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Chemical Composition and Evaluation of the Acaricidal Activity of Two Essential Oils and Their Formulations against the Two-spotted Spider Mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAFW and HMT designed the study, wrote the protocol, wrote the first draft of the manuscript, analyses of the study and managed the literature searches. Author HHFH collected the experimental data in the laboratory and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The synthetic acaricides were used to control two-spotted spider mite. The using increase of biocide cause problems such as environmental pollution and pesticide resistance, these needs increasing the sustainable, environmentally-friendly control methods. The essential oils' biodegradable with acaricidal properties referring as green pesticides. The study was aimed to determine the chemical composition and acaricidal activity of two essential oils extracted by hydro-distillation of the Basil (*Ocimum basilicum* L.), Caraway seeds (*Carum Carvi* L.) and their formulations which were prepared as emulsifiable concentrates (ECs) against the two-spotted spider mite as a model under laboratory conditions. These ECs Physico-chemical properties were studied. The percentages of the main components of the extracted essential oils were identified by gas chromatography-mass spectrometry (GC/MS) which were: Estragole (30.17%), Methyl eugenol (13.81%) and Linalool (12.66%) for basil and Carvone (66.72%), D-limonene (31.46%) for caraway as the main compounds. The result of the present study show that formulated tested essential oils has high

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toxicity on *T. urticae* than non-formulated tested essential oils. Also, the results of the acaricidal activity showed that the formulated essential oil from basil has a highly toxic effect against *T. urticae* with LC_{50} of 2259.162 ppm than caraway with LC_{50} of 8283.269 ppm. Our findings revealed that the formulated essential oils can be used as alternatives to chemical pesticides for controlling this pest.

Keywords: Tetranychus urticae; Tetranychidae; essential oils; chemical composition; oil formulations; acaricidal activity.

1. INTRODUCTION

The two-spotted spider mite is a highly polyphagous pest that can destroy many crops [1]. More than 900 species of plant-hosts like citrus, avocado, beans, cotton, apples, pears, plums and many other horticultural and ornamental's crops are destroyed by, *Tetranychus urticae* Koch (Acari: Tetranychidae), [2]. It is especially control in intensive, high-yield cropping systems. It affects yields by feeding on leaves thereby decrease the area of photosynthetic activity [3,4]. When there are severe infestations, it causes leaves abscission. The most important pests *Tetranychus urticae* Koch (Acari: Tetranychidae), are distributed worldwide in terms of the damage and control cost [5]. Farmer applied synthetic acaricides to decrease losses. However, the increasing use of the synthetic acaricides caused the development of resistance. *Tetranychus urticae* pesticide-resistant populations where been recorded in more than 40 countries in both greenhouses and open field crops [6-9]. The rapid development of resistance in *T. urticae* is favored by its high reproductive potential, extremely short life cycle [2,10]. For commercial acaricides, like organophosphates, dicofol and organotins, fail in the chemical control of spider mites caused by resistance where been reported only a few years after their introduction [11-14]. The present alternative strategies based on the use of eco-chemical control plant-insect relationships that considered one of the most promising methods aiming to reduce the chemical pesticides [15]. Indeed, higher plants produce different bioactive secondary metabolites participate in their natural resistance to pathogens, insects and mites. The essential oil is present in plants as secondary metabolites that are concentrated in the leaves, bark, or fruit of aromatic plants [16,17]. Plant resistance to insect pests can result from the excretion of these essential oils [18]. The essential oils were recorded some problems although, their promising activities against many pests, for example, the essential oils' volatility, water solubility and oxidation playing an important role in the essential oils' activity, application and persistent. Therefore, these problems must be resolved before using the essential oils as an alternative to synthetic pesticides for the pest control [19]. The formulations can resolve these problems. A formulation that allows protecting the essential oil from high temperatures, oxidation and UV light must be found. For this purpose, the essential oils' agrochemical formulations shall be physically stable in the long term and shall increase the agrochemical biological efficiency [20]. There are different agrochemical formulations and the most common formulations are still emulsifiable concentrates for oil-soluble chemicals. Emulsifiable concentrates (ECs) is one of the most widely used formulations because it has many advantages like good storage stability, easy applicability and high biological activity etc. [21,22]. In a recent trend increasingly need safer and specified nature pesticide formulations instead of the conventional pesticides which cause various problems in plants and the environment.

The aim of the present work was to identify the major constituents of the essential oil distilled from Basil (*Ocimum basilicum* L.), Caraway seed (*Carum carvi* L.) and determined the toxicity of the extracted two essential oils and their formulations on the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Plant Material

Seeds of caraway (*Carum carvi* L.*)* used in this study were obtained from the herbal market in Egypt while fresh basil (*Ocimum basilicum* L.*)* was obtained from a farmer in Qalyubia government.

2.2 Chemical Used

A - Surfactants

Arkopal N 100: 4-Nonylphenyl-polyethylene glycol.

