Chemical Composition of Essential Oils Extracted from Aleppo Pine *(Pinus halepensis* Miller*)* and Their Termiticidal and Fungicidal Effects

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ABSTRACT

The essential oils used in this study were isolated from Aleppo pine. The chemical composition was investigated using GC-MS. Thirty-seven and thirty-two compounds, representing 91.01and 99.82% from leaves and cones respectively, of the total collective essential oils were identified. Essential oils were dominated by hydrocarbon compounds especially monoterpenes. The major compounds from oils stations were: caryophyllene (40.78%), α-pinene (10.30%); H-cycloprop [e]azulene, decahydro-1,1,7-trimethyl-4-methylene-,[1aR-(1aà,4aà,7à,7aá,7bà)] (9.53) and 3-carene (7.58%) from needles and caryophyllene (38.26%); 3-carene (31.78%); bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl) (11.74%); 1,4-cyclohexadiene and 1- methyl-4- (1-methylethyl)-(3.01%) from cones. The essential oils revealed an interesting termiticidal and fungicide activity effects against dry-wood termites *Kalotermes flavicollis* and damping-off fungi (*Fusarium solani* and *Rhizoctonia solani*). Results of the present study indicate that the needles and cones of *Pinus halepensis* are promising potential sources of green pesticides and safe environmental applications for human, animal and plant health.

Key words: Chemical composition; Termiticidal effects; fungicide effects; essential oils; *Pinus halepensis*.

INTRODUCTION

There is considerable interest in evaluating non-traditional methods of insect and fungal protection. Among these systems are oils derived from foliage, seeds, wood and bark of many plants. A variety of plant-derived oils have been explored for protecting wood against fungi and insects. There are a wide array of possible plant-derived oil candidates including, oils of *Jatropha*, *Eucalyptus*, neem and pines. A large number of plant essential oils have been reported to have antifungal and phytotoxic activities (Amri *et al*., 2012a).

Crop loss due to fungal species also remains a serious problem (Pimentel *et al*., 2001). Emerging resistance of these species is seriously decreasing the number of effective antimicrobials (Ghasemi *et al*., 2007; Koudou *et al*., 2008; Shonouda *et al*., 2008 and Nour *et al*., 2009. Plants and their essential oils are potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many types of microbes, including phyto-pathogenic fungi, (Amri *et al*., 2011).The main constituents of essential oils were mono and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, acetates and ketones, which are responsible for their biological activity. Aromatic and medicinal plants produce a wide variety of volatile terpenes and their oxygenated derivatives. The antimicrobial properties of the essential oils are well recognized and their preparations are the founder applications as naturally occurring antimicrobial agents in pharmacology, pharmaceutical botany, phyto-pathology, medical and food preservation. In an attempt to reduce the use of synthetic pesticides, extensive investigations towards the possible exploitation of plant compounds as natural commercial products, that are safe for humans and the environment, were conducted

The genus *Pinus* belongs to the family Pinaceae and comprises about 250 species. It is the largest genus of conifers existed naturally in the northern hemisphere, especially in the Mediterranean region, Caribbean area, Asia, Europe, North and Central American. The genus *Pinus* has been planted in the temperate regions of the southern hemisphere. They are evergreen and resinous trees growing to 3-80 m tall with needle-like gray-green leaves that grow in pairs (Fuentes *et al*., 2006).

The medicinal and aromatic properties of the chemical compounds (e.g., turpentine, resins and essential oil….) of pine make it one of the most popular plants concomitant with all civilizations. Pine is also still widely used in traditional therapeutic practice in the world and has economic importance (Baba Aissa, 1991 and Dob *et al.*, 2005 and 2007)

There are many classified compounds within *Pinus halepensis*, the first being monoterpene 3-carene, α-pinenes and caryophyllene, which acts as anti-fungal and anti-bacterial (Deba *et al*., 2008 and Amri *et al*., 2013), and have key roles as an insect repellent, which have been reported by (Hossaina *et al*., 2008 and Nikitina *et al*., 2012).

Pinus halpensis have many hydrocarbonic compounds, especially monoterpenes. myrcene, α-pinene, E-β-caryophyllene, terpinolene, 2-phenyl ethyl isovalerate, terpinene-4-ol and sabinene . According to their chemical composition, they revealed antimicrobial activities (Ashafa *et al*., 2008 and Fekih *et al*., 2014).

