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Article

Field application of six commercial essential oils against Date Palm mite, *Phyllotetranychus aegypticus* (Acari: Tenuipalpidae) in Egypt

Sally Farouk Allam, Marwa Abd El-Aziz Mahmoud, Mourad Fahmy Hassan, Ayman Hefny Mabrouk*

Zoology and Agricultural Nematology Department, Faculty of Agriculture, Cairo University, Gamaa St., 12613 Giza, Giza, Egypt; E-mails: sallyfmallam@gmail.com, marwa_abdelaziz_m@yahoo.com, mfhassan51@yahoo.com

* Deceased

ABSTRACT
Six commercial essential oils including thyme (*Thymus vulgaris*), clove (*Syzyium oromaticum*), camphor (*Cinnamomum camphora*), jasmine (*Jasminum officinale*), banana (*Musa* spp.) and spearmint (*Mentho spicata*) were evaluated in field application against the date palm mite, *Phyllotetranychus aegypticus* Sayed in eight successive months, April to November 2018. All the essential oils had toxic effects on *P. aegypticus*. *Thymus vulgaris* (Lamiaceae) and *S. aromaticum* (Myrtaceae) showed relatively higher levels of toxicity when compared to *J. officinale* (Oleaceae) and *Musa* spp. (Musaceae) as evident through their lower effective values. However, toxicity levels exhibited by the other two oil samples, *M. spicata* (Lamiaceae) and *C. camphora* (Lauraceae) were also found enticing, as the toxicity levels of these oils were found comparatively lower. During spring period, no significant difference was found among spearmint, jasmine, banana, camphor and clove, but there was a significant difference between thyme and all other oils. During summer, there was no significant difference between “thyme and clove oils” and “camphor and spearmint”due to high temperature’s effect on the components of oils. During autumn, thyme and clove oils possessed the highest toxicity against *P. aegypticus*.

KEY WORDS: Banana; camphor; clove; field application; jasmine; spearmint; thyme.

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INTRODUCTION

Phyllotetranychus aegypticus Sayed was originally described from *Phoenix dactylifera* L. (Arecaceae) in Egypt (Sayed 1938), and later by (Wafa *et al*. 1969; Zaher and Yousef 1969; Zaher *et al*. 1969; Radwan and Attia 2013; Nawar *et al*. 2014; Mahmoud 2015). In the Middle East, it was found on Wadi Araba, Jordan (Smith-Meyer and Gerson 1981), and Iran (Khanjani *et al.* 2013).

Essential oils are defined according to the International Standard Organization (ISO) as products originated from plant parts. The compounds found in each essential oil vary according to the species, structure, and part from which the oil is extracted (Santurio *et al*. 2007). Essential oils are a combination of volatile, lipophilic, commonly odoriferous and liquid compounds. Chemically, they consist of terpenes, mainly monoterpenes and sesquiterpenes, and phenylpropanoids. These

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chemical components play a role in the responses of plants and perform different functions such as attracting pollinators, protecting against predators, water loss, UV rays, and inhibiting germination (Simões and Spitzer 2002; Oliveira *et al*. 2006; Hüsnü *et al*. 2007; Scherer *et al*. 2009; Silva *et al*. 2009, 2013).

Further advantages in using essential oils in integrated pest management (IPM) applications are their rapid degradation due to their high volatility, which reduces environmental contamination and side effects on non-target organisms (Isman 2006). In addition, compounds present in the essential oils can act at different sites of action, consequently reducing the chance of ending with resistant arthropod biotypes (Bomford and Isman 1996).

In an *in vitro* experiment (unpublished data), it was concluded that using commercial essential oils in high concentrations (150 ml/L) under laboratory conditions was toxic and would be effective as well as promising for limiting the palm pest *P. aegyptiacus* field infestations. Therefore, current study was aimed to evaluate this theory using commercial essential oils of thyme, clove, banana, spearmint, jasmine and camphor, along with temperature on the *P. aegypticus* population, during the whole experimental period in a field application on infested date palm trees located in Giza Governorate, Egypt, during spring, summer and autumn 2018.

MATERIALS AND METHODS

Tested materials

Six commercial essential oils were used: thyme, clove, camphor, jasmine, banana and spearmint. Essential oils were packed in 30 ml tubes (Harraz© for Food Industry & Natural Products, https://www.harraznatural.com). Table 1 shows the plant parts used, extraction method, and major compound percentage.

