Academic Journal of Life Sciences
ISSN(e): 2415-2137, ISSN(p): 2415-5217
Vol. 5, Issue. 8, pp: 48-54, 2019
URL: https://arpgweb.com/journal/journal/18
DOI: https://doi.org/10.32861/ajls.58.48.54

Academic Research Publishing Group

Original Research **Open Access**

Fumigant Toxicity of Rue Essential Oil, *Ruta Graveolens* L. on *Tetranychus Urticae* Koch (Acari: Tetranychidae)

Fatma Sh. Kalmosh*
Plant Protection Research Institute, Agricultural Research Center, Dokki, 44516 Giza, Egypt

Samah N. El Shafiey
Plant Protection Research Institute, Agricultural Research Center, Dokki, 44516 Giza, Egypt

Hany M. G. El-Kawas
Plant Protection Research Institute, Agricultural Research Center, Dokki, 44516 Giza, Egypt

Abstract

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pests affecting agricultural and ornamental crops worldwide. In fact many of natural plant (bioactive compounds) have fewer side effects compared with chemical pesticides. So, this study aimed to evaluate the effect of rue leaves essential oil, *Ruta graveolens* L. as fumigant toxicity (Sapindales: Rutaceae) against eggs and adult females of *T. urticae*. Gas chromatography–mass spectrometry (GC-MS) data cleared that the basic constituents were 2-undecanone (60.54%) and 2-nonaone (17.71%) which belonged to methyl ketones that represented by 81.65% of the total oil. The ovicidal activity of rue essential oil mentioned that according to LC50 values, the one-day-old eggs were more susceptible than three -days-old eggs recording 0.008 and 0.011 µl/ml air after 7 days post fumigant for 24 h, respectively. Furthermore, the adult female mortality recorded 0.018 and 0.0724 µl/ml air for LC50 and LC90, respectively. Additionally, results demonstrated that significant reduction in the mean number of deposited eggs/female/day recorded 3.182 eggs compared to 6.561 eggs for control. Finally, a significant reduction in acid phosphatase for eggs and Aacetylcholine esterase (AChE) for adults of *T. urticae* were recorded.

Keywords: *Tetranychus urticae*; Fumigant toxicity; Essential oil; *Ruta graveolens*; Biochemical studies; Chemical profile.

1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch is one of the most important plant pests which can cause significant loss of yield many plant species in greenhouses, orchards and field crops [1-3]. This mite infests many greenhouses plantations causing great problems. Because greenhouses are usually cultivated with valuable crops, this loss is amplified. In addition, high-density plantation applied in greenhouses provides shelters for pests which may prevent the pesticides to reach all the pest individuals [4].

The widespread application of acaricides for controlling *T. urticae* has a serious impact on the natural balance of the ecosystem. These problems prompted researchers to look for a new application to control this pest. Therefore, one of the new suitable approaches for the greenhouse may be the use of natural fumigants because of their safety and their ability to reach every point in the high-density plantation [5]. The fumigant toxicity of several volatile oils has been investigated against various pests [4, 6-11].

The efficacy of essential oils has been demonstrated to act as afumigant for the pests of stored product [12-15] and spider mite Amizadeh, *et al.* [16] and Ebadollahi, *et al.* [1]. As well as parasitic mites of honey bee [12, 17]. So, the essential oils may be effective and safe alternatives to traditional industrial fumigation. The genus *Ruta* features Shrubby plants that are native to the Mediterranean region and are represented by 40 species in the world [18], which were used as traditional medicine in many countries to treat a variety of diseases such as rheumatism, neuralgia, menstrual bleeding, fever, arthritis, hepatic diseases, antifertility, and gastrointestinal disorders [19]. Also, recent studies [20-22], revealed that rue species have analgesic, antipyretic, anti-inflammatory, antifungal, emmenagogue, insect repellent, molluscicidal, nematicidal, antimicrobial, anthelmintic, sedative, and antiplatelet properties. *R. graveolens* is a small aromatic shrub cultivate in Egypt and known for its medicinal aromatic properties, since ancient times [23]. Essential oil extracted from the aerial part is a CNS inhibitor and has become a narcotic at high doses Claus [24] and Miguel [25].

Thus, the main purpose of this investigation is to determine the chemical composition of essential oil of fresh leaves from rue, *R. graveolens* oil grows in Egypt and to evaluate its fumigant toxicity against eggs and adults of *T. urticae*. Furthermore, biochemical induced by rue oil was studied on eggs and adults of *T. urticae*.

2. Materials and Methods

2.1. Plant Material and Essential Oil Extraction

A sample of *R. graveolens* leaves were collected from Abo Hamad, Sharkia Governorate, Egypt in May 2016 then subjected to air-drying for 5 days at room temperature, and the leaves were crushed. Essential oil was extracted

*Corresponding Author

by hydrodistillation for 3 h using the Clevenger apparatus. Anhydrous sodium sulphate was used to remove water after extraction [26]. The essential oil was transferred to glass vials covered with aluminum foil and stored in the refrigerator at 4∘C until it was used for the current experiments.