Termites play an important role in ecosystems. There are more than 2300, termite species all over the world (Edwards and Mill, 1986). One hundred and forty seven of these species belong to the group of subterranean termites and about 28 species are of economic importance (Su and Schefferahn 1998). A new strategy under investigation by Myles (1996) involves coating termites to transmit toxicants such as growth regulators, and microbial pathogens by trophallaxis and grooming. Essential oils are also being examined as green alternatives to commonly used synthetic pesticides. Root-rot caused by *Fusarium solani* and *Rhizoctonia solani* is considered among the most deleterious diseases, which caused significant losses in many places of the world (Celar, 2000 and Nawar, 2007)

Fusarium root rot is one of the most common diseases of trees seedlings in the world (Smith, 1975 and Bloomberg, 1981). *Fusarium solani* wilt affects many different horticultural plants and it is considered the most important pathological problem of plants grown in artificial growing media (Couteaudier and Alabouvette, 1981).

Rhizoctonia solani (teleomorph: Thanatephorus spp.) is a plant pathogenic fungus with a wide host range and worldwide distribution. This plant pathogen was discovered more than 100 years ago. *R. solani* frequently exists as thread-like growth on plants or in culture, and it is considered a soil-borne pathogen. *R. solani* is best known to cause various plant diseases such as collar rot, root rot, damping off and wire stem. *R. solani* attacks its hosts when they are in their juvenile stages of development such as seeds and seedlings, which are typically found in the soil. It makes sense then that this saprophytic pathogen would live and survive in the soil and attack the part of its hosts that reside there (Parmeter, 1970).

The pinpoint the composition of aim of this study was to access the essential oils from *Pinus halepensis* needles and cones essential oil study different concentrations effects, on dry-wood termites *K. flavicollis* and fungi damping-off fungi (*F. solani* and *R. solani*).

MATERIALS AND METHODS

Plant Material

The needles and cones were collected from *P. halepensis* trees growing in many gardens at Alexandria City in October, then cut into small pieces and placed in a 2l- flask containing water. The oils were isolated by hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia (Maisonneune, 1975). The distillation period was 2^{hr} (fresh samples). The yield was measured with respect to the fresh weight of the sample (0.27 ml, and 14 ml per 100 g fresh weight of needles and cones, respectively). The oil was kept dry in sealed Eppendorf tubes with aluminum sheets and stored at 4 °C prior to chemical analysis according (Salem *et al*., 2013).

Collection and breeding termites

The experiment was carried out in the laboratory of insects (Department of Entomology, Faculty of Agriculture, Alexandria University). Termites were obtained in the fall of 2012 from the trees of *Casuarina* spp planted at the garden of Agricultural Research Center, Alexandria. and the termites have been retained in the sample insects in the laboratory. A laboratory culture of various termite castes of *K. flavicollis* fabr. was kept at 23-29°C and 71-86% RH in blackened glass jars on wood shavings or wafers (ca. 0.1cm thick) of Casuarina *spp* according to (Alfazairy *et al*., 2001).

Test of essential oils against termites

the wafers (ca. 2g in weight and 1 mm thick of each) of the Casuarina *spp* wood were placed in a clean and sterile Petri dish (9 by 1.5 cm) containing the tested concentration of the essential oil dissolved in dimethyl sulfoxide (DMSO; E. Merck, Germany). Concentrations of 100, 200 and 400 µm/ml were used. The wood wafers were thoroughly steeped with each test concentration by means of a sterile forceps and manual shaking to ensure uniform exposure of wafers to the essential oils .Samples were immersed for 6 hours in the essential oils. The wafers were air dried for 30min. until it had been completely evaporated. Ten termite individuals were introduced in each test Petri dish. Control group of wafers was treated with the solvent only and tested after the solvent had completely evaporated too. Three replications were used. All Petri dishes were kept between two black paper sheets to avoid exposure of insect to light. The number of dead termites was recorded daily. In addition, , both the loss or any damage in the test wood shavings due to termite feeding, and the faecal pellet production were visually estimated.

In vitro antifungal activity of essential oils

The essential oils of the needles and cones of *P. halepensis* were prepared by dilution in 10% dimethyl sulfoxide (DMSO; E. Merck, Germany) and Tween 80 (Sigma-Aldrich, USA) (10:1 v/v) at a concentration of 2000 µl/ml. The antifungal

activities against the growth of damping-off fungi (*F. solani* and *R. solani*) were assessed.

The fungi, *F. solani* and *R. solani* obtained from Plant Pathology Department, Faculty of Agriculture, Alexandria University, El-Shatby and they were used to study the impact of obtained extract against them.