Table 1. Commercial essential oils used in the field application experiment against the Date Palm mite, *Phyllotetranychus aegypticus*.

Essential oil	Plant	Family	Part used	Extraction method	Major compound*
Thyme	*Thymus vulgaris* L.	Lamiaceae	Leaf	Steam distillation	Thymol (34.6%)
Clove	*Syzyium oromaticum* (L.) Merr. & Perry	Myrtaceae	Band	Steam distillation	Eugenol (59.3%)
Camphor	*Cinnamomum camphora* (L.) Presl.	Lauraceae	Leaf	Steam distillation	Eugenol (76.1%)
Jasmine	*Jasminum officinale* L.	Oleaceae	Flower	Steam distillation	N/A
Banana	*Musa paradisiaca* L.	Musaceae	Banana peel	Steam distillation	N/A
Spearmint	*Mentha veridis* L.	Lamiaceae	Leaf	Steam distillation	Carvone (54.7%)

* Major compound and extraction methods as provided from Harraz company.

Experimental design

The experiment field was located inside the faculty of agriculture experimental station farm, Cairo University (30° 01' 13.7" N, 31° 11' 50.3" E Giza, Egypt). Seven palm complexes were selected; each group consisted of four palms 1–1.5 m in height as replicates for each commercial essential oil treatment (the seventh was treated with water as control). Tested essential oils were sprayed onto the leaves with a KDGPUM® hand sprayer (3L hand pressure compressed air sprayer with pump pressure sprayer) from April to November 2018.

Experimental procedure

A preliminary experiment was run *in vitro* to detect the most effective concentration of oils to apply. 50, 100, and 150 ml/L of each oil were tested. Results of this trial recommended 150 ml/L concentration to be applied for field application (unpublished data). Spray application was done after investigating the mite populations (individual/ 50 leaflet) in April 2018. Concentrations were prepared in the laboratory, 150 ml of oil per one-liter distilled water; triton X-100 (1 ml/L) was used for reducing surface tension of the mixture for ease of use. Populations were counted weekly after and before spray application for each treated palm complex until the end of the experiment in November 2018.

Also, temperature (high, low, and optimum) was recorded weekly during the experiment to determine if temperature had an impact on oil efficiency.

Fifty leaflets of each group (complex) were examined after application in the laboratory using a stereo-microscope, number of active stages was counted and calculated, and data were tabulated for statistical analysis to determine the response of commercial oils treatment on mite populations.

Data analysis

Original data of mites and the response to essential oils were not transformed; data were grouped into three main categories (spring, summer, and autumn). Comparing means was done by IBM SPSS© Statistics for Windows version 20.00. Data were analyzed by one-way analysis of variance *F*-test (ANOVA), means ± std. error (SE) for groups in homogeneous subsets analyzed by Tukey at $P \leq 0.05$ using harmonic mean sample size (n) for each group (spring = 20, summer and autumn = 26). Pearson correlation and regression (R) were analyzed at probability level $P \leq 0.05$ to measure the impact of more than one factor on the targeted pest. Linear regression graph was designed by IBM SPSS statistics 20 at confidence level 95%.

RESULTS

In order to achieve the main idea of research, investigating the impact of oils and temperature on the mite population, during the whole experimental period, we divided application weeks into three groups according to temperature, spring, summer, and autumn. This was done to show which oil(s) has/have the toxic effect on targeted pest, also, to recommend when to use oil application in IPM protocols.

Effect of commercial essential oils treatments in P. aegyptiacus *abundance*

The null hypothesis was designed as H_0 = all the variables are not affected by using the tested commercial essential oils at $P = 0.05$, and the alternative hypothesis H_1 was designed as commercial essential oils have significant differentiations and *P. aegyptiacus* affected by the treatments.

Results did not statistically confirm H_0, and the alternative theory H_1 was accepted. Data were grouped in three categories; according to time of application; spring, summer, and autumn as presented in Table 2. Spring had a highly significant effect of using commercial oils in the mite populations (F = 27.562, $P = 0.000$) compared with control group (only water-spraying treatment). Thyme commercial oil recorded the lowest mean number of mites (25.34 ± 4.81), the highest number was recorded from jasmine (41.31 ± 5.70) and banana (41.03 ± 7.17) oils, respectively.

Mite populations during summer application were significantly affected (F = 206.215, $P = 0.000$). Reduction of mite populations was obvious in thyme (43.48 ± 8.98), and clove (49.96 ± 5.53) while, jasmine (83.24 ± 18.39) had the lowest influence on the mites.

