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Original article (Orijinal araştırma)

Incidence and economic impact of the mint aphid, *Eucarazzia elegans* (Ferrari) (Hemiptera: Aphididae) on common sage[1]

Adaçayında nane yaprakbiti *Eucarazzia elegans* (Ferrari) (Hemiptera: Aphididae)'ın zararı ve ekonomik etkisi

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Summary

A survey and laboratory experiments were conducted on incidence rates and economic impact of *Eucarazzia elegans* (Ferrari) (Hemiptera: Aphididae) in *Salvia officinalis* L. (Lamiaceae). The investigations were purposively performed on conventionally grown common sage in Bornova and Menemen, Izmir-Turkey. Experiments were set up with zero, normal and double population densities of the aphid. The corresponding treatments 0 (no exposure), 2, 4, 6, 8, 10 and 12 weeks (complete exposure) were used for evaluating economic impact. *Eucarazzia elegans* infested old leaves with an incidence rate of 9.5% in the early spring and then migrated to young leaves and blossom at the beginning of the summer with the incidence rate of 1.1%. The seasonal distribution of the aphid was affected more by temperature than humidity and rainfall. Although the aphid infestation had no differences on the quantity of the essential oil, some of the essential oil components were markedly different from the control. The total plant weights (fresh and dry) with the normal and double infestation rate were decreased about a third and more than a half compared to the control, respectively. Aphids feeding on common sage beyond 8-10 weeks caused more than 50% yield loss. The highest cost-benefit ratio was achieved in plots where six insecticide applications were made.

Keywords: Economic impact, *Eucarazzia elegans*, incidence rate, *Salvia officinalis*

Özet

Eucarazzia elegans (Ferrari) (Hemiptera: Aphididae)'ın *Salvia officinalis L.* (Lamiaceae) üzerindeki zarar oranını ve ekonomik etkisini belirlemek üzere sürvey ve laboratuvar çalışmaları gerçekleştirilmiştir. Çalışmalar Bornova ve Menemen'de bilimsel amaçlı kurulmuş parsellerde gerçekleştirilmiştir. Denemeler yaprakbitinin sıfır, normal ve iki katı olmak üzere üç farklı popülasyon yoğunluğunda kurulmuştur. Çalışmada zararlının ekonomik etkisini belirlemek için karşılaştırma yapmak üzere 0 (salım öncesi), 2, 4, 6, 8, 10 ve 12 haftalar boyunca yapılan sayımlardan elde edilen veriler kullanılmıştır. Çalışma sonuçlarına göre, *E. elegans*'ın, erken ilkbahar döneminde %9.5 zarar oranı ile bir önceki yıldan kalan kışlamış yapraklar üzerinde beslendiği belirlenmiş ve daha sonra yaz başında %1.1 zarar oranı ile genç yapraklara ve çiçeklere göç ettiği saptanmıştır. Yaprakbiti mevsimsel dağılımının nem ve yağışa göre sıcaklıktan daha fazla etkilendiği görülmüştür. Yaprakbiti zararı uçucu yağ miktarı üzerinde bir farklılık oluşturmamasına rağmen, uçucu yağ bileşenlerinden bazıları kontrol grubuna göre büyük ölçüde değişime uğramıştır. Toplam yaş ve kuru yaprak ağırlık artışı iki kat popülasyon seviyesinde sırasıyla kontrol ve normal yaprakbiti popülasyon seviyesinde 1/3 ve 1/2 oranında azalmıştır. Yaprakbitlerinin, adaçayında 8-10 haftadan fazla beslenmesi sonrasında %50'den fazla ürün kaybına neden olduğu saptanmıştır. En yüksek maliyet yarar oranı altı kez insektisit uygulanmış parsellerde gerçekleşmiştir.

Anahtar sözcükler: Ekonomik etki, *Eucarazzia elegans*, zarar oranı, *Salvia officinalis*

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Introduction

The mint aphid, *Eucarazzia elegans* (Ferrari, 1872) (Hemiptera: Aphididae) is one of the important aphid species feeding on aromatic and herbal plants in the family Asteraceae, Solanaceae and Lamiaceae, such as *Mentha, Salvia, Lavandula, Coleus, Melissa, Nepeta* and *Origanum* (Blackman & Eastop, 2006). It is a small green aphid which lives generally on the undersides of leaves, shoots and flowers (Stoetzel, 1985; Hales et al., 2009). The alatae of *E. elegans* is easily identifiable by observing the siphunculi which are dark, narrow at the base and swollen towards the tip. The swollen part is more than two times the diameter of the stem of the pale cylindrical base. The wings have dark triangular spots at the ends of all the veins (Stoetzel, 1985; Blackman & Eastop, 2006).