2.2. Analysis and Identification of Essential Oil Components

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analysis the rue essential oil, HP 6890 Series A (Agilent) equipped with column (Thermo Scientific (TR-5MS), 5% Phenyl Polysil Phenylene Siloxane; 30 m x 0.25 mm i.d.; 0.25 µm film thickness). The column flow rate was 1.00 ml He/min. Temperature program: initial temperature 50˚C for 5 min., temperature rate 4˚C/min, and final temperature 250˚C. The injector temperature was 250˚C; injection volume 1µl.

Tentative identification of essential oil components was conducted by comparing their relative retention times (RT) and relative retention index (RRI) with a series of n-alkanes mass spectrum matching was aided by commercial libraries; Replib, wiley9, and mainlib.

2.3. Mite Culture

The mite individuals used throughout the study was obtained from the culture of *T. urticae* maintained for one year without any pesticide exposure in Acarology Dept. at Plant Protection Research Institute (PPRI), (Sharqia Branch), Egypt. Lima bean (*Phaseolus vulgaris* L.) seeds were planted in plastic jars (12 cm. diameter) at a rate of 6-7 seeds per jar, and incubated under muslin cage to prevent any infestation. Jars containing Lima bean seedlings (15 cm. long) were taken to the laboratory, then infested leaves of *T. urticae* were transferred to these plants and left to reproduce under laboratory conditions at 26 ± 1˚C and 65 ± 5% R.H.

2.4. Fumigant Assay

The toxicity test of modified fumigation has been moved abroad to determine the toxicity of *R. graveolens* essential oil against egg and adult stages of *T. urticae*. Plastic containers (5 liters capacity) were used as fumigation chambers tidily cover with plastic cling wrap. Each container containing two Petri dishes, the first dish representing the mulberry discs with mite individuals and the second dish including filter papers discs (2.5 cm in diameter) dropped by rue oil using micro applicator [4]. Various concentrations 20, 40, 60, 80 and 100µl of essential oil were prepared. The concentration per (ml air) calculated by lose of filter papers discs weight which dropped by tested essential oil. Each concentrate was repeated five times.

2.5. Acaricidal Activity

To evaluate the toxic activity of *R. graveolens* essential oil against adult females of *T. urticae*; twenty adult females were transferred on the lower surface of mulberry leaf discs (2.5 cm diameter) by using a fine brush. Discs were placed separately upside-down on moist cotton wool in small dishes. In order to, reduce the evaporation of the water in the test jars, the selected dishes were chosen slightly larger the discs, which were fixed on the bottom of the test jars for the fumigation [4]. Blank Petri dishes serves as controls. The jars were closed and kept under constant temperature of 26±5˚C for 24 h. The mortality percentages of *T. urticae* adult females were calculated. Also, the number of deposited eggs per treated females was counted during six days post treatment.

2.6. Ovicidal Effect

The ovicidal effect of *R. gravelones* oil against eggs (1and 3 days-old), was studied. Ten adult females of *T. urticae* were placed on mulberry leaf disc (2.5 cm) which was put on wet cotton wool in a Petri dish and incubated for 24 h to deposit eggs, and then females were removed from the leaf. Each disc carrying 50 eggs (one-day-old) were placed separately upside-down on moist cotton wool in small diches and subjected to series concentrations of the volatile oil vapors of *R. gravelones* for 24 h, as described above. Treated eggs were incubated at 26±1˚C and 65±5 % R.H. for six days till hatching and the hatching percentages were determined. The same procedure was carried out for the 3 days-old eggs. Mortality percentages were corrected by Abbott [27].

2.7. Biochemical Studies

Adult females and eggs (1 and 3 days-old) of *T. urticae* used for biochemical assays, which collected after 24 h post fumigant by LC_{50} of tested rue oil. Samples were collected in 1.5 ml Eppendorf tube surrounded with a jacket of crushed ice and homogenized in cold distilled water using an Eppendorf plastic pestle for 3 min. Homogenates were centrifuged at 4000 rpm for 10 min at 5˚C. Supernatants were subjected to the biochemical analysis.

The activity of acid phosphatase in egg stage were determined using the method of Powell and Smith [28], while the method described by Simpson, *et al.* [29] was used for determination of Acetylcholinesterase (AchE) activity in adults using acetylcholine bromide (AchBr) as substrate. Finally, Total soluble protein (TSP) was measured according to Gornall, *et al.* [30] in all samples.

2.8. Statistical Analysis

The percentages of untreated and treated eggs and adults of *T. urticae* were recorded from which the average mortality percentages that calculated per each concentration and corrected using Abbott [27]. The LC_{50} and LC_{90} of tested oil were statistically analyzed according Finny [31].