Autumn weekly applications recorded highly significant effects in reducing mite numbers/50 leaflets of each date complex (F = 33.547, $P = 0.000$). Commercial oil of clove resulted in the lowest number of *P. agyptiacus*/leaflets (16.42 ± 13.46), while the highest mean number of *P. agyptiacus*/leaflets was reported by the jasmine commercial oil (70.72 ± 31.23).

Means for the effect of commercial oils treatment in homogeneous subsets were statistically different at $P = 0.05$, although using harmonic sample size within each group (spring, summer, and autumn) the variance was F = 13.394 when $P = 0.000$ for thyme, for spearmint (F = 10.207, $P = 0.000$), for jasmine (F = 21.047, $P = 0.000$), for banana (F= 39.645, P= 0.000), for camphor (F = 12.283, $P = 0.000$), and for clove (F = 82.976, $P = 0.000$; Table 2).

Table 2. Mean number of mite *Phyllotetranychus aegyptiacus* (mean ± SD) on palm tree leaflet after application of commercial essential oils spray during 2018 in Giza.

Application	Spring 2018^{Y}	Summer 2018^{Z}	Autumn 2018^{Z}	$F_{df\,(2, 71)}$	$P \leq 0.05$
	$N^{X} = 20$	$N^{X} = 26$	$N^{X} = 26$		
Thyme	25.34 ± 4.81 Bc	43.48 ± 8.98 Ae	31.29 ± 17.80 Bcd	13.394	0.000**
Spearmint	38.59 ± 4.43 Bb	54.30 ± 8.39 Ad	35.51 ± 24.76 Bc	10.207	0.000**
Jasmine	41.31 ± 5.70 Bb	83.24 ± 18.39 Ab	70.72 ± 31.23 Ab	21.047	0.000**
Banana	41.03 ± 7.17 Bb	67.43 ± 6.78 Ac	37.41 ± 19.70 Bc	39.645	0.000**
Camphor	37.38 ± 6.24 Bb	53.22 ± 7.89 Ade	36.42 ± 19.48 Bc	12.283	0.000**
Clove	37.62 ± 6.93 Bb	49.96 ± 5.53 Ae	16.42 ± 13.46 Cd	82.976	0.000**
Control	63.70 ± 21.34 Ca	151.50 ± 29.74 Aa	119.85 ± 61.34 Ba		
F	27.562**	206.215**	33.547**		

^{Y} F df = (6, 139), $P \leq 0.05$
^{Z} F df = (6, 181), $P \leq 0.05$
^{X} N= number of intervals per season
Within rows, means followed by the same letter are not significantly different (Tukey, $P \leq 0.05$).
Within columns, means followed by the same letter are not significantly different (Tukey, $P \leq 0.05$).
ns = not significant, *significant, **highly significant

Effect of temperature in using commercial essential oil treatments against P. aegyptiacus

Here, the null hypothesis was H_0 = all the variables are not affected by temperature of the experimental period (spring, summer, autumn) at $P = 0.05$, and the alternative hypothesis H_1 as there were significant effects. Results agreed with H_1, meaning statistical significant effects were noted between temperature and the mite population (untreated and treated with commercial essential oils), as presented in the linear model of the regression relationship (Fig. 1), and also as recorded in the field application (Fig. 2). To test the hypothesis and explain this relationship, the linear model had three contents of variables (Fig. 3), which are statistically differentiated; season (treated *P. aegypticus* with the six commercial essential oils) (F = 36.94, df = (6, 18), $R^2 = 0.828$, $P = 0.005$), temperature (F = 22.72, df = (6, 18), $R^2 = 0.841$, $P = 0.202$), and control (untreated *P. aegypticus*) (F = 101.85, df = (6, 18), $R^2 = 0.980$, $P = 0.000$) (Table 3).

Table 3. Model of the relationship across temperature, untreated mite population (control), and treatments of commercial oils during season of 2018.

Model	Model Fit statistics				
	R-squared	MAE	F	DF	Sig.
Season Model	0.828	0.275	36.936	18	0.005*
Temp Model	0.841	0.916	22.716	18	0.202^{ns}
Control Model	0.980	6.565	101.846	18	0.000*

According to this relation, independent-samples median test (Kolmogorov-Smirnov Test) was performed, which resulted in the median distribution and statistics significance of each commercial oil across the three categories of season (spring, summer, and autumn); thyme (*P* = 0.001), spearmint (*P* = 0.001), jasmine (*P* = 0.002), banana (*P* = 0.000), camphor (*P* = 0.000), and clove (*P* = 0.000) (Table 4, Fig. 5).