Eucarazzia elegans has been recorded indigenously in the Mediterranean region (Stoetzel, 1985) and has spread to the Madeira (Aguiar & Ilharco, 1997), Middle East (Hussain et al., 2015), Central Asia (Moktari et al., 2012), Pakistan, India, Australia (Hales et al., 2009), Africa (Muller & Scholl, 1958), western USA (Stoetzel, 1985) and South America (Bolivia, Argentina and Brazil) (Avila et al., 2014). The genus of *Eucarazzia* has only two or three species (Blackman & Eastop, 1984), but only *E. elegans* has spread (Blackman & Eastop, 2006). This aphid species has demonstrated the capacity to spread readily via modern transport pathways (Hales et al., 2009).

Research on *E. elegans* infestations, especially on common sage, is limited. In Turkey, the first *E. elegans* record was by Bodenheimer & Swirski (1957), however, there are no reports of economic impacts and losses for most host plants. This aphid species is a well-known pest of *Salvia splendens* F. Sellow ex Roem. & Schult. (Özdemir & Toros, 1997) and *Mentha* sp. (Özdemir, 2004). Ülgentürk et al. (2013) reported *E. elegans* as a honeydew producer on shrubs and herbaceous plants in Aegean, Marmara and Mediterranean Regions, but did not report on damage and yield loss.

In this study, we evaluated *E. elegans* populations to predict incidence rates on *S. officinalis* by survey and its economic impact by laboratory experiments. These data were also used to determine control required for optimal common sage production.

Material and Methods

Field experiment

Seasonal distribution of the mint aphid, *E. elegans* was determined for January to December 2016. The common sage fields used were 0.5 and 1 ha plantings of the experimental farms of the Field Crops Department, Faculty of Agriculture, Ege University Bornova and Aegean Agricultural Research Institute, Menemen, respectively, in Izmir, Turkey. A total 50 blocks from the two plantings were monitored using the incident random sampling. Aphid populations were recorded every 2 weeks for three selected clumps of plant per block. The aphids were counted each infested leaf in each clump. Weather data was obtained for the Second Region of Izmir from the Turkish State Meteorological Service. The relationships between temperature, humidity and rainfall, and seasonal changes in aphid population were determined by multiple regression analysis. Principal component analysis was used to explore the relationship between variables observed.

Laboratory experiment

The experiment had a randomized complete design with 21 replications of three treatments *i.e* zero, normal and double aphid population densities on 2-month old *S. officinalis.* The plants were grown in 15-cm diameter plastic pots under laboratory condition. The zero-population density had no aphids with aphids controlled by azadirachtin insecticide 0.3 g/L EC at the recommended field dose of 5 ml/L applied as a spray every 2 week. The normal population density was 100-200 aphids/plant and the double population density was 300-400 aphids/plant. Final instar or adult aptera aphids reared on *S. officinalis* in the laboratory were used and the aphid population densities were predicted by pre-experimental observations. The aphid populations were maintained every week by reinfesting or removal by hand using a brush. The experiment was conducted for 90 d under the laboratory conditions of 25±2ºC, 65±5% RH and 16:8 h L:D photoperiod. The plant parameters measured were fresh and dry weight, and essential oil concentration. Data were analyzed by repeated measures mixed model analyses of variance.

Water distillation and gas chromatography were used to determine the essential oil concentration and quality, respectively. The essential oil was separated by hydrodistillation for 3 h using a Clevenger-type apparatus, according to the procedure described in the German Pharmacopoeia (Wichtl, 1971). The components of essential oil were determined using an Agilent 6890 N gas chromatograph equipped with a flame ionization detector (GC-FID) at the Central Laboratory, Ege University. Compounds were separated on a high-polarity capillary column (DB-Wax, 30 m x 0.25 mm, 0.25 µm film thickness), with helium as the carrier gas, at a constant flow rate of 1 ml/min. The oven temperature program was as follows: hold for 2 min at 45ºC, the ramp from 45 to 250ºC at 3ºC/min, and hold at 250ºC for 34 min. The injector and detector temperatures were 250ºC. The GC-FID was calibrated using authentic standards (Sigma-Aldrich).

Economic impact of *Eucarazzia elegans*

A randomized complete block experiment was conducted under laboratory conditions with 21 replications of 2-month-old *S. officinalis* plants grown in 15-cm diameter plastic pot. The plants were infested at the normal aphid population density (as above) and were sprayed with azadirachtin applied at 2-weekly intervals either 0, 1, 2, 3, 4, 5 or 6 times to give aphid exposure periods from 12 to 0 weeks. Aphid populations were counted a day before application of the insecticide. The percentages of aphid infestation were measured by determining the ratio of fallen and remaining leaves per plant. At harvest, yields were recorded for each replicate. The plant parameter measured were fresh and dry weight, and plant height. The yield data were subjected to analysis of variance to determine the minimum number of sprays required to produce the maximum yield. Yield data and number of aphids per plant within each treatment were used to calculate the linear regression: $y = a - bx$, where y is the potential yield, a is the expected yield loss at zero infestation level, b is the regression coefficient or yield loss in g/plant caused by one aphid per plant, and x is the percentage infestation.