Span 80: Sorbitan monostearate.
Span 20: Sorbitan laurate
Tween 80: Sorbitan monooleate ethoxylate

All surfactants were supplied by: Kafr El- Zayaat Pesticides and Chemical Co., Kafr El- Zayaat, Egypt.

B – Solvents

Solvesso 100 was supplied by: Kafr El- Zayaat Pesticides and Chemical Co., Kafr El- Zayaat, Egypt.

2.3 Isolation of Essential Oil

The collected materials were dried, crashed and then grinded. The oil percentage was determined using the hydro-distillation method by Clevenger apparatus according to Guenther [23]. A known weight of powder (100 g) was placed in a flask for distillation, and an adequate amount of water was added. A proper essential oil trap and condenser were attached to the flask and enough water was added to fill the trap. The distillation continued for 3 hours until no further increase in the oil was observed. After finishing the distillation process the apparatus was left to be cooled, and the essential oil percentage was estimated as follows:

Yield (%) = (Oil (mL))/ (Plant (g)) × 100

The oil was dried by sodium sulphate anhydrous

2.4 Physicochemical Properties of Extracted Essential Oils

2.4.1 Refractive index

To measure the refractive index of oil, ATAGO Refractometer DR-AI. was used according to the ASTM D 1218-12 [24] method.

2.4.2 Density and specific gravity

It was assessed according to the ASTM D4052-11 [25] by Rodulph Densitometer DDM 2910.

2.4.3 Acid value

Acid value was calculated using the following formula according to AOAC [26].

Acid number = V×N× 56.1 / W

Where:

V = volume of potassium hydroxide used
N = normality of Potassium hydroxide
W = weighting of the sample
56.1 is potassium hydroxide molar mass

2.5 Gas Chromatography-mass Spectrometry

The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at the Central Laboratories Network, National Research Centre, Cairo, Egypt was used for identification of the chemical structure of essential oils. The samples were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 *μ*m film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:30, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4°C /min to 150°C and held for 6 min; rising at 4°C/min to 210°C and held for 1 min. The injector and detector were held at 280 °C and 220°C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of m/z 50-550. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

2.6 Acaricidal Effect

2.6.1 Mite rearing

The stock colony of *Tetranychus urticae* was obtained from the Department of Mite Research, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. All mites were cultured on mulberry leaves (*Morus alba* L.) placed on moistened cotton pads resting on sponges in plastic boxes. The mulberry leaves were examined every few days and replaced with fresh ones when over-crowding of mites and yellow leaves were observed. The colonies were maintained at room temperature (25 ± 2°c and 70% ± 5 R.H.). Adult mites were placed on new leaves for 10 days and newly emerged adults were used for each experiment. All bioassays were conducted and carried out under the same environmental conditions as the culture.

2.6.2 Toxicity of extracted essential oils against two spotted spider mites (*Tetranychus urticae*) under laboratory conditions

Insecticidal activity of the extracted essential oils on *T. urticae* adults was determined according

method described by **Sokeli and Karaka, [36].** A leaf-dipping method was used for bioassays with the extracted essential oils. Each experimental unit was kept in a glass petri dish (7 cm in diameter) and consisted of a treated mulberry leaf disc (5 cm in diameter) placed on cotton pads that were soaked in water as a source of moisture and to prevent mite escaping. Stock solution of each extract essential oil was made prior to use (essential oils + 0.2% Tween 80). Five serial concentrations were prepared for each extract. The mulberry leaf discs were dipped individually in the insecticidal essential oil solution for 20 seconds and dried at ambient temperature in the laboratory for 20 min, then placed upside down on the cotton pad in the Petri dish. Ten adult mites were placed on the lower surface of the mulberry leaf disc with a fine brush. Three replications were made for each concentration. The experimental units were incubated in a chamber at 25 ± 2°c and 70% ± 5 R.H. Numbers of live and dead two spotted spider mite adults were counted at 24 hrs post-treatment. Two spotted spider mite adults were considered dead if no movement was apparent after probing with the tip of a fine brush.

The percentages of the mortalities were corrected by using Abbot Formula [37]. The mortality data were subjected to statistical analysis.

Corrected mortality % = (Observed mortality % - Control mortality %)/(100 - Control mortality%) x 100

Statistical analysis and toxicity index

Mortality values 24 hr after exposure were analyzed by probit analysis (LCP line) to obtain LC_{50} and slope for each extract according to the method adopted by Finney [38].

The toxicity index of the tested compounds was determined according to Sun [39] as follow:

Toxicity Index = (LC_{50} of the most effective compound) / **(**LC_{50} of the compound used) x 100

In this equation, the most toxic compound has been given 100 units on the toxicity index scale.