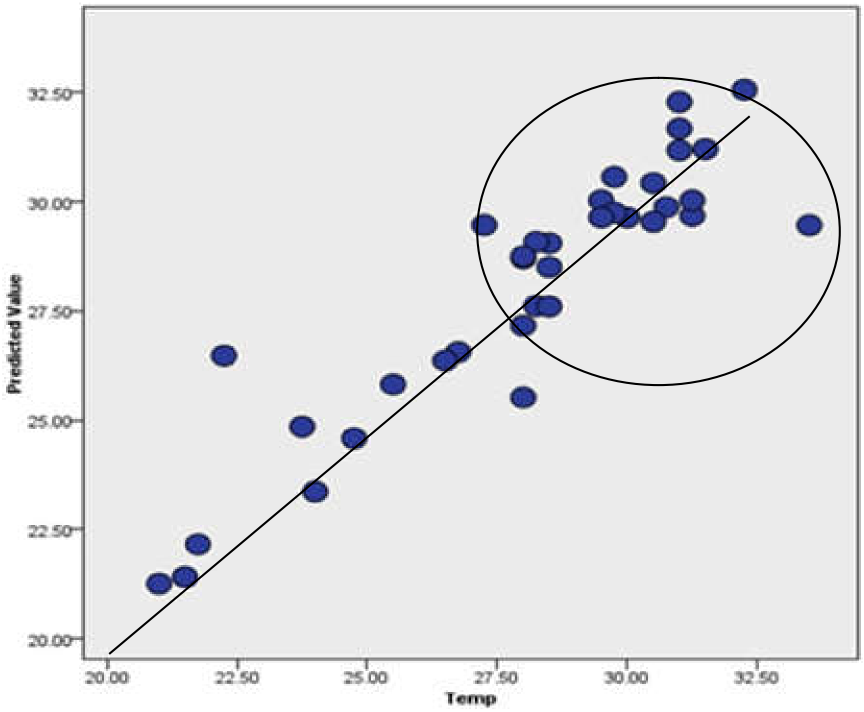

Figure 1. The relationship (regression modeling at *P* = 0.05) between X (temperature recorded in spring, summer, and autumn), and Y (predicted value which representing mite populations before and after oil treatments). The line is the linear model, significant part till temperature ≤ 27 (which represents spring and autumn treatments). While the circle in the end part, temperature > 27.50 (representing effect of oil in summer) is not significant, $P = 0.202^{ns}$.

Another test was performed to discuss the relation between oil treatment and the temperature; the test of ordered alternatives (Jonckheere-Terpstra Test) at significance level of 95%. The test results showed high significant impact of temperature on the efficiency of oil usage including thyme (*P* = 0.002), spearmint (*P* = 0.000), banana (*P* = 0.000), camphor (*P* = 0.000), clove (*P* = 0.000), a jasmine oil which was the least significant (*P* = 0.01; Table 4, Fig. 5).

DISCUSSION

Despite the high concentration used in the current experiment (150 ml/L), there was a significant impact of lethal toxicity of used commercial essential oils. Thyme and clove oils shown a significantly higher level of toxicity when compared with jasmine and banana. However, toxicity levels of spearmint and camphor oils were only slightly significant. These differences could be explained as some essential oils have low persistence under field conditions because of their volatility (Cantrell *et al.* 2012).

Figure 2. The relationship between numbers of *Phyllotetranychus aegyptiacus* active stages/50 leaflets of palm trees (treated and control), and the optimum temperature recorded (°C) during spring, summer, and autumn 2018 in Giza.

Thyme oil has been reported to have an insecticidal activity (Ranger *et al.* 2013). *Thymus serpyllum* is rich in phenols, thymol, and carvacrol (Isman 2000). The current study showed that thyme treatment has the highest toxic effect in reducing the palm mite population among three seasons (spring, summer, and autumn) compared with non-treated palms (*P* = 0.000). Thyme components might be the reason of mite's mortality.As mentioned in Regnault-Roger *et al*. (1993), the chemical analysis and main components of *T. serpyllum* were thymol 30.4%, carvacrol 28.9%, *p*-cymene 10%, and citra 4.2%. Also, for *T. vulgaris* main components were thymol 47.5 and 26.5%, *p*-cymene 17.3 and 18.4%, β-caryophyllene 6.1, and 3.5%. Toxicity of thymol as the main element of thyme oil was shown as larvicide of *Anopheles dirus* and *Aedes aegypti* mosquito (Pitasawat *et al.* 2007). Mortality was 99% of *Rhipicephalus microplus* larvae after exposure to 0.1% thyme solution for 24 hours (Koc *et al.* 2013).