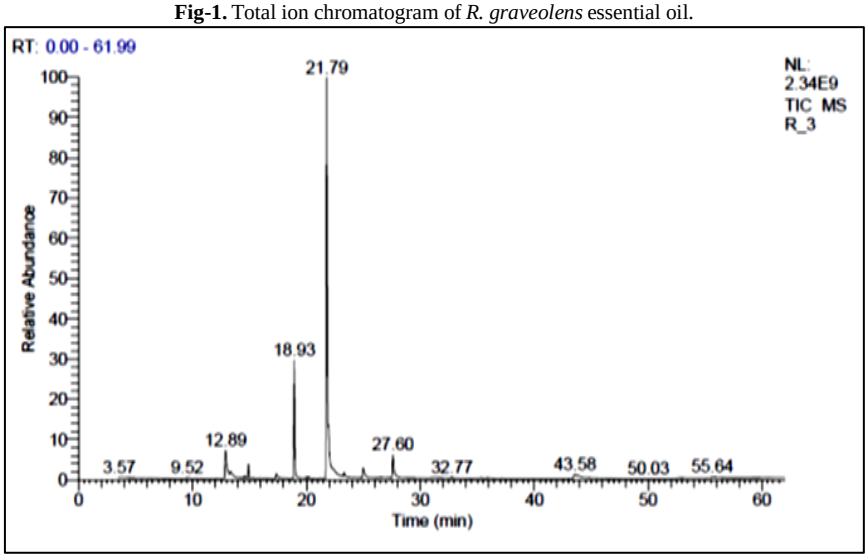
3. Results and Discussion

3.1. Chemical Composition of *R. Graveolens* Essential Oil

Hydrodistillation of the air-dried aerial parts of *R. graveolens* yielded 0.6 % (v/w) of strong odor yellowish oil. GC-MS data in Fig 1, showed the total ion chromatogram (TIM) of oil components represented 97.9 % of the oil which was majorly composed by 2-Undecanone (60.54%), 2-Nonaone (17.71%), 1-Nonene (4.90%) and 1-Undecanolacetate (3.84%) (Table-1).

Both 2-Undecanone and 2-Nonaone belonged to methyl ketones which estimated with 81.65% of total oil and considered a chemotype of *R. graveolens* oils from different parts of the world Ferhat, *et al.* [32]. This be through with, De Feo, *et al.* [33] and Rustaiyan, *et al.* [34], when profiled the oil extracted from *R. graveolens* growing in Venezuelan Andes (Merida) with 2-Undecanone and 2-Nonanone as major components.

Fig-1. Total ion chromatogram of *R. graveolens* essential oil.



On the other hand, twenty minor compounds were identified and listed in Table (1) and estimated totally with 14.75% of total oil beside 2.1% unknowns. All components detected in the current study falls in the range previously reported. Quantitative differences likely came from the frequency of plant parts used, harvesting time, environmental factors like the soil nature and the climate.

Table-1. Chemical composition of R. graveolens essential oil

R.T.*	Common name	Area %	CAS-No
9.53	3-Methyl-3-heptanol	0.31	5582-82-1
12.08	3,3,3-Tri methoxy propanenitrile	0.09	70138-31-7
12.89	1-Nonene	4.90	821-55-6
13.35	2-Nonanol	0.59	628-99-9
14.58	2-Octanol acetate	0.39	2051-50-5
14.91	Geijerene	2.06	6902-73-4
15.35	8-methyl-2-nonanone	0.18	4026-05-5
17.35	2-Decanone	1.15	693-54-9
18.93	2-Nonanone	17.71	112-17-4
20.00	Pregeijerene	0.24	20082-17-1
20.18	1-Undecanol	0.32	112-42-5
21.17	1,2-Epoxyhexadecane	0.10	7320-37-8
21.78	2-Undecanone	60.54	112-12-9
23.32	2-Acetoxytridecane	0.67	136376-56-2
24.97	2-Dodecanone	2.07	6175-49-1
27.11	α-Acorenol	0.14	28400-11-5
27.59	1-Undecanolacetate	3.84	1731-81-3
30.99	2-tridecanone	0.21	593-08-8
32.76	Hedycaryol	0.26	475-20-7
35.36	α-cedrole	0.32	77-53-2
37.14	β-Eudesmol	0.10	473-15-4
43.55	Tumeronol B	1.32	131651-38-2
55.64	Isomaturnin	0.39	62706-44-9
	Total methyl ketones	81.65	-
	Unknowns	2.1	-

R.T. = Retention time (min.)

3.2. Acaricidal Activity of *R. Graveolens* Essential Oil

The data in Table (2) showed the toxicity fumigation of *R. graveolens* essential oil against *T. urticae* adult females. The LC_{50} and LC_{90} values recorded 0.018 and 0.075 µl/ml air, respectively. Toxicity results cleared the efficiency of *R. graveolens* essential oil that increased with the increasing of vapor concentration.