In vitro antifungal activity of extracts and the essential oils were prepared at different concentrations of (100, 200 and 400 µm/ml). The concentrated oils were used to evaluate their inhibitory effects in terms of linear growth of the tested fungus *F. solani* and *R. solani* .The different concentrations of oils were added to a known amount of Potato Dextrose Agar (PDA) medium immediately before solidification and poured to a depth of 5.0 mm (about 15 ml medium) into the plates (9 cm diameter) and solidified for 30 min.

Mixture of dissolution in 10% dimethyl sulfoxide (DMSO; E. Merck, Germany) and Tween 80 (Sigma-Aldrich, USA) (10:1 v/v) with concentration of 2000 µl/ml, was used as a control sample. The treated and control plates were inoculated individually with a disc (5 mm diameter) for each of the fungi *F. solani* and *R. solani*. Three replicates were used for each particular treatment and all plates were incubated at a 26 ± 1 ^{0}C until the fungal mycelium reached the edges of their solvent control dishes.

The antifungal index expressed as % inhibition and was calculated by the following formula,

Antifungal index % = $(D_2- D_1)/ D_2\times100$

where D_1 is the diameter of growth zone in the experimental dish and D_2 is the diameter of the growth zone in the solvent control dish.

GC/MS analysis of the essential oils

The chemical constituents of essential oils of cones and needles from *P. halepensis* were analyzed using the Trace GC Ultra/Mass Spectrophotometer ISQ (Thermo Scientific) (GC/MS) apparatus (Institute of Graduate Studies and Researches - Alexandria University, Alexandria, Egypt). The GC-MS was equipped with a ZB-5MS Zebron capillary column (95% dimethyl polysiloxane + 5% diphenyl, length 30 m × 0.32 mm internal diameters, 0.25 µm film thicknesses). Helium (average velocity 39 cm/s at constant flow rate of 1 mL/min) was used as carrier gas, and the oven temperature was held at 45 °C for 2 min and increased from 45 °C to 165 °C at 4 °C/min, and 165 °C to 280 °C at 15 °C/min and held for 5 min. Samples (2 µl) were injected at 250 °C with a split less setting for 1 min then with split at flow ratio of 1:10.

All mass spectra were recorded using electron impact ionization (EI) at 70 EV with ion source temperature of 220 °C. The mass spectrometer was scanned from *m/z* 40 to 500 (mass ratio) at five scans per second. Peak area percentage was used to obtain quantitative data using the GC with HP-ChemStation software without correction factors according (Elansary and Ashmawy, 2013). Identification of the constituents was performed on the basis of MS library searches (NIST and Wiley) (Davies 1990 and Adams 2001). Retention indices (RIs) were calculated using a generalized equation for all components using a mixture of aliphatic hydrocarbons (C8-C32; Sigma-Aldrich, USA) which were co-injected at the temperature program mentioned above used in GC/MS for the essential oils samples and computer matching with Wiley 7 n.l library.

Statistical analysis

The experimental design used was complete randomized design (CRD) and the data were statistically analyzed as a split split plot method for termites, where the main plot was for source of plant-part extract, sub plot was for extract concentration and sub-sub plot was for day and a factorial arrangement for fungi. The data were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, inc, 1996).

RESULTS AND DISCUSSION

Effect of *Pinus halepensis* essential oils on mortality of termites:

After one day of exposure of termite to the extract, it has been found that the mortality increased with increasing concentration of all oils tested with few differences among the treatments. Mortality virtually nearly complete after 3 days of exposure, indicating that all the extracts were lethal to the workers.

The present study revealed beneficial activities of the tested oils for the management of termites, particularly the volatile oils distilled from the needles of *P. halepensis* (27.2%) and cones (8.13.%). Bioassay results showed a promising termiticidal efficacy of these essential oils on *K. flavicollis* termites based on the recorded cumulative percentage termite mortality. Survival of the dry wood termite *K. flavicollis* fabr. was adversely affected by the *P. halepensis* cones extract with concentrations (100,200 and 400 µm/ml/2g), wood shavings inundation wafers within 1-3 days post treatment, data for termite mortality rates, are listed in Table (1). Due to the no-choice feeding on the test wood wafers or the casuarina chips, in respect steeped separately with 30 min of *P. halepensis* needles and cones essential oil showed 83-100% mortality of termites individuals. Concentration of 400 µm/ml essential oil for both cones and needles showed the best results one day post application and they recorded 100% mortality (Table 5). However concentration of 200 µm/ml of essential oil of cones essential oil was better than the same concentration for needles, the mortality reached 100% one day post application with cones and 100% two days post application with needles essential oil. In both cones

and needles essential oil at concentration 100 µm/ml the mortality of *K. flavicollis* termites recorded 100% three days post application, (Al fazairy *et al*., 1994).