Relative potency = LC_{50} of lowest toxic oil/ LC_{50} of tested oil

2.7 Preparation of Plant Essential Oil as a Suitable Formulation

According to WHO [27] and FAO/WHO [28] specification, successful emulsifiable concentrate (ECs) shall not show any solid or oily separation when it cooled at 0°C for seven days. Its flash point shall not be lower than 22.8°C. The emulsion shall not show any oil separation and the creamy separation or precipitation shall not exceed 2 ml when it emulsified with hard and soft water at the rate of 5%. The foam shall not exceed 5 ml after one minute. The free acidity or alkalinity shall not exceed 0.3%. The emulsifiable concentrate subjected to accelerated storage at 54°C for 14 days shall be chemically and physically stable.

The formulated extracts' essential oils were prepared weight/volume (w/v) in the form of an emulsifiable concentrate according to the following procedure Thakur [29]. The emulsifiable concentrate (ECs) was developed by dissolving active ingredients (extracted essential oils) in a petroleum-based solvent like Solvesso 100 with suitable emulsifiers. Extracted essential oils were dissolved in a fixed quantity of solvent, suitable emulsifier or a blend of emulsifiers after screening was added to make it clear solution and transferred quantitatively to 100 ml glass stoppered volumetric flask. The flask was shaken vigorously to assure homogeneity and was kept in tightly closed vials then the formulation was ready for examination. The emulsion stability was carried out according to CIPAC MT.32 [30].

General formula for EC development is given below in the following table.

2.7.1 Determination of physico-chemical properties of locally prepared formulated essential oils

a- Emulsion stability test: This property was measured according to the guidelines of FAO/WHO recommendation [28] using CIPAC MT 32 [30]. The standards of hard and soft water

S. No.	Active ingredients	Amount % (W/V)
1	Extracted essential oils	80-85
2	Emulsifier	5
3	Solvent	q .s.

were used. Hard water of 342 ppm as calcium carbonate according to CIPAC MT 18 [31], was prepared by dissolving 4.0217 gm of calcium chloride (anhydrous) and 1.388 gm of magnesium chloride hexahydrate ($MgCl_2$. $6H_2O$) in distilled water and makeup to 1000 ml. The soft water was prepared by mixing one volume of hard water with five volumes of distilled water to provide water hardness of 57 ppm. The volume of self-emulsion formed by pouring 5ml of pesticides' formulations Tof emulsifiable concentrates (EC) into a gradual cylinder containing 95 ml of hard or soft water was recorded as spontaneity. The cylinder was stoppered and inverted 30 times at the rate of complete cycle for two minutes. The contents were allowed to stand undisturbed for half-hour. The cream or oil layer found at either top or bottom of the cylinder and it was used as an indicator of emulsion stability. The amount of cream formed either at the top or the bottom of the cylinder for the successful formulation should not exceed than 2 ml as total volume.

b- Persistent foam: Persistent foam is a measure of the amount of foam likely to be present in a spray tank after dilution of the product with water. The specified amount of the material was added to prepared standard water (soft and hard) (95 ml) in the measuring cylinder and made up to the mark. The cylinder was stoppered and inverted 30 times. The cylinder was left to stand undisturbed on the bench for 5 min. The volume of the foam was recorded according to WHO [27] and CIPAC MT 47.1 [31].

c- Free acidity or alkalinity: It was determined according to the guidelines of FAO/WHO [28] specification and according to CIPAC MT 31.1 [30] by weighing 10 grams of material and introduce it to 100 ml of distilled water (if the material is soluble or emulsified in water) or introduce it into 25 ml of acetone and 75ml of distilled water, then filter it (if the material is immiscible in water). Then the solution was titrated immediately with 0.02N NaOH or 0.02N HCL using methyl red as an indicator.

Free acidity as % H_2SO_4= 0.0098x volume of 0.02N NaOH.

Free alkalinity as % NaOH= 0.008x volume of 0.02 N HCL.

d- Flash point: The flash point was measured by using a Kolchler closed cup Flash Tester and glass syringe ASTM D3828-12a [32].

e - Storage stability:

Cold storage: It was determined according to CIPAC MT 39.3 [33], the method carried out by transferred about 50 ml of the formulated EC in a sealed glass container to a refrigerator and remained at 0 ± 1°C for 7 days. At the end of 7 days, the container was removed from the refrigerator, and allowed to remain undisturbed at room temperature for 3 hours. The volume of any separated material of the container was subsequently recorded.

Accelerated storage: It was determined according to CIPAC MT 46.3 [33], 50 ml of the formulated EC was kept for 14 days at a temperature of 54 ± 1°C in a sealed glass container to avoid loss of volatile solvents. After storage period, the sample was cooled to a room temperature**,** and then emulsion stability test was carried out in soft and hard water.