Results and Discussion

Seasonal distribution and incidence of *Eucarazzia elegans*

The mint aphid appeared in both Bornova and Menemen in the first week of February (the end of winter, Figure 1). The aphids had started to establish in the fields by early spring and then migrated to young leaves and blossom by early summer. The more aphids were captured at Bornova than Menemen ($T = 0.935$; $df = 41.5$; $P < 0.05$), with two distinct peaks of aphid flights observed at Bornova. These were between late February to March and late October to mid-November. The peak observed in late winter to early summer was the highest.

The population dynamics and incidence of *E. elegans* at Bornova were different from Menemen but similar in pattern (Figure 1). At Bornova, the population of total aphids was relatively high from February until the last week of March compared to other periods. However, a sharp decline in the density of aphid population was observed until the beginning of September with the number of aphids captured at this period was lower compared to Menemen. The minimum and maximum incident rate of the aphid was from 1.1 to 9.5% and 0.9 to 8.2% for Bornova and Menemen, respectively. The aphid incidence patterns in both areas were similar with the highest density in March and the lowest in August. This was probably due to the presence of the large populations of natural enemies of aphids, such as Coccinellidae (Coleoptera), Chrysopidae (Neuroptera), Cecidomyiidae and Syrphidae (Diptera) species found feeding on the aphids in spring. Also, considerable variation in weather occurred in the following season.

The aphid population dynamics were strongly affected by weather variations (Figures 2 to 4). The analysis of variance of multiple regression revealed that temperature and humidity had a strong effect on the changes in populations of aphids for both locations (Bornova, R = 0.682, F = 3.136, df = 5, P < 0.033 and Menemen, R = 0.628, F = 3.094, df = 5, P < 0.040). Moreover, it was also clear that at both locations the changes in aphid population was affected more by maximum temperature than humidity (Bornova, P < 0.007 and Menemen, P < 0.038) and minimum temperature (Bornova, P < 0.010 and Menemen, P < 0.039). In contrast, the variation in the aphid populations at both locations was not significantly correlated with rainfall (Bornova, P > 0.088 and Menemen, P > 0.154).