The most reasonable results (ca. 46.9-100% of mortality according to Finney, 1952) in the present bioassay were obtained at 1, 2 and 3 day after treatment in case of the *P. halepensis* needles and cones essential oil.

Table 1: Cumulative percent mortality of *K. flavicollis* termite individuals on casuarina wood chips treated with different concentrations of essential oils from needles and cones of *P. halepensis*

Tree part	Conc (µm/ml)	Mortality % on indicated three days post treatment		
		1	2	3
Needles	100	96.6	96.6	100
	200	86.6	100	-
	400	100	-	-
Cones	100	46.6	83.3	100
	200	100	-	-
	400	100	-	-

Efficacy of methanol extracts against dry-wood termites, *Kalotermes flavicollis*:

The analysis variance revealed that there were significant effects for each of the essential oil from needles and cones of *Pinus halepensis* tree as well as their concentrations and the effect of interaction between them on the survival of the dry-wood termites *K. flavicollis*.

Table (2) showed that the essential oil from the needles using concentration of 100 and 400 µm/ml gave the best results (97.77 and 100%) mortality, respectively, however The essential oil of the cones at concentration of 200 ppm produced the best result (100%) mortality, compared with the control (0.00 %) mortality.

Table (3) showed that the essential oil from the needles at day 1, 2 and 3 induced the highest mortality of termites (94. 44; 98. 88 and 100%) mortality, respectively, compared with the control (0.0% mortality).

The concentration of (400 µm/ml) has brought the highest mortality of termites after one day the (50%) mortality (Table 4); while (200 µm/ml) induced highest mortality of termites two days post treatment (50% mortality). The concentration (100, 200 and 400 µm/ml) recorded after (three days) of the exposure the extracts (50%) mortality (Table 4).

On the other hand the concentration 100 µm/ml of the essential oil from the needles has brought the highest mortality of termites after 1, 2 and 3 days of the treatment (96.66, 96.66 and 100 %) mortality, respectively (Table 5). However, 100 µm/ml of essential oil from the cones gave the highest mortality of termites after 3 days post treatment vs. 0.00 for the control. Concentration 200 µm/ml of essential oil from the cones after one day of the treatment resulted; gave the highest mortality (100%) Whilst, the same concentration of essential oil from the needles and cones after 2 and 3 days produced the highest mortality (100 %).

Table 2: Effect of source and concentration of oil extracted from *Pinus halepensis* on mortality (%) of *K. flavicollis* after 1 day of the treatment.

Concentration (µm/ml)	Mortality (%)				Means (b)
	Extracted oil				
	Control free	Blank	Needles	Cones	
100	0.00	0.00	97.77	73.33	42.77
200	0.00	0.00	95.55	100	48.88
400	0.00	0.00	100	90	47.50
Means a	0.00	0.00	97.77	87.77	
$LSD_{0.05}$ (a)			7.9006		
$LSD_{0.05}$ (b)			4.0798		
$LSD_{0.05}$ (ab)			19.89		

Table 3: Effect of source of oil extracted from *Pinus halepensis* on mortality of *K. flavicollis* after 1, 2 and 3 days of the treatment.

Day	Mortality %				Means c
	Extracted oil				
	Control free	Blank	Needles	Cones	
1	0.00	0.00	94.44	80.00	43.61
2	0.00	0.00	98.88	83.33	45.55
3	0.00	0.00	100.0	100.0	50.00
Means a	0.00	0.00	97.77	87.77	
$LSD_{0.05}$ (a)			7.9006		
$LSD_{0.05}$ (c)			4.229		
$LSD_{0.05}$ (ac)			14.81		

Table 4: Effect of concentrations essential oil of *Pinus halepensis* on mortality of termites after 1, 2 and 3 days.

Day	Mortality %				Means c
	Concentration (µm/ml)				
	100	200	400	100	
1	34.16	46.66	50.00	34.16	43.61
2	44.16	50.00	42.50	44.16	45.55
3	50.00	50.00	50.00	50.00	50.00
Means a	42.77	48.88	47.50	42.77	
LSD (b)			4.0798		
LSD (c)			4.229		
LSD (bc)			25. 817		

After 1, 2 and 3 days of the highest mortality of termites, the percentage of death (100%) was obtained using (400 µm/ml) of essential oils of needles and cones, compared with the control (0.0%) mortality (Table 5).