Table-2. Susceptibility of T. urticae adult female to R. graveolens essential oil fumigant

LC_{50} (lower – upper) (µl/ml air)	LC_{90} (lower – upper) (µl/ml air)	Slope	P
0.018 (0.014-0.036)	0.075 (0.073-0.982)	2.066	0.290

Previous studies revealed that the acaricidal effects of plant essential oils against *T. urticae* are related to their main components [35, 36]. So, the fumigation toxicity of *R. graveolens* essential oil may be related to its methyl ketones abundance especially 2-Undecanone (60.54%). This compound was developed to become a commercial arthropods repellent registered with the US EPA (Environmental Protection Agency), BioUD® for use on skin and clothing to control mosquitoes and ticks. It was more than two to four times more repellent than N,N-diethyl-meta-toluamide (DEET) against ixodid ticks, *Amblyomma americanum*, *Dermacentor variabilis* and *Ixodes scapularis* and showed highest repellency for 5 weeks on cotton cheese cloth against *A. americanum* [37, 38].

Moreover, Zhu, *et al.* [39] explored the insecticidal activity of four different methyl ketones (2-Undecanone, 2-Nonanone, 2-Octanone and 2-Heptanone) as fumigants, all compounds were found to be effective in eliciting mortality of fire ants. 2-Undecanone also had fumigant effects against adult German cockroaches, adult red flour beetles and the eggs of the tobacco budworm.

Generally, the fumigant toxicity depends on the respiratory rate of the target pest, meaning that the highest respiratory rate of the organism is the high sensitivity [40]. The mode of action of 2-undecanone is unknown but it is assumed to act directly on the cuticular surface of insects and plants, provided that breathing is not merely a target [41].

3.3. Ovicidal Activity of *R. Graveolens* Essential Oil

Data in Table (3) showed the ovicidal activity of *R. graveolens* essential oil against 1, 3 days old eggs of *T. urticae* with LC_{50} (0.005 & 0.009 µl/ml air, respectively). The corresponding LC_{90} values were 0.022 and 0.021 µl/ml air, respectively. Accordingly, a marked reduction in egg hatchability was observed with the increase concentration of the oil vapors. The one-day- old eggs were more susceptible than three days old eggs. The oil toxicity may be returned to the large quantity of 2-methyl ketone compounds which recently used as eco-friendly bio-fumigants according to Zhu, *et al.* [39]**,** more propped data presented by Perera, *et al.* [42]**,** when rated out the fumigant ovicidal activity of *R. graveolens* essential oil and four authentic methyl ketones against the freshly laid eggs of *Corcyra cephalonica* and found that the oil and even-chain methyl ketone compounds were more toxic than the odd-chaine methyl ketones. Also, Hong, *et al.* [43] recorded that *Bradysia procera* egg hatching was inhibited by 2-Nonanone, the 2nd major compound of *R. graveolens.*

Table-3. Susceptibility of *T. urticae* egg stage to *R. graveolens* essential oil fumigant

Tested eggs	LC_{50} (lower-upper) (µl/ml air)	LC_{90} (lower-upper) (µl/ml air)	Slope	P
one day-old eggs	0.005 (0.002-0.006)	0.022 (0.017-0.047)	1.98	0.820
three days-old eggs	0.009(0.007-0.010)	0.021(0.017-0.034)	4.036	0.489