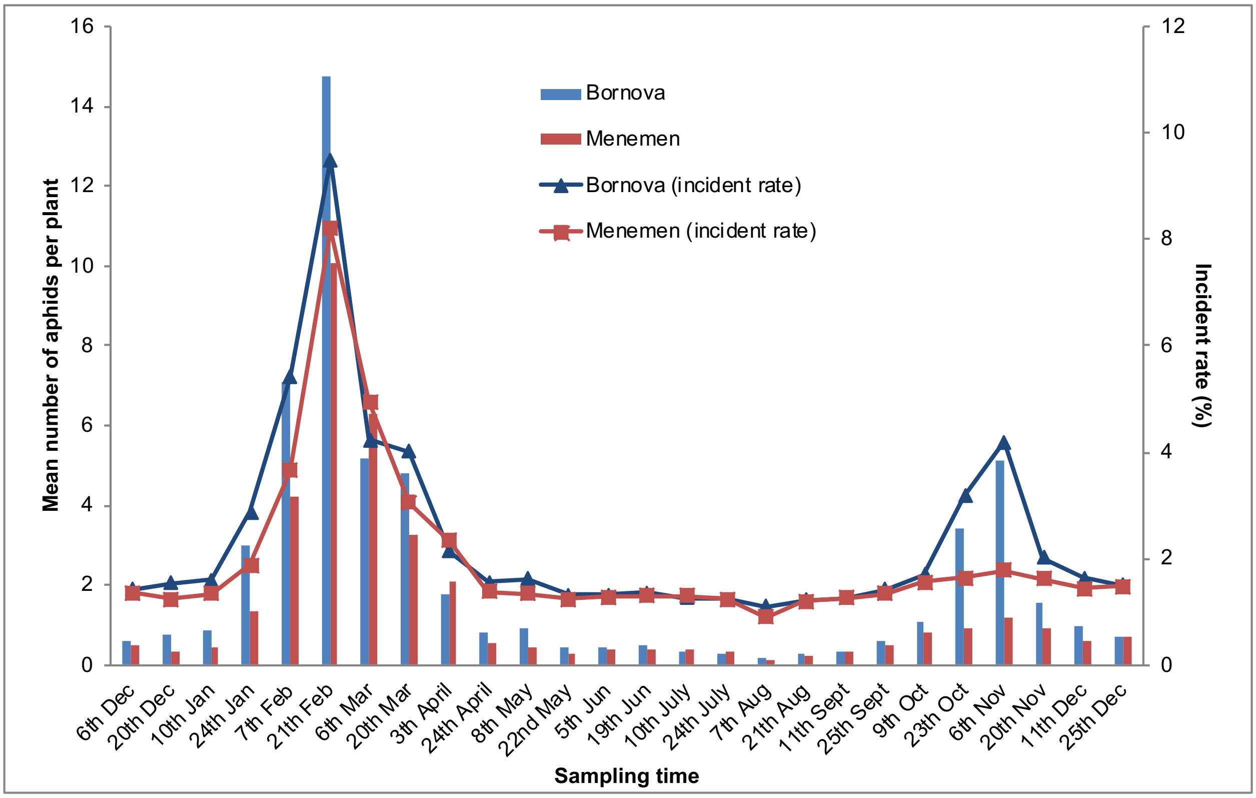

Figure 1. Seasonal distribution and incident rates of *Eucarazzia elegans* on *Salvia officinalis* at Bornova and Menemen in 2016.

The analysis of seasonal changes of aphid population density at Bornova and Menemen showed that peak and low aphid densities were significantly different, however, this was not due variation in rainfall. Whereas, minimum and maximum temperatures appeared to be drivers of aphid population change (Figures 2 to 4). When the two locations were compared, rainfall had a less effect on weekly mean aphid numbers at Menemen than Bornova, but this was not sufficient to reduce the population density. In this study, there was no clear effect of rainfall, which contrasts with several studies that found a negative relationship between rainfall and aphid population density (Mann et al., 1995). Rainfall mainly washes aphids off plants and effects to flight activity, restricting their ability to move within and between plants (Wains et al., 2010; Alyokhin et al., 2011). *Salvia officinalis* has a large, dense canopy which prevents the penetration of raindrops. In fact, mint aphids are able to easily crawl across and between the plants. *Eucarazzia elegans* is also temperate species (Stoetzel, 1985), which is active and develops faster at low temperatures than tropical species. Also, its rate of development at high temperatures allows its population to increase and range to expand when the low-temperature limitation abates (Parry et al., 2006; Hazell et al., 2010; Brabec et al., 2014). Therefore, early emergence in the late winter can lead to an outbreak, if the population of natural enemies in early spring is low.

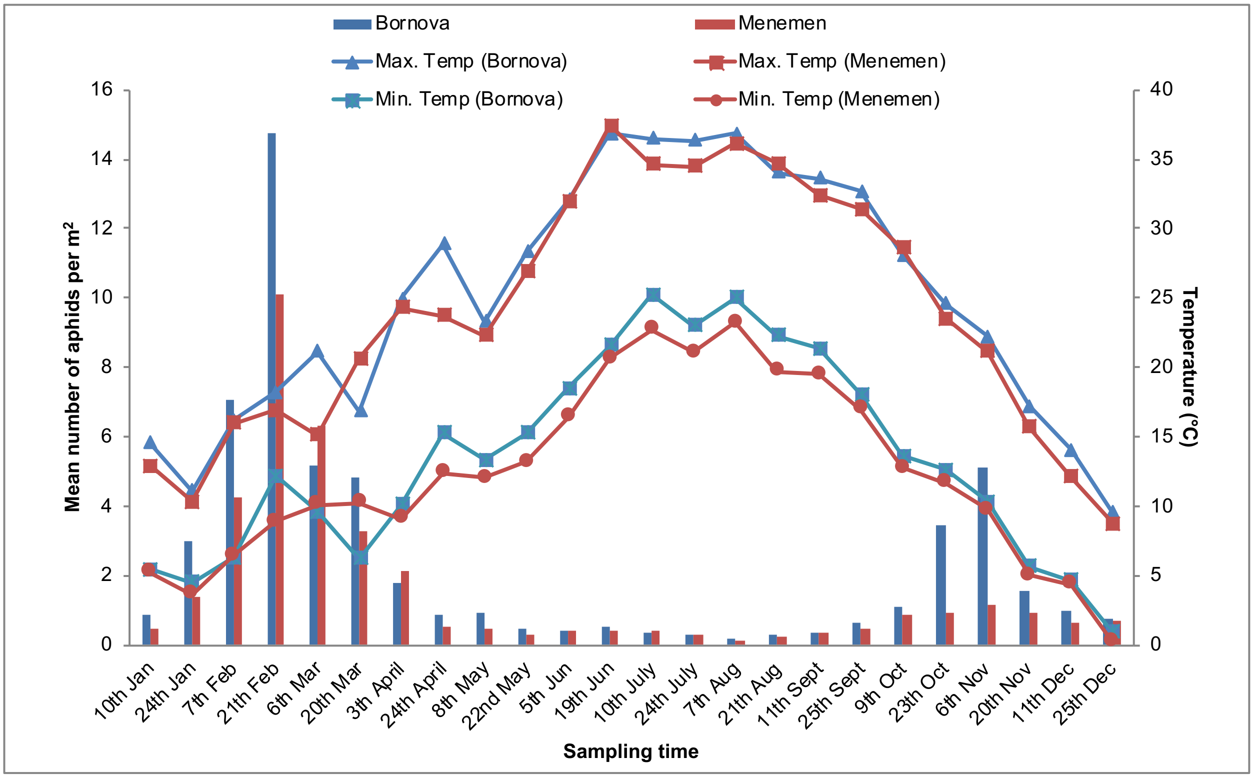

Figure 2. Weekly mean population density of *Eucarazzia elegans,* and maximum and minimum temperatures at Bornova and Menemen in 2016.