2.7.2 Determination of physico-chemical properties of spray solutions of the locally formulated essential oils:

The spray solutions of formulated essential oils for measuring physical proprieties were made up under dilution rate 5% for both soft and hard water.

a- Persistent foam: It was determined using WHO [27] as mentioned before**.**

b- Emulsion stability test: It was determined according to CIPAC MT. 32 [30] as mentioned before.

c- pH Measurement: pH value of prepared solutions was measured using a pH Meter (Model: Jeway 3510). When 30 minutes after shaking passed well again making homogenous solution, taking a constant volume (about 30 ml) and measuring the pH value, and taking the reading after 1 minute or when the reading was stable CIPAC MT 75.3 [33].

d- Electrical Conductivity and Salinity: The conductivity of spray solutions was measured by Conductivity and Salinity meter "Thermo Orion model 115A^+, USA". where µMHOS is the unite of the electrical conductivity CIPAC MT 32 [31]. When 30 minutes after shaking passed well again to make a homogenous solution, take a constant volume (about 80ml) to measure the conductivity of the solution and take the reading after 1 minute or when the reading was stable.

e- Viscosity: The viscosity of the spray solution was measured using Brookfield DV II+ PRO″ digital Viscometer (Brookfield, USA), UL rotational adaptor (ULA) ASTM D2196-15, [34].

f- Surface tension: The surface tension of the spray solution was measured using Force tensiomate sigma 700. USA by du Nouy method, a platinum-iridium ring ASTM D1331-14, [35].

2.8 Toxicological tests

The biological activities of the two-formulated extracted essential oils against the *T. urticae* were studied. The experiment of bioassay was repeated in the same manner as mentioned before with extracted essential oils and all parameters were observed. The LC_{50}, slope, toxicity index and Relative potency were represented as mentioned before.

3. RESULTS AND DISCUSSION

3.1 Essential Oil Yield

The percent yield of hydro-distillated essential oils in the present study was 1.195%, 0.8% for caraway and basil oil respectively.

3.2 Physicochemical Properties of Extracted Essential Oils

The data in table (1) showed that, the values of refractive index, density, specific gravity and acid value for hydro-distillated essential oils. The refractive index of the tested essential oil of basil and caraway at 25°C were 1.5142 and 1.4914 respectively. All density and specific gravity less than one at 25°C. The density and specific gravity observed for the oil samples in this study were 0.9647 gm/cm^3, 0.9679 for basil and 0.9497 gm/cm^3, 0.9526 for caraway respectively. The acid values were 0.676 and 1.122 for essential oil of basil and caraway respectively.

3.3 Chemical Compositions of Extracted Essential Oils

The results of gas chromatography/mass spectrometric (GC-MS) analysis of the tested essential oil (EO) of basil are presented in Table (2) and Figure (1). 33 compounds were identified, accounting for (99.84% of total oil). Methyl chavicol (Estragole) (30.17%), methyl eugenol (13.81%), linalool (12.66%) and eucalyptol (8.36%) were recorded as the most abundant components in *Ocimum basilicum* oil. Five components were identified in essential oil of caraway as shown in Table (3) and Figure (2), which represents 98.81% of the total essential oil. It consists of carvone (66.72%) and D-limonene (31.46%) as the main components. These results agree with those reported by Altantsetseg [40] who found that the main components of common basil were methyl chavicol (52.1%) and linalool (23.9%). Marotti [41] who reported that the presence of the linalool, methyl chavicol, and eugenol as major constituents of Italian basil essential oil (EO). Additionally, the essential oils from Madagascar [42], Iran [43] and Thailand [44] was rich in methyl chavicol.

Previous reports indicate that limonene and carvone were the main components of caraway essential oil [45]. Also, the result of our analysis was in agreement with the other literatures that reported carvone (47-62%) and limonene (34-50%) as the main component in the essential oil of caraway seeds [46]. Also, an essential oil of caraway collected in Qinghai and China contained (*R*)-carvone (51.62%) and D-limonene (38.26%) as its two major constituents [47]. (*R*)-carvone and D-limonene were also found to be the two main components in the essential oils collected from Europe and North America [48-52].

3.4 Toxicity Testing of Non-formulated Extracted Essential Oils against Two Spotted Spider Mite *Tetranychus urticae* under Laboratory Conditions

From the data recorded in Table (4) and Figs. (3), it was observed that, the two extracted essential oils had an effect on the two-spotted spider mite *Tetranychus urticae*. Generally, the percentages of mortality were more of Basil (*Ocimum basilicum*) essential oil than Caraway (*Carum carvi*) essential oil. The LC_{50} values were 4155.979 and 13437.825 ppm for basil and caraway oils, respectively. In the present investigation, the assays of toxicity index are also very important to evaluate the toxicity of each extracted essential oils. However, the least toxicity index value was attained with the effect Caraway oil (30.927) and the higher value was given to the effect of basil oil (100). On the other hand, the extracted basil oil was the highest toxic effect with relative potency (3.2) while the

extracted caraway oil was the least with relative potency (1.0).