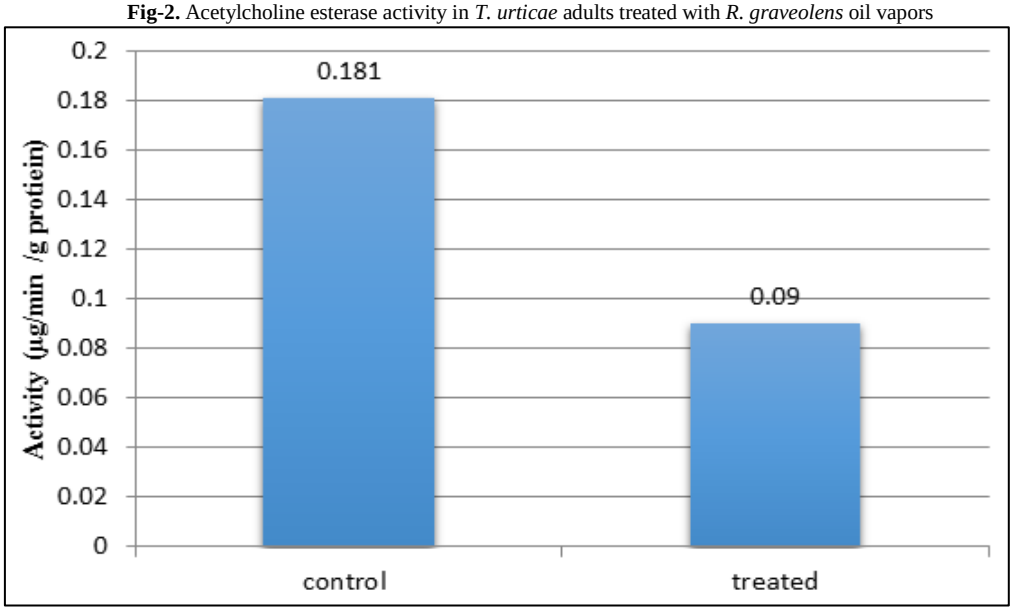
3.4. Biochemical Changes

The changes in the content of total soluble protein and enzymatic activities on eggs (1 and 3 days-old) and adults of *T. urticae* as response of fumigant by the potent LC_{50} of rue oil and control after 24 h of treatment were detected.

3.5. Aacetylcholine Esterase (AChE)

Acetylcholine esterase (AChE) activity in *T. urticae* adult females was negatively affected by fumigation with *R. graveolens* essential oil, (Fig. 2). The LC_{50} concentration led to decay in AChE activity reach to (0.09µg/min/mg protein) compared with the control (0.181µg/min/mg protein). This weak inhibition doesn't match with the high toxicity results. According to the oil composition, *R. graveolens* essential oil poor in monoterpenes that mainly response of the AChE inhibitory activity of essential oil [44]

Fig-2. Acetylcholine esterase activity in *T. urticae* adults treated with *R. graveolens* oil vapors



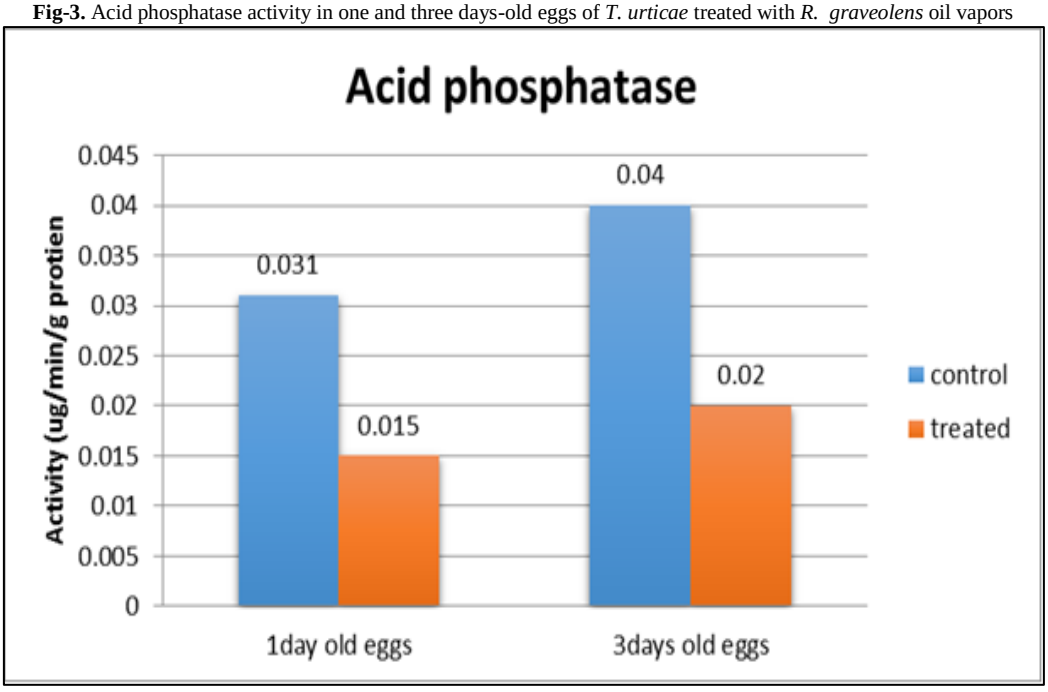
The high content of methyl ketones may provide another hypothesis consistent with Abdelgaleil, *et al.* [44], in addition that ketones compounds had weak AChE inhibitory activity in spite of its high toxicity. Moreover, [Lee, *et al.* [45], 46] did not find a direct correlation between insect toxicity and AChE inhibition by menthone or β-pinene. These may suggest that AChE is not the only target for *R. graveolens* essential oil effect and may there be another targets.

3.6. Acid Phosphatases Activity

In general acid phosphatase activity during egg development is a general requirement for differentiating Vitellin (VT); the main component of yolk platelets through its processing systems, Sappington and Raikhel [47]. Moreover, phosphatases should be involved in VT digestion later with embryo development. Yolk platelet-associated hydrolases Nussenzveig, *et al.* [48].