The obtained results confirmed the termiticidal activity of conifer essential oils, suggested that toxicity of constituents is related to the octopaminergic nervous system of insects. Relatively few studies have been done on insecticidal activity or fumigant toxicity of α-pinene and caryophyllene. Its high toxicity may result from the inhibition of the mitochondrial electron transport system, changes in the concentration of oxygen or carbon dioxide may affect respiration rate of the insect, so that, eliciting fumigant toxicity effects, (Emekci *et al*., 2002, 2004; Qi, 2009 and Ebadollahi, 2013).

Effect of type and concentrations of essential oils on fungi:

The analysis of variance showed that there were significant effects for each of the essential oil from needles and cones of *P. halepensis* tree, their concentrations and the significant interaction between them on the growth of *F. solani*, and *R. solani*.

The essential oil extracted from needles was applied to growth media at 400 and 100 µm/ml induced fungal growth (colony) diameter of (0.00 and 6.80 cm respectively). The essential oil of the cones at 400 and 200 µm/ml gave the lowest growth diameter of (0.00 and 4.13 cm), respectively, vs. the control (Table 6).

All the concentrations of essential oil from the needles and cones ceased fungal growth totally (0.00 cm), compared with the control (8 cm) (Table7).

Table 5: Effect of essential oil source and its concentrations of *Pinus halepensis* extracts on mortality (%) of termites after 1, 2 and 3 days.

Concentration (µm/l)	Day	Mortality (%)			
		Extracts oil			
		Control free	Blank	Needles	Cones
100	1	0.00	0.00	96.66	40
	2	0.00	0.00	96.66	80
	3	0.00	0.00	100	100
200	1	0.00	0.00	86	100
	2	0.00	0.00	100	100
	3	0.00	0.00	100	100
400	1	0.00	0.00	100	100
	2	0.00	0.00	100	100
	3	0.00	0.00	100	100

Table 6: Effect of essential oil source and its concentration of *pinus halepensis against Fusarium solani* growth.

Concentration (µm/ml)	Growth hyphal				Means (b)
	Essential oil				
	Control free	Blank	Needles	Cones	
100	8.00	8.00	6.80	7.00	7.45a
200	8.00	8.00	4.83	4.13	6.24b
400	8.00	8.00	0.00	0.00	4.00c
Means a	8.00a	8.00a	3.87b	3.71b	
$LSD_{0.05}$(a)			0.2262		
$LSD_{0.05}$(b)			0.1959		

Table 7: Effect of essential oil source and its concentration *pinus halepensis against Rhizoctonia solani* growth.

Concentration (µm/ml)	Growth hyphal				Means (b)
	Essential oil				
	Control free	Blank	Needles	Cones	
100	8.00	8.00	0.00	0.00	4.00
200	8.00	8.00	0.00	0.00	4.00
400	8.00	8.00	0.00	0.00	4.00
Means a	8.00	8.00	0.00	0.00	
$LSD_{0.05}$ (a)	0				
$LSD_{0.05}$ (b)	0				

The obtained results confirmed the antifungal activity of Alippo pine essential oils. It is shown that the high level of monoterpene 3-carene, α-pinenes and caryophyllene, which is existed in the resin ducts of conifers of displayed antifungal properties, (Magiatis *et al*., 1999; Hossain *et al*., 2008 and Nikitina *et al*., 2012). The anti-fungal activities of these compounds have been reported by others (Costa *et al*., 2000; Hammer *et al*., 2003; Filipowicz *et al*., 2003; Do *et al*., 2003; Sacchetti *et al*., 2005; Deba *et al*., 2008 and Amri *et al* 2013). Lis-Balchin *et al*. (1998) correlated lack of the antifungal activity of the needle pine oil with the high content of α-pinenes and caryophyllene