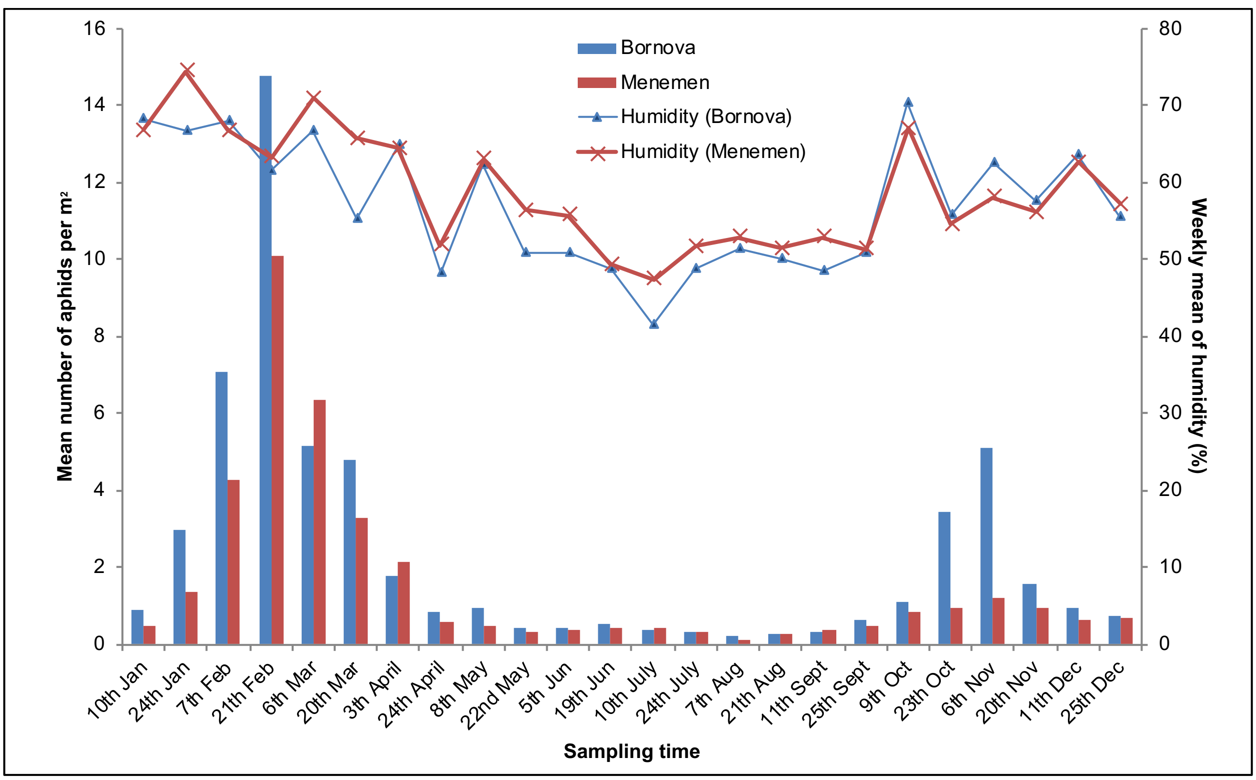

Figure 3. Weekly mean population density of *Eucarazzia elegans* and humidity at Bornova and Menemen in 2016.