Data in Fig. (3) mentioned that the acid phosphatases activity during eggs development after fumigation with *R. graveolens* essential oil, a notable increase was detected from one to three days old control eggs (0.031 to 0. 04 µg/min/mg protein), respectively, similarly with Fialho, *et al.* [49] who noticed a 5 fold increase at 6 days after oocyte fertilization and after 6th day acid phosphatases activity reaches a plateau and the enzyme is kept activated up to the end of embryogenesis**.** In contrast, the treatment with LC_{50} of oil vapors cause highly inhibition in enzyme activity in both tested ages but activity still higher in three days old (0.015 & 0.02 µg /min/mg protein), respectively, that may refer to the acid phosphatases may be a target of oil components fumes in egg and its inhibition mostly was one of the ovicidal cause. Also, the efficacy in early egg stages was higher and a later stage was less susceptible.

Fig-3. Acid phosphatase activity in one and three days-old eggs of *T. urticae* treated with *R. graveolens* oil vapors



4. Conclusion

The developments of natural inseticides can help reduce the negative effects of synthetic pesticides, such as residues, resistance, and environmental pollution. According to the current study, *Ruta graveolens* essential oil is an effective, natural, low cost and environmental friendly fumigation agent against eggs and adults of *T. urticae* because of the potential role of fumigants in reduction proportion of treated pest especially under conditions as a candidate under similar conditions in green houses and stored products. It should be noted that the technique used is simple and can be modified and developed.

References

[1] Ebadollahi, A., Sendi, J. J., Aliakbar, A., and Razmjou, J., 2015. "Acaricidal activities of essential oils from satureja hortensis (l.) and teucrium polium (l.) against the two-spotted spider mite, tetranychus urticae koch acari: Tetranychidae." *Egyptian Journal of Biological Pest Control,* vol. 25, pp. 171-176.

[2] Stumpf, N., Nebitz, C. P. W., Kraus, W., Moores, G. D., and Nauen, R., 2001. "Resistance to organophosphates and biochemical genotyping of Acetylcholinesterases in Tetranychus urticae Acari: Tetranychidae." *Pest Biochem. Physiol.,* vol. 69, pp. 131-142.

[3] Van Leeuwen, T., Van Pottelberge, S., Nauen, R., and Tirry, L., 2007. "Organophosphate insecticides and acaricides antagonise bifenazate toxicity through esterase inhibition in Tetranychus urticae." *Pest Manag. Sci.,* vol. 63, pp. 1172-1177.

[4] Bakr, E. M., 2006. "Fumigant toxicity of some volatile oils belonging to Apiaceae plants against Tetranychus urticae Koch." *J. Pest. Cont. Environ. Sci.,* vol. 14, pp. 83-89.

[5] Mansour, F., Azaizeh, H. H., Saad, B., Tadmor, Y., Abo-Moch, F., and Said, O., 2004. "The potential of Middle Eastern flora as a source of new safe bio-acaricides to control Tetranychus cinnabarinus, the carmine spider mite." *Phytoparasitica,* vol. 32, pp. 66-72.

[6] Don-Pedro, K. N., 1996. "Fumigant toxicity of citruspeel oils against adult and immature stages of storage insect pests." *Pestic. Sci.,* vol. 47, pp. 213-223.

[7] Khedr, M. and El-Kawas, H. M. G., 2013. "Control of Spodoptera littoralis (Boid.) (Lepidoptera: Noctuidae) and Tetranychus urticae Koch (Acari: Tetranychidae) by coriander essential oil." *Journal of Entomology,* vol. 10, pp. 170-181.

[8] Reddy, A. V. and Singh, R. P., 1998. "Fumigant toxicity of neem (Azadirachta indica A. Juss) seed oil volatiles against pulse beetle, Callosobruchus maculatus Fab. Col., Bruchidiae." *J. Appl, Entomol,* vol. 122, pp. 607-611.

[9] Sammataro, D., Drgrandi-Hoffman, G., Needham, G., and Wardell, G., 1998. "Some volatile plant oils as potential control agents for varroa mites (Acari: Vrroidae) in honey bee colonies Hymenoptra: Apidae." *Am. Bee. J.,* vol. 138, pp. 681-685.

[10] Stamopoulos, D. C., 1991. "Effect of four essential oil vapours on the oviposition and fecundity of Acanthoscelides obtecus (Say) (Coleoptra: Bruchidae): laboratory evaluation." *J. Stored Prod. Res.,* vol. 27, pp. 199-203.

[11] Weaver, D. K., Dunkel, F. V., Potter, R. C., and Ntezurubanza, L., 1994. "Contact and fumigant efficacy of powdered and intact Ocimum canum Sims (Lamiales: Lamiaceae) against Zabrotes subfasciatus (Boheman) adult Coleoptra: Bruchidae." *J. Stored Prod. Res.,* vol. 30, pp. 243-252.

[12] Calderone, N. M. and Spival, M., 1995. "Plant extracts for control of parasitic mite Varroa jacobsoni (Acari: Varroidae) in colonies of the Western honey bee Hymenoptera: Apidae." *J. Econ. Entomol.,* vol. 88, pp. 1211-1215.