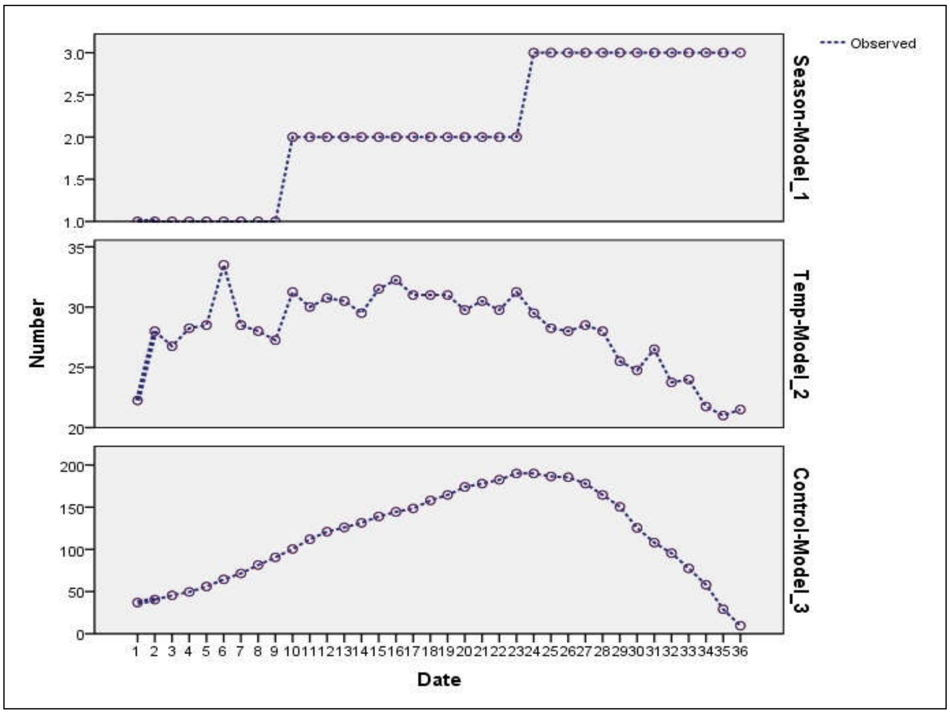

Figure 3. Regression linear modeling of the relationship across temperature, untreated mite population (control), and treatments of commercial oils during season of 2018.

The efficacy of clove essential oil as a control method for *Varroa destructor* mites was investigated showing that high concentrations of 0.75 gm caused a 96% mortality of *V. destructor* (Gashout and Guzmán-Novoa 2009; Girisgin *et al*. 2014). De Melo *et al*. (2019) results showed high mortality of *Tenuipalpus heveae* (Acari: Teuinpalpidae) treated with clove, cedar, and neem essential oils. These results showed that clove oil is promising in the IPM applications of this mite. In the current study, the clove essential oil showed a potential impact on the investigated mite's populations, the probability level was highly significant at confidence level of 95%. In autumn, the highest palm mite mortality was caused by clove oil. Mahakittikun *et al*. (2014) studied the constituents of clove essential oil. Phenylpropanoids, which are the compounds synthesized by plants from phenylalanine amino acid, of which eugenol was the main component of this oil. The clove oil is antimicrobial, antioxidant, antifungal, antiviral, anti-inflammatory, cytotoxic, and acaricidal. Kim *et al.* (2003) showed that methyleugenol, isoeugenol, eugenol, acetylyeugenol, and benzyl benzoate are the most to least toxic components, respectively, for the House Dust Mites (HDM) *Dermatophagoides pteronyssinus* and *D. farinae.*