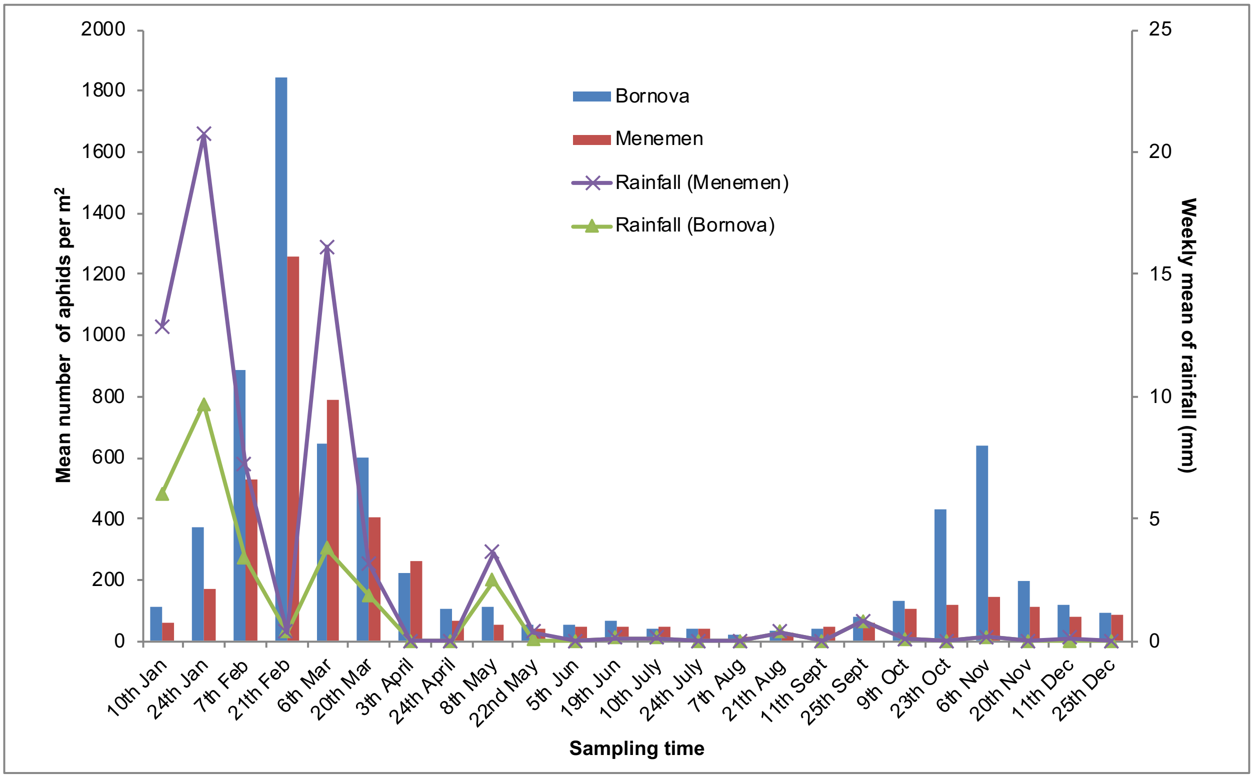

Figure 4. Weekly mean population density of *Eucarazzia elegans* and rainfall at Bornova and Menemen in 2016.

Impact of normal and double *Eucarazzia elegans* population densities on *Salvia officinalis*

There was significant difference between the effect of normal and double density aphid infestations on fresh and dry weight, and essential oil concentrations of *S. officinalis* compared to the control (Table 1). The fresh and dry weight loss caused by aphid populations was about 30 and 60% for normal and double densities, respectively, compared to the control. However, there was no significant difference in essential oil production between normal and double density aphid infestations, they were both about 20% less than the control.

Table 1. The impact of *Eucarazzia elegans* infestation on the fresh and dry weight, and essential oil plant production

Treatments	Fresh weight±SEM* (g)	Dry weight±SEM (g)	Essential oil concentration±SEM (%)
Double Population	0.79±0.04 a**	0.56±0.02 a	1.25±0.90 a
Normal Population	1.53±0.08 b	1.07±0.06 b	1.30±0.41 a
Zero Population (Positive Control)	2.17±0.10 c	1.52±0.07 c	1.59±0.97 b

* SEM: Standard error of the mean;
** Means in a column followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test).

The aphids tend to infest old leaves and cause serious defoliated. The resultant leaf fall, as a plant defense mechanism, contributes to the magnitude of the plant weight losses (Matsuki, 2004; Ballhorn et al., 2008; Gong & Zhang, 2014). In the laboratory experiment, infestation of about 50-80 aphids/leaf was enough to cause leaf fall within 5-7 days. Moreover, over two months a normal population density increased to a double density and caused leaf fall over the next 1-2 months. This clearly demonstrates that *E. elegans* has the potential to be a serious pest of common sage if there are no factors limiting population growth, such as environmental conditions, availability of resources and impact of natural enemies, to keep the population below an economic threshold.

The quality of the sage essential oil analyzed by GC-FID showed that normal and double aphid infestation levels influenced the concentrations the oil's components (Table 2). Some essential oil components, such as camphor and camphene, were greatly reduced, while other components, such as borneol, thymol, β-caryophyllen and limonene, were only slightly decreased. In contrast, α,β-thujone, β-pinene and bornyl acetate were increased, in case some components such as linalool and 1,8-cineol were either stable or showed no consistent response. The differences in percentage of essential oil components may reflect resistance and tolerance traits in plant defense mechanisms (Gong & Zhang, 2014), or impact of degraded development resulting from plant cell disruption (Steinbauer et al., 2014). Changes in chemical components, such as terpenoids; phenolic compounds; nitrogen compounds; tannins, lignin and cellulose; plant hormones and lectin; protease inhibitors; and volatile compounds, are made to defend plants against herbivores, and can be used as indicators of chemical defense capacity (Fürstenberg-Hägg et al., 2013; Schiestl et al., 2014).