The present results of basil oil are in agreement with those documented by Sajjadi, [53] who found that the basil oil showed the highest insecticidal activity against two spotted spider mites. Also, he reported that the high insecticidal activity of this oil probably related to the presence of active compounds of methyl chavicol and linalool.

The results of this study are compatible with several studies reported that insecticidal and acaricidal activities of essential oils derived from *Ocimum basilicum* [54-58]. The results obtained are compatible with Fang [59] who reported that, caraway essential oil have insecticidal activity against different species of insects and mites, like the two-spotted spider mite (*T. urticae*), rice weevil (S. oryzae), flat grain beetle (Cryptolestes pusillus) and German cockroaches (Blattella germanica). He suggested that the insecticidal activity of the essential oil of caraway was due to its containing a several components like carvone and limonene.

According to previous studies the toxicity of essential oils from aromatic plants against insect and mite is regarding to the presence of the main components, which were: ϒ- terpinene, pulegone, anethole, carvacrol, thymol, cymol, α-terpinene, linalool, eugenol, methyl eugenol and methyl chavicol [60-64]. Differences in chemical composition of the oils may probably explain why plant extract differs in resistance induction [65-67].

3.5 Physico-chemical Properties of Locally Prepared Plant Oils as Emulsifiable Concentrates (EC's)

The data in table (5) demonstrated the Physico-chemical properties of locally prepared two plant oils as emulsifiable concentrates (Basil 80% EC and Caraway 85% EC). On the basis of the thickness of the creamy layer that does not exceed 2 ml with the absence of oil separation at the rate of 5%. All prepared essential oils as EC's pre and after accelerated storage at 54˚C for 14 days passed successfully in emulsion stability test according to the FAO/WHO specifications. Also, all the formulations passed successfully in the cold test at 0˚C for 7 days since it didn't show any separation or sedimentation. On the other hand, the formulated caraway oil does not record any foam while formulated basil recorded 3 ml in hard and soft water. For the free acidity or alkalinity test, it shall not exceed 0.3%. All prepared formulations have acidic nature. The formulated caraway recorded the highest acidic value 0.258 while the formulated basil recorded 0.0598 before storage. The free acidity of both two prepared formulations was slightly increased after accelerated storage. Both of two oils prepared as EC's passed successfully through a flash point test since they showed gave high flash point values than 22.8°C.

3.6 Physico-chemical Properties of Spray Solution of the Locally Prepared Plant Oils as EC's

Data in table (6) demonstrated the physical properties of the spray solution of the two prepared formulations in different types of water. All two prepared formulations were succeeded in emulsion stability test and there is no oily separation or creamy layer in hard and soft water. The formulated caraway oil does not record any foam while the formulated basil recorded 3 ml in hard and soft water. The pH values in hard and soft water were (4.69 and 4.64) for caraway and (3.47 and 3.41) for basil indicating that the formulations have acidic character. As the maximum values of surface tension of formulated caraway oil and basil oil were 31.990 and 28.650 dyne/cm in hard water, respectively. The viscosity of formulated basil oil gave the highest value 4.18 cp. in H.W. than formulated caraway oil was 2.23 cp. The conductivity was (502 and 131 µs) and (682 and

Table 1. Physicochemical properties of extracted essential oils obtained by hydro-distillation

Characteristic	Value	
	Basil oil	Caraway oil
Refractive index at 25°C	1.5142	1.4914
density (gm/cm$^{3)}$ at 25°C	0.9647	0.9497
specific gravity at 25°C	0.9679	0.9526
acid value	0.673	1.122

287 µs) in hard and soft water. The salinity of the two prepared EC gave the same values in hard and soft water. The decrease pH value of spray solution would lead to the deionization of insecticides with an increase in its deposit's and penetration into the tested surface with a consequent increase in their insecticidal efficiency [68]. It was also shown that the decrease in surface tension of the pesticides spray solution gives a prediction of increasing wettability and spreading over the tested surface with increasing pesticidal efficiency [69]. Also increasing the viscosity of spray solution cause reduction drift and an increase in retention sticking and insecticidal efficiency [70]. According to El-Attal [71] increasing electric conductivity of the formulated extracts was coupled with increased mortality rate due to increased deposition and penetration of the formulated extracted particles.

3.7 Toxicity of Formulated Essential Oils against Two Spotted Spider Mites *Tetranychus urticae* under Laboratory Conditions

The present study was also extended to evaluate the effect of extracted basil and caraway oils post formulation. On the basis of LC_{50} value and in comparison, to the values recorded previously. The formulated extracts of basil and caraway oils were toxic against two spotted spider mites as shown in Table (7) and illustrated in Fig. (4). From data recorded in table (7) it was observed that, the formulated extract of basil oil was more toxic than caraway. The calculated LC_{50} values were 2259.162 and 8283.269 ppm for basil and caraway oils, respectively. As regards to the calculated value of toxicity indices, the higher toxicity index was (100) for the formulated extract of basil oil while the values recorded for the formulated extract of the caraway oil was 27.274. On the other hand, the lower slope was that of caraway oil (1.712) formulated extract and of the basil oil (1.414). On the other hand, the formulated extract of basil oil was the highest toxic effect with relative potency (3.7) while formulated extract of caraway oil was the least with relative potency (1.0).