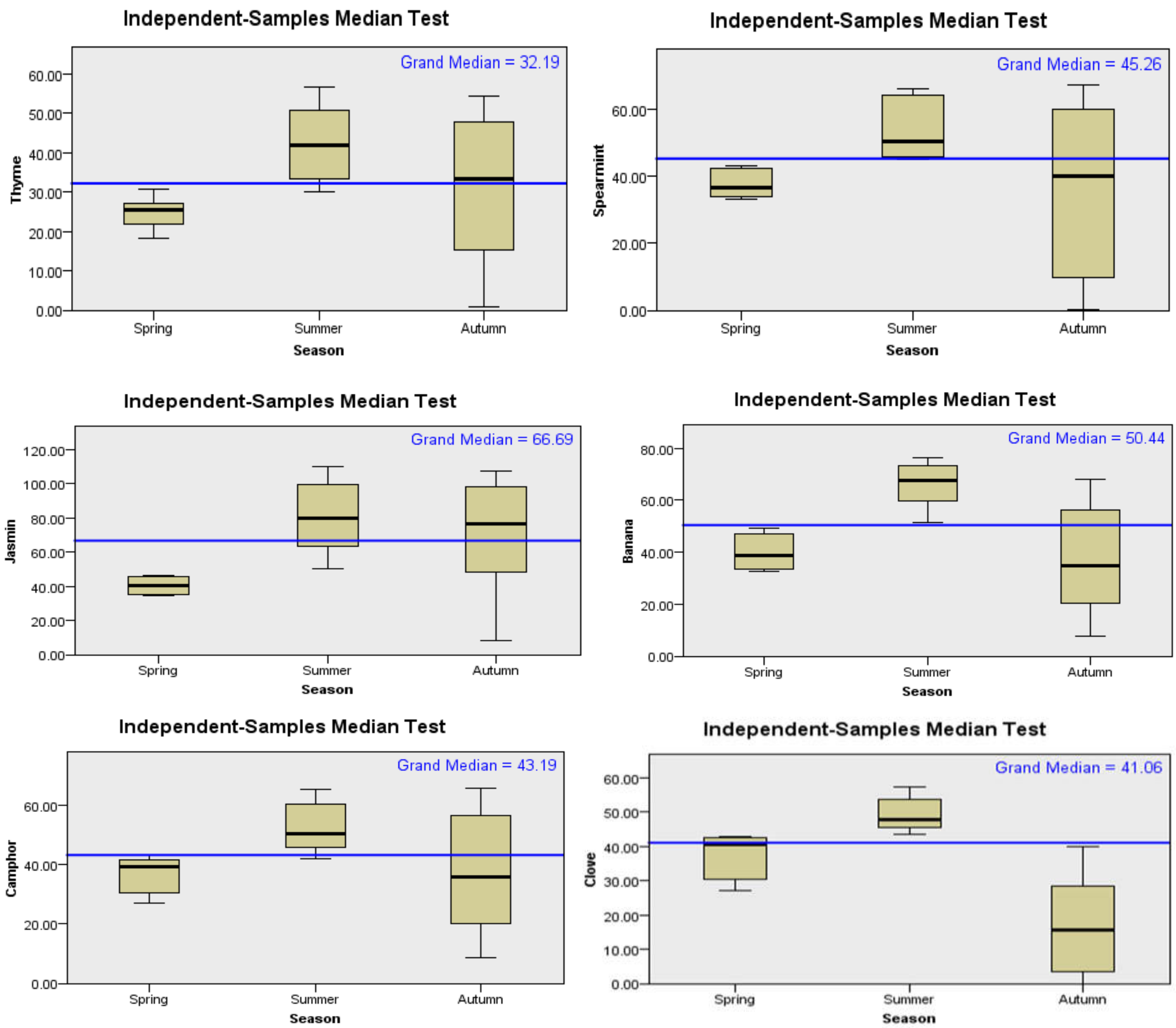

Figure 4. Independent-samples median (Kolmogorov-Smirnov Test) resulted the distribution and the median of tested commercial essential oils of thyme, spearmint, jasmine, banana, camphor and clove across the three categories of season (spring, summer, and autumn), at 0.05 significance level.

Table 4. Independent-samples of distribution and significance effects of median test (Kolmogorov-Smirnov Test) and the test of ordered alternatives (Jonckheere-Terpstra Test) on the relation between oils-season and oils-temperature according to H_1 at significance level of 95%.

Commercial essential oil	N	Independent-samples median test (Kolmogorov-Smirnov Test)[Z]				Test of ordered alternatives (Jonckheere-Terpstra Test)[Y]	
		Median	test statistics	df	*P*	test statistics	*P*
Thyme	36	32.19	13.68	2	0.001	191.00	0.002
Spearmint	36	45.26	16.22	2	0.000	164.50	0.000
Jasmine	36	66.7	12.26	2	0.002	211.50	0.01
Banana	36	50.44	24.92	2	0.000	118.50	0.000
Camphor	36	43.19	19.98	2	0.000	152.50	0.000
Clove	36	41.07	27.11	2	0.000	83.00	0.000

[Z] Relationship of commercial essential oils across three season categories (spring, summer, autumn), sig. 95%.
[Y] Relationship of commercial oils and the recorded temperature in the field during the experimental period (N= 36), sig. 95%.