Table 2. The impact of *Eucarazzia elegans* infestation on the essential oil components

Essential Oil Components	Zero Population (Positive Control) (%)	Normal Population (%)	Double Population (%)
α,β-Thujone	51.47	58.03	61.55
Camphor	16.95	12.04	11.70
Camphene	9.24	6.75	6.36
1,8-Cineole	5.62	8.32	4.34
Borneol	2.58	1.86	1.34
Limonene	2.14	1.49	1.50
β-Pinene	1.45	1.82	1.50
Thymol	0.94	0.75	0.68
β-Caryophyllene	0.49	0.35	0.30
Linalool	0.06	0.11	0.06
Bornyl acetate	0.08	0.18	1.44

The effect of the series insecticide applications against *E. elegans* is shown in Table 3. The aphid population increased significantly when period of exposure (i.e. the period without insecticide application) exceeded 4 weeks. A single insecticide application had no significant effect on plant weight. Also, 3-5 insecticide applications showed no significant differences for all parameters. Six insecticide applications caused a reduction in plant dry weight. However, the plant height was almost the same with three to five insecticide applications. The greatest plant height occurred with complete exposure (no insecticide application) due to the positive response to the substantial leaf fall caused by aphid infestation.

The benefit-per-unit cost of insecticide varied with exposure period, which influenced the yield and determined the number of sprays. The highest yield was obtained when plants were sprayed every two weeks. Allowing aphids to feed on common sage beyond 4 weeks resulted yield loss from 25 to over 64% (Table 3). The highest benefit-to-cost ratio was obtained maximum with a 4-week aphid exposure. Then, the highest gross profit was evident when common sage was kept free of aphids and decreased with an increase in aphid exposure period in both seasons.

Table 3. Effects of insecticide application on development of *Eucarazzia elegans*

Aphid exposure period (weeks)	Number of insecticide sprays	Fresh weight±SEM* (g)		Dry weight±SEM (g)		Plant height±SEM (cm)		Aphid infestation±SEM (%)	
0	6	2.78±0.15	a**	1.60±0.09	a	19.02±0.81	bc	0.79±0.27	a
2	5	2.16±0.15	b	1.24±0.09	b	19.35±0.80	b	4.89±0.37	b
4	4	2.07±0.12	b	1.19±0.07	b	18.21±0.84	bc	32.94±0.66	b
6	3	1.97±0.11	bc	1.13±0.06	bc	17.50±0.73	bc	46.56±0.62	bc
8	2	1.65±0.13	c	0.95±0.07	c	16.83±0.40	c	66.14±1.42	c
10	1	1.13±0.07	d	0.65±0.04	d	19.10±0.71	bc	84.39±0.71	d
Complete	0	1.00±0.05	d	0.57±0.03	d	21.91±0.71	a	86.90±0.46	d

* SEM: Standard error of the mean;
** Means in a column followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test).

The relationship between aphids' infestation and dry weight production is described by a regression equation: $y = 1.488 - 0.010x$ (Figure 5). This formula revealed that the aphid infestation inflicted significant reductions on sage yield as the number of aphids per plant increased. The reduction in yield and yield components are attributed to the feeding activities of aphids. This activity increased with duration of aphid exposure, with complete aphid exposure still permitting 30% plant development, though in some cases it caused plant mortality. The conversion of aphid population per plant to percentage aphid infestation follow the formula $y = 19.8x$ (Figure 6), which every percentage of aphid infestation being about 19-20 aphid per plant. These equations are useful for pest control policy given that this aphid species continues to be accidentally spread and introduced to other countries (Hales et al., 2009).