Table 2. Identification of chemical composition of basil essential oil by GC/MS

Peak	Compound	RT, min	Area, %
1	Alpha-Pinene	8.001	0.67
2	Sabinene	9.341	0.32
3	Beta-Pinene	9.417	1.15
4	Beta-Myrcene	10	0.34
5	D-Limonene	11.282	0.42
6	Eucalyptol	11.376	8.36
7	Fenchone	13.398	0.37
8	Linalool	13.998	12.66
9	Camphor	15.403	0.45
10	Terpinen-4-ol	16.614	0.96
11	Alpha-Terpineol	17.163	1.52
12	Estragole	17.554	30.17
13	Carvone	18.954	1.63
14	Eugenol	22.782	4.37
15	unknown	23.872	0.59
16	Methyl-eugenol	24.397	13.81
17	Caryophyllene	24.729	1.75
18	Trans-alpha-bergamotene	25.26	2.71
19	Humulene	25.79	1.14
20	Cis-Beta-Farnesene	25.918	0.34
21	Germacrene D	26.641	1.23
22	Aromandendrene	26.757	0.67
23	unknown	27.107	0.53
24	Alpha-Bulnesene	27.393	0.39
25	Gamma-Muurolene	27.643	1.79
26	Cis-Calamenene	27.906	0.36
27	Trans-Alpha-Bisabolene	28.495	1.31
28	Spathulenol	29.584	1.06
29	Caryophyllene oxide	29.759	1.09
30	unknown	30.68	0.46
31	Epicubenol	30.896	0.97
32	.tau.-Cadinol	31.951	5.94
33	Alpha-Cadinol	32.493	0.31

Table 3. Identification of chemical composition of caraway essential oil by GC/MS

Peak	Compound	RT, min	Area, %
1	Beta-Myrcene	10.012	0.56
2	D-Limonene	11.329	31.46
3	Cis-Dihydrocarvone	17.327	0.56
4	Cyclohexanone,2-methyl-5-(1-methylethenyl)-	17.577	0.69
5	Carvone	19.25	66.72