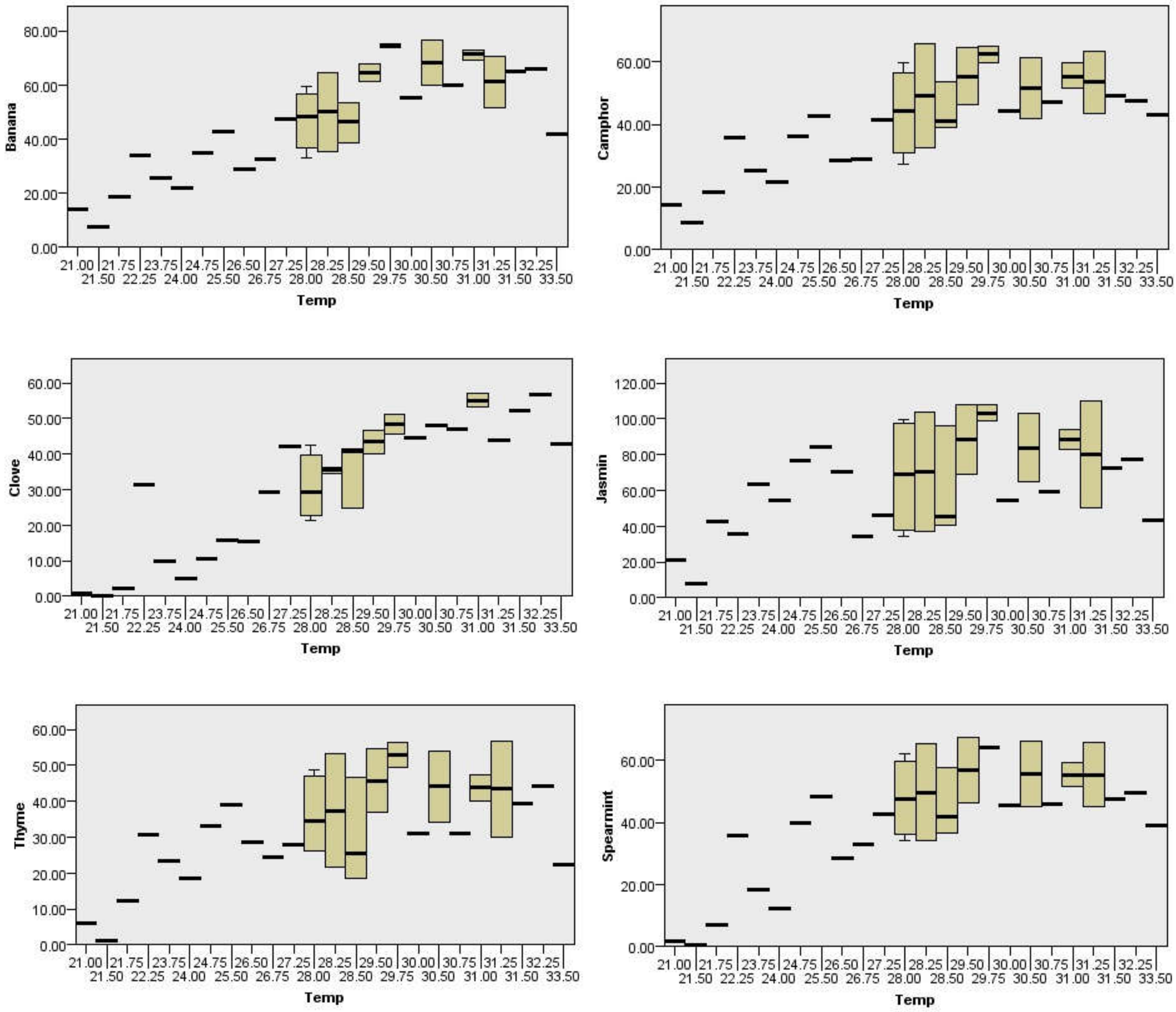

Figure 5. Independent-samples test of ordered alternatives (Jonckheere-Terpstra Test) on the relation between tested commercial essential oils of thyme, spearmint, jasmine, banana, camphor and clove across the temperature recorded in the field application 2018, at 0.05 significance level.