[13] El-Nahal, A. K. M., Schimidt, G. H., and Risha, E. M., 1989. "Vapours of Acarus calamus oil- a space treatment for stored-product insects." *J. Stored. Prod. Res.,* vol. 25, pp. 211-216.

[14] Rice, P. J. and Coats, J. R., 1994. "Insecticidal properties of several monoterpenoids to the housefly (Diptera: Muscidae), red flourbeetle (Coleoptera: Tenebrionidae) and southern corn rootworm Coleoptera: Chrysomelidae." *J. Econ. Entomol,* vol. 87, pp. 1172-1179.

[15] Shaaya, E., Ravid, U., Paster, N., Juven, B., Zisman, U., and Pissarev, V., 1991. "Fumigant toxicity of essential oils against four major stored-product insects." *J. Chem. Ecol.,* vol. 17, pp. 499-504.

[16] Amizadeh, M., Hejazi, M. J., and Saryazdi, G. A., 2013. "Fumigant toxicity of some essential oils on Tetranychus urticae Acari: Tetranychidae." *Internat. J. Acarol.,* vol. 39, pp. 285-289.

[17] Delaplane, K. S., 1992. "Controlling tracheal mites (Acari: Tarsonemidae) in colonies of honey bees (Hymenoptera: Apidae) with vegetable oil and menthol." *J. Econ. Entomol.,* vol. 85, pp. 2118-2124.

[18] Daniel, M., 2006. *Medicinal plants: chemistry and properties*. Enfiled, NH, USA: Science Publishers. p. 250.

[19] Gonzalez-Trujano, M. E., Carrera, D., Ventura-Martinez, R., Cedillo-Portugal, E., and Navarrete, A., 2006. "Neuropharmacological profile of an ethanol extract of Ruta chalepensis L. in mice." *J. Ethnopharmacology,* vol. 106, pp. 129-135.

[20] Gunaydin, K. and Savci, S., 2005. "Phytochemical studies on Ruta chalepensis (Lam.) lamarck." *Nat. Prod. Res.,* vol. 19, pp. 203-210.

[21] Ntalli, N. G., Ferrari, F., Giannakou, I., and Menkissoglu-Spiroudi, U., 2011. "Synergistic and antagonistic interactions of terpenes against Meloidogyne incognita and the nematicidal activity of essential oils from seven plants indigenous to Greece." *Pest, Manag. Sci.,* vol. 67, pp. 341-351.