Table 8: Essential oils extracted from needles of *P. halepensis*

RT	Compound name	Area %
6.93	3-Carene	7.58
8.25	α-Phellandrene	1.99
8.94	α-Pinene	10.30
9.48	3-Methylene-bicyclo[3.2.1]oct-6-en-8-ol	4.07
10.12	2-Methyl-1-nonene-3-yne	0.75
10.88	1,3,6-Octatriene, 3,7-dimethyl-, (E)-	0.62
11.20	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0.25
12.29	Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl-	3.08
12.74	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	0.37
15.51	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	0.83
15.80	Benzenemethanol, à,à,4-trimethyl-	0.26
16.00	3-Cyclohexene-1-methanol, à,à,4-trimethyl-, (S)-	0.45
19.41	endo-Borneol	0.20
21.61	Copaene	0.23
21.79	6-Octen-1-ol, 3,7-dimethyl-, acetate	0.24
22.50	Naphthalene,1,2,3,4,4a,5,6,8a -octahydro-7-methy l-4-methylene-1-(1-methylethyl)-, (1à,4aá,8aà)-	0.75
22.88	2-Naphthalenol, decahydro-	0.52
24.26	Caryophyllene	40.78
25.14	1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4-methylene-,[1aR-(1aà,4aà,7à,7aá,7bà)]-	9.53
25.74	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1à,4aà,8aà)-	0.16
25.88	1HCyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-,[3aS-(3aà,3bá,4á,7à,7aS*)]-	0.36
26.06	Oxalic acid, pentyl 2-phenylethyl ester	0.27
26.25	Butylphosphonic acid, di(2-phenylethyl) ester	3.48
26.50	Isoledene	0.57
26.72	à-Farnesene	0.16
26.94	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	0.59
27.19	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	0.95
27.74	Di-epi-à-cedrene	0.22
27.93	Cyclohexanemethanol,4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-,[1R-(1à,3à,4á)]-	0.20
28.93	Caryophyllene oxide	1.79
29.36	Guaiol	0.78
29.66	Cyclohexene, 4-methyl-1-(1-methylethyl)-	0.27
30.21	Methyl 5,7-hexadecadiynoate	0.16
31.01	1H-Cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1aà,4à,4aá,7bà)]-	0.27
35.67	1,3,6,10-Cyclotetradecatetraene,3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	1.30
35.80	1,3,6,10-Cyclotetradecatetraene,3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	0.97
35.96	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol,4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]-	0.68
36.79	Thunbergol	4.03

Table 9: Essential oils extracted from cones of *P. halepensi*

RT	Compound name	Area %
6.96	3-Carene	31.78
8. 90	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl)-	11.74
9.46	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	3.01
10.11	α-Phellandrene	0.95
12.27	1,3-Cyclohexadiene, 1,5,5,6-tetramethyl-	0.62
12.74	Terpineol, cis-á-	0.17
15.06	Borneol	0.20
19.41	Bornyl acetate	0.87
21.20	Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-,(3R-trans)-	0.19
22.49	Isoledene	0.47
23.04	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1methylethenyl)-, [1S-(1à,2á,4á)]-	0.15
24.08	Caryophyllene	38.26
25.12	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	0.39
25.85	Naphthalene,decahydro-1,6-bis(methylene)-4-(1-methylethyl)-,(4à,4aà,8aà)-	0.20
26.17	Naphthalene,1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1à,4aá,8aà)-(ñ)-	1.09
26.46	à-Muurolene	0.37
26.90	Ethanone,1-[1-hydroxy-3,3-dimethyl-2-(3-methyl-1,3-butadienyl)cyclopentyl]-, [1à,2à(E)]-	0.46
27.91	Cyclohexanemethanol,4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-,[1R-(1à,3à,4á)]-	0.44
29.34	Guaiol	0.78
31.00	2-Naphthalenemethanol,1,2,3,4,4a,5,6,7-octahydro-à,à,4a,8-tetramethyl-, (2R-cis)-	0.30
35.45	Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol,4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]-	0.66
36.45	Naphthalene,decahydro-1,1,4a-trimethyl-6-methylene-5-(3-methylene-4-pentenyl)-, [4aS-(4aà,5à,8aá)]-	0.35
36.50	17-Norkaur-15-ene, 13-methyl-, (8á,13á)-	0.37
36.76	Thunbergol	0.18
37.03	1,3,6,10-Cyclotetradecatetraene,3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	0.52
37.27	[5,5-Dimethyl-6-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]hept-1-yl]-methanol	0.22
37.45	Podocarp-7-en-3-one, 13á-methyl-13-vinyl-	0.67
37.55	Acetic acid,10-hydroxy-12a-methyl-7-oxo-1,2,3,3a,3b,4,5,7,8,9,10,11,12,12a-tetradecahydro-benzo[c]cyclopenta[h]azulen-1-yl ester	0.71
38.08	Podocarp-7-en-3-one, 13á-methyl-13-vinyl-	0.29
38.19	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,4b,5,9,10,10 a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-, methyl ester, [1R-(1à,4aá,4bà,10aà)]-	1.64
38.64	Retinol	0.89
39.06	Podocarp