Temperature and time of exposure have an impact on yield quality (of oil harvested), essential oil percentage, and the toxicity on the targeted pest (Ebrahimi *et al.* 2011; Veeraphant *et al*. 2011). In the current study, the temperature had a highly significant effect shown in the linear model of the relationship between mite population, temperature, and the reduction effect of essential oils (Fig. 3). The high temperatures recorded in the summer season resulted in low mortality and mite populations were not affected. While the low temperatures in both autumn and spring presented a significant effect of essential oil usage.

Some investigations have explained why essential oils have low persistence under field conditions; it is due to their volatility. Essential oils are secretions in glands or special parts in plants. Due to the natural origin of these secondary metabolites, they are easily degraded by natural degradation mechanisms such external factors (temperature, daylight, relative humidity, wind speed, and plant domatia) and/or internal pest factors (metabolism, pest stage, pest's cuticle structure, and pest's digestive enzymes content) (Regnault-Roger *et al*. 1993; Isman 2006; Pavela 2008; Hernández-Carlos and Gamboa-Angulo 2019; Ebadollahi *et al*. 2020).

Further studies are needed to study the efficiency of each oil and its content, and also, to explain what is the commercial oils mode of action, and how it affects the targeted mites. Also, to discuss which toxicity criteria (repellent, antifeedants, sterilizers, anti-molting, anti-depositing, toxic, etc.) might they belong to?

CONCLUSION

The study suggested that using commercial oils, has a significant effect in spring and autumn, in the low mite population of palm trees. Summer application is useless, due to oil degradation, temperature, relative humidity, mite digestive enzymes, high pest population, etc.

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کاربرد صحرایی شش اسانس روغنی تجاری روی کنهٔ درخت خرما، *Phyllotetranychus aegypticus* (Acari: Tenuipalpidae) در مصر

سالی فاروق علام، مروة عبدالعزیز محمود، مراد فهمی حسن و أیمن حفنی مبروک*

گروه جانورشناسی و نماتودشناسی کشاورزی، دانشکدهٔ کشاورزی، دانشگاه قاهره، خیابان جمعه، غزه ۱۲۶۱۳، غزه، مصر؛ رایانامه‌ها: sallyfmallam@gmail.com، marwa_abdelaziz_m@yahoo.com، mfhassan51@yahoo.com

* مرحوم شدند

چکیده

شش اسانس روغنی تجاری شامل آویشن (*Thymus vulgaris*)، میخک (*Syzyium oromaticum*)، کافور (*Cinnamomum camphora*)، یاس (*Jasminum officinale*)، موز (*Musa* spp.) و نعناع (*Mentho spicata*) در کاربرد صحرایی روی کنهٔ درخت خرما، *Phyllotetranychus aegypticus* Sayed طی هشت ماه پیاپی از آوریل تا نوامبر ۲۰۱۸ ارزیابی شدند. همهٔ اسانس‌های روغنی روی *P. aegypticus* اثر سمی داشتند. گیاهان *Thymus vulgaris* (Lamiaceae) و *S. aromaticum* (Myrtaceae) در مقایسه با *J. officinale* (Oleaceae) و *Musa* spp. (Musaceae) سمیت نسبی بیشتری داشتند همانطور که از مقادیر موثر پایین نشان داده شده توسط آنها مشخص است. اما سطح سمیت نشان داده شده دو اسانس روغنی دیگر، *M. spicata* (Lamiaceae) و *C. camphora* (Lauraceae) نیز دلگرم‌کننده به نظر می رسید، هرچند میزان سمیت این روغن‌ها به نسبت کمتر بود. در بهار، اختلاف معنی‌داری بین نعناع، یاس، موز، کافور و میخک پیدا نشد اما بین آویشن و دیگر اسانس‌های روغنی اختلاف معنی‌دار وجود داشت. در طول تابستان، بین اسانس‌های روغنی «نعناع و میخک» و «کافور و آویشن» به دلیل اثر دمای زیاد بر ترکیبات اسانس‌های روغنی اختلاف معنی‌داری وجود نداشت. در پاییز، اسانس‌های روغنی آویشن و میخک بیشترین سمیت را روی *P. aegypticus* داشتند.

واژگان کلیدی: موز؛ کافور؛ میخک؛ کاربرد صحرایی؛ یاس؛ نعناع؛ آویشن.

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