[22] Shehadeh, M. B., Afifi, F. U., and Abu-Hamdah, S. M., 2007. "Platelet aggregation inhibitors from aerial parts of Ruta chalepensis grown in Jordan." *Integrative Medicin Insights,* vol. 2, pp. 35-39.
[23] Aboutabl, E. A., Elazzouny, A. A., and Hammerschmidt, F., 1988. "The essential oil of Ruta graveolens L. growing in Egypt." *J. Sci. Pharm.,* vol. 56, pp. 121-124.
[24] Claus, E. P., 1961. *Pharmacognosy*. Philadelphia, U.S.A.: Lea and Febger.
[25] Miguel, S., 2003. "Rue (Ruta L., Rutaceae) in traditional Spain: Frequency and distribution of its medicinal and symbolic application." *Economic Botany,* vol. 57, pp. 231-244.
[26] Handa, S. S., 2008. *An overview of extraction techniques for medicinal and aromatic plants. In: Extraction technologies for medicinal and aromatic plants. Handa, S. S., S. P. S. Khanuja, G. Longo and D. D. Rakesh (Eds)*. Italia: International Centre for Science and High Technology. pp. 21-54.
[27] Abbott, W. S., 1925. "A method of computing the effectiveness of an insecticide." *J. Econ. Entomol.,* vol. 18, pp. 265-267.
[28] Powell, M. E. A. and Smith, M. J. H., 1954. "The determination of serum acid and alkaline phosphatase activity with 4-aminoantipyrine (A.A.P.)." *J. Clinical Pathology,* vol. 7, pp. 245-248.
[29] Simpson, D. R., Bulland, D. L., and A., L. D., 1964. "A semi micro technique for estimation of cholinesterase activity in boll weevils." *Ann. Ent. Soc. Amer.,* vol. 57, pp. 367-371.
[30] Gornall, A. G., Bardawill, C. J., and David, M. M., 1949. "Determination of serum proteins by means of biuret reaction." *J. Bio. Chem.,* vol. 177, pp. 751-766.
[31] Finny, D. I., 1971. *Propit analysis*. 3rd ed ed. London: Cambrige Univ. Press.
[32] Ferhat, M., Kabouche, A., and Kabouche, Z., 2014. "Comparative compositions of essential oils of three Ruta species growing in different soils." *J. Mater. Environ. Sci.,* vol. 5, pp. 735-738.
[33] De Feo, V., De Simone, F., and Senatore, F., 2002. "Potential allelochemicals from the essential oil of Ruta graveolens." *Phytochemistry,* vol. 61, pp. 573-578.
[34] Rustaiyan, A., Khossravi, M., Sultani-Lotfabadi, F., Yari, M., Masoudi, S., and Monfared, A., 2002. "Constituents of the essential oil of Ruta chalepensis L. from Iran." *J. Essent Oil Res.,* vol. 14, pp. 378-379.
[35] Badawy, M. E., El-Arami, S. A., and Abdelgaleil, S. A., 2010. "Acaricidal and quantitative structure activity relationship of monoterpenes against the two-spotted spider mite, Tetranychus urticae." *Exp. Appl. Acarol.,* vol. 52, pp. 261-274.
[36] Roh, H. S., Lee, B. H., and Park, C. G., 2013. "Acaricidal and repellent effects of myrtacean essential oils and their major constituents against Tetranychus urticae Tetranychidae." *J. Asi. Pac. Entomol.,* vol. 16, pp. 245-249.
[37] Bissinger, B. W., Apperson, C. S., Sonenshine, D. E., Watson, D. W., and Roe, R. M., 2009a. "Efficacy of the new repellent BioUD® against three species of ixodid ticks." *Exp. Appl. Acarol.,* vol. 48, pp. 239-250.
[38] Bissinger, B. W., Jiwei, Z., Apperson, C. S., Sonenshine, D. E., Watson, D. W., and Roe, R. M., 2009b. "Comparative efficacy of BioUD to other commercially available arthropod repellents against the ticks Amblyomma americanum and Dermacentor variabilis on cotton cloth." *Am. J. Trop. Med. Hyg.,* vol. 81, pp. 685-690.
[39] Zhu, J., Dhammi, A. B., van Kretschmar, J., Vargo, E. L., Apperson, C. S., and Roe, R., 2018. "Novel use of aliphatic n-methyl ketones as a fumigant and alternative to methyl bromide for insect control." *Pest Manag. Sci.,* vol. 74, pp. 648–657.
[40] USDA-APHIS, 2013. *Methyl bromide treatment manual. US department of agriculture animal and plant health inspection service*. Riverdale, MD: USDA-APHIS.
[41] Roe, R. M. and Kennedy, G., 2011. "Compositions and methods of use for ketones and aldehydes as herbicides." *US Patent Application,* vol. 61, pp. 575,553.
[42] Perera, A. G. W. U., Karunaratne, M. M. S. C., and Chinthaka, S. D. M., 2017. *Fumigant toxic potential of Ruta graveolens essential oil and its 2-methlyl ketone constituents on the eggs of Corcyra cephalonica. Internat Res Symposium on Pure and Applied Sciences*. Fac Sci Univ Kelaniya Sri Lanka, p. 25.
[43] Hong, T. K., Perumalsamy, H., Jang, K. H., Na, E. S., and Ahn, Y. J., 2018. "Ovicidal and larvicidal activity and possible mode of action of phenylpropanoids and ketone identified in Syzygium aromaticum bud against Bradysia procera." *Pesticide Biochemistry and Physiology,* vol. 145, pp. 29-38.
[44] Abdelgaleil, S. A. M., Mohamed, M. I. E., Badawy, M. E. I., and El-Arami, S. A. A., 2009. "Fumigant and contact toxicities of monoterpenes to sitophilus oryzae (l.) and tribolium castaneum (herbst) and their inhibitory effects on acetylcholinesterase activity." *J. Chem. Ecol.,* vol. 35, pp. 518-525.
[45] Lee, Choi, W. S., Lee, S. E., and Park, B. S., 2001a. "Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, Sitophilus oryzae." *Crop Protect,* vol. 20, pp. 317-320.
[46] Lee, Lee, B. H., Choi, W. S., Park, B. S., Kim, J. G., and Campbell, B. C., 2001b. "Fumigant toxicity of volatile natural products from Korean spices and medicinal plants towards the rice weevil, Sitophilus oryzae (L)." *Pest Manag Sci.,* vol. 57, pp. 548–553.
[47] Sappington, T. W. and Raikhel, A. S., 1998. "Molecular characteristics of insect vitellogenins and vitellogenin receptors." *Insect Biochem. Mol. Biol.,* vol. 28, pp. 277-300.
[48] Nussenzveig, R. H., Oliveira, P. L., and Masuda, H., 1992. "Identification of yolk platelet-associated hydrolases in the oocytes of Rhodnius prolixus " *Arch Insect Biochem Physiol.,* vol. 21, pp. 253-262.
[49] Fialho, E., Silveira, A. B., Masuda, H., and Silva-Neto, M. A. C., 2002. "Oocyte fertilization triggers acid phosphatase activity during Rhodnius prolixus embryogenesis." *Insect Biochem. Mol. Biol.,* vol. 32, pp. 871-880.