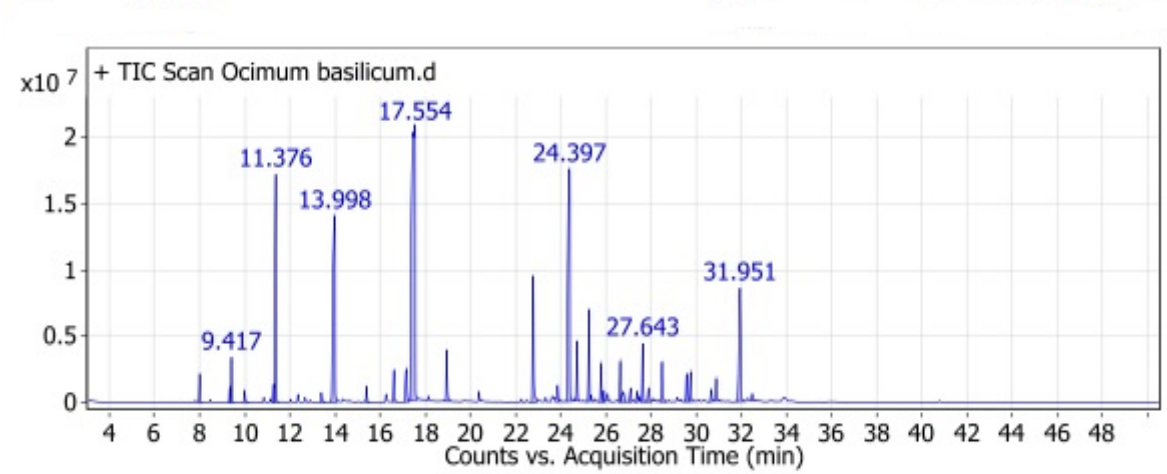

Fig. 1. GC-MS Chromatogram of Basil (*Ocimum basilicum*) essential oil

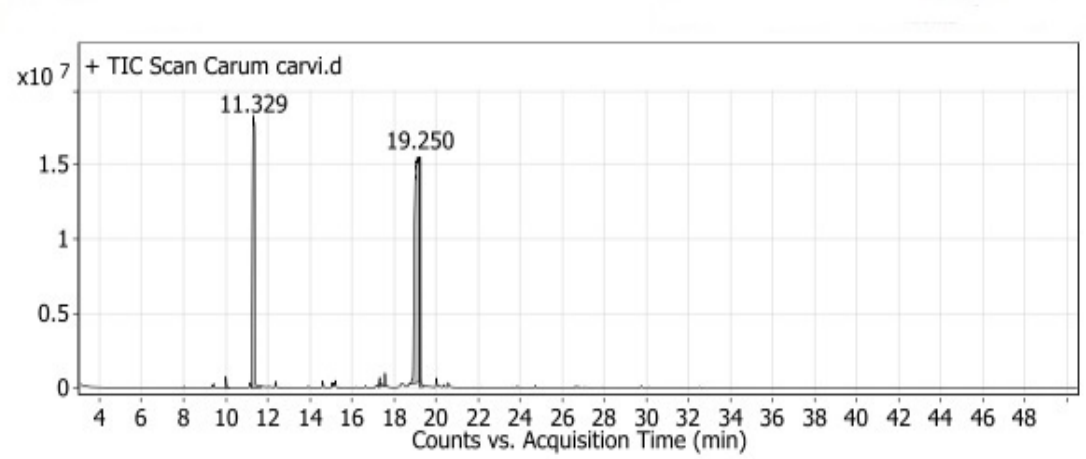

Fig. 2. GC-MS chromatogram of caraway (*Carum carvi*) essential oil

Data in table (8) showed the role of the formulation in increasing the effectiveness of the tested extracted essential oils. According to LC_{50} values EC formulation was more effective than its extracted essential oils. The increase in effectiveness of the formulation was 84% and 62% for the basil oil formulated extract and caraway oil, respectively. This means increased extracts essential oils toxicity by formulation. The contrasting results obtained by using the tested, formulated extracts can be explained on the basis of physico-chemical changes that contributed to formulated extract. Whereas the ability of the additives (surfactants) of the formulation to increase penetration of the active ingredient inside plant component with a consequent increase in its efficiency. The surfactants also enhance retention, uptake, and penetration of tested insecticides and to increase the spread of spray droplet on the target leaf surface.

Table 4.Toxicity of non-formulated extracted essential oils against *Tetranychus urticae*

Treatments	LC_{50} (ppm)	Lower limits	upper limits	Slope value	Toxicity index	Relative potency at LC_{50}
Basil oil	4155.979	3164.003	5239.866	1.152	100	3.2
Caraway oil	13437.825	11061.07	17387.094	1.589	30.927	1

Table 5. Physicochemical properties of locally prepared plant oils as emulsifiable concentrate (EC) formulation

Plant oil prepared as (EC's)	Emulsion stability (ml.cream.sep.)		Persistent Foam (ml.)		Acidity as % H_2SO_4	Cold storage at 0˚C for 7 days	Flash point °C	Accelerate storage		
	HW	SW	HW	SW				Emulsion stability (ml. cream. sep.)		Acidity as % H_2SO_4 at 54˚C for 14 days
								HW	SW	
Basil 80% EC	0.00	0.00	3	3	0.0598	passed	>50 °C	0.00	0.00	0.097
Caraway 85% EC	0.00	0.00	-	-	0.258	passed	>50 °C	0.00	0.00	0.289

S.W. =Soft Water
H.W. = Hard Water

Table 6. Physico-chemical properties of the spray solution of the locally prepared formulations of two essential oils

Plant oil prepared as (EC's)	Caraway 85% EC		Basil 80% EC	
	H.W	S.W	H.W	S.W
Persistent foam (ml)	-	-	3	3
Emulsion stability (ml. Cream. Sep.)	passed	passed	passed	passed
pH	4.69	4.64	3.47	3.41
Conductivity (µs)	502	131	682	287
Salinity (0/1000)	0.3	0.1	0.3	0.1
Viscosity (cp.)	2.23	2.14	4.18	3.15
Surface Tension (Dyne/cm)	31.990	31.013	28.650	28.436

