınlı & Mennan (2016). The absence of *M. javanica* in open fields in Samsun Province supports the results of previous studies (Bora, 1970; Yüksel, 1974; Mennan & Ecevit, 1996) and suggests that this species does not survive in open fields but does survive in the greenhouses in this region. However, *M. javanica* was detected in open fields in a different region of Turkey (Aydın, Aegean Region) that was more temperate than the Black Sea Region during the winter period (Kaşkavalcı & Öncüer, 1999). Van Gundy (1985) reviewed the environmental factors affecting survival of *Meloidogyne* spp. and noted that *M. javanica* had a lower resistance to cold stress than both *M. incognita* and *M. arenaria*. In the present study, *M. incognita* was collected in only one field, in contrast to the finding of Mennan & Ecevit (1996) who reported a wide distribution of this nematode in the same area. Differences between the results of the study of Mennan & Ecevit (1996) and the present study in terms of the distribution of this species were also reported in the greenhouse studies of Katı & Mennan (2006) and Aydınlı & Mennan (2016). A likely reason for this is the similarity of the perineal pattern of *M. incognita* and *M. luci*.

The *M. luci* specific esterase enzyme phenotype (L3) is the best tool for differentiating this species from other *Meloidogyne* species (Carneiro et al., 2014; Gerič Stare et al., 2017; Maleita et al., 2018). Additionally, esterase studies can be supported with data from mtDNA regions. The efficacy of mtDNA markers was previously reported by Gerič Stare et al. (2017) and Maleita et al. (2018). Janssen et al. (2016) also showed usefulness of mtDNA regions for tropical root-knot nematodes differentiation. Gerič Stare et al. (2017) reported that in phylogenetic analysis of the COII/IRNA region of mtDNA, *M. luci* formed a monophyletic clade and allowed a clear separation of this recently described species. In addition, Maleita et al. (2018) stated that the mtDNA COI was a useful region for the differentiation of *M. luci* from *M. ethiopica* but it could not be used to differentiate *M. arenaria, M. ethiopica, M. incognita, M. inornata* and *M. javanica*.

In conclusion, *M. luci* was detected for the first time in open vegetable fields of Turkey. This nematode was previously reported from open fields associated with kiwifruit and maize in Greece, and potatoes in Portugal in the EPPO region (Conceicão et al., 2012; Maleita et al., 2018), cucumber, lettuce, broccoli, okra, green bean, yakon, kiwifruit and lavender in Brazil, grapevine in Chili in South America (reviewed in Carneiro et al., 2014), rose, snapdragon and sedum in Iran in Asia (Carneiro et al., 2014). Moreover, Strajnar et al. (2011) reported that it survived in the winter in open fields in both sub-Mediterranean regions and also in the continental climates of regions in Slovenia, despite temperatures below zero. Therefore, *M. luci* appears to constitute a real threat to both open field and protected field crops in the world due to its wide host range and ability to persist in different climatic zones.

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