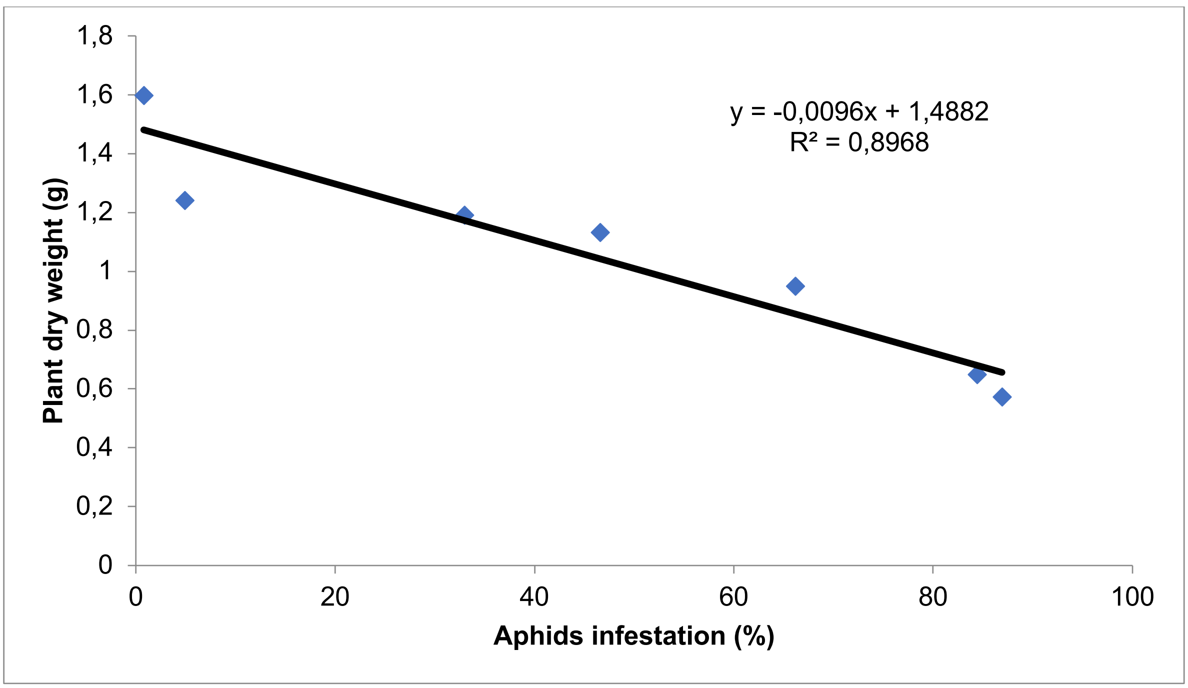

Figure 5. Regression line shows the relationship between plant dry weight and percentage of *Eucarazzia elegans* infestation.

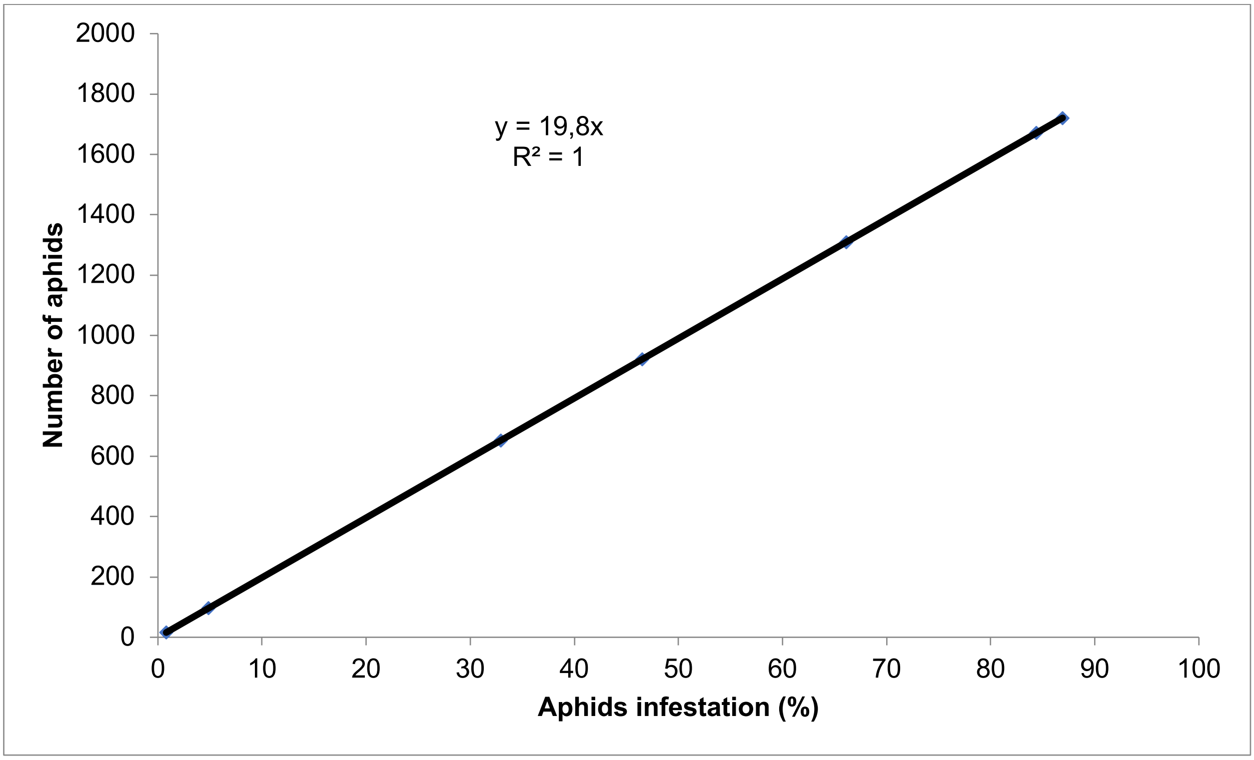

Figure 6. Regression line shows the relationship between number of aphids/plant and percentage of *Eucarazzia elegans* infestation.

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