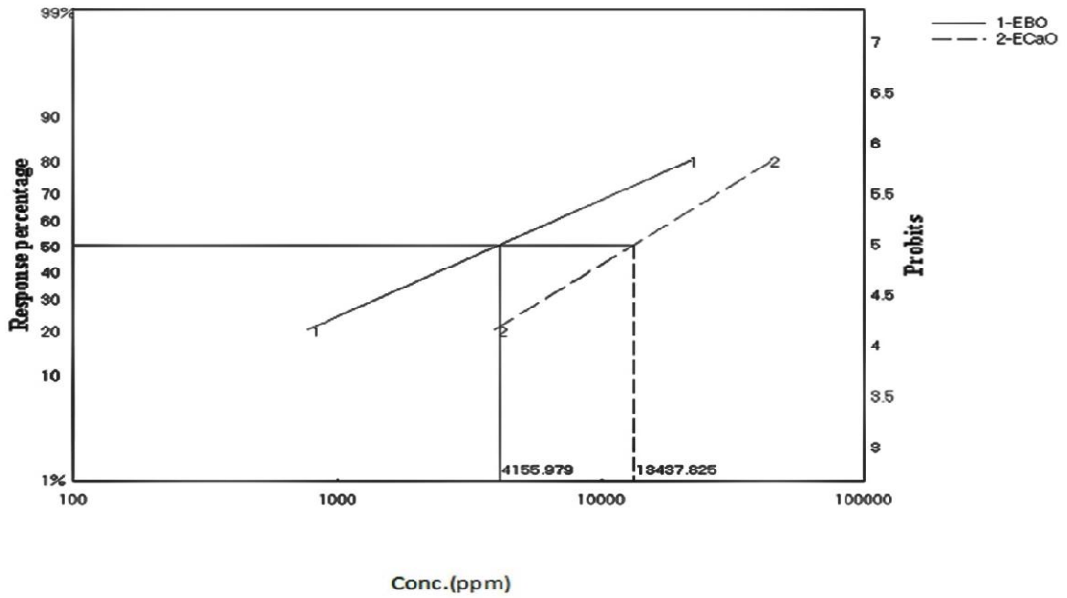

Fig. 3. Toxicity lines for two spotted spider mite due to the effect of extracted essential oils of basil and caraway (1(EBO)=extracted basil oil, 2(ECaO) = extracted caraway oil)

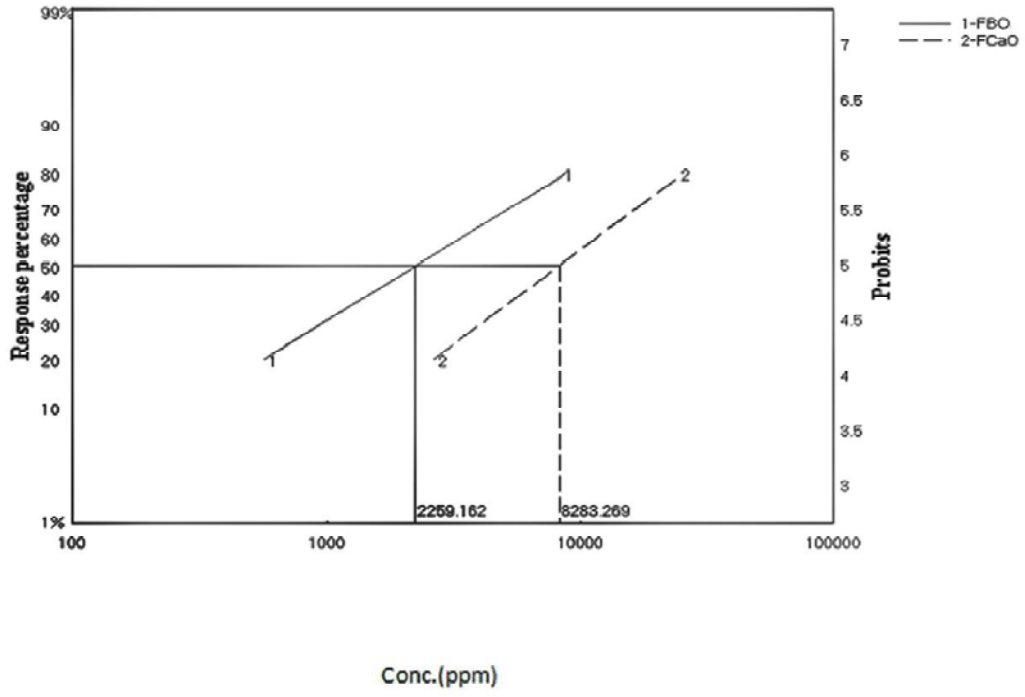

Fig. 4. Toxicity lines for two spotted spider mite due to the effect of formulated essential oils of basil and caraway (1(FBO)= formulated basil oil, 2(FCaO) = formulated caraway oil)

Table 7. Toxicity of the locally prepared plant oils EC's against *Tetranychus urticae*

Treatments	LC_{50} (ppm)	Lower limits	upper limits	Slope value	Toxicity index	Relative potency at LC_{50}
Basil 80% EC	2259.162	1676.231	2846.513	1.414	100	3.7
Caraway 85% EC	8283.269	7045.154	9847.834	1.711	27.274	1

Table 8. LC_{50} values for extracted essential oils (Basil and caraway oils), it's LC_{50} formulation and the percentage in increase in efficiency

	Basil oil	Basil 80% EC	Caraway oil	Caraway 85% EC
LC_{50}	4155.979	2259.162	13437.825	8283.269
Increase in effectiveness	84%		62%	

4. CONCLUSION

In conclusion, it could be considered that both of the two-tested plant essential oils prepared as emulsifiable concentrates (ECs) formulation showed a high acaricidal activity against the two-spotted spider mite (*T. urticae*) as compared to the extracted plant essential oils may be due to the role of the surfactants, and it could be used as a safer alternative in food and economic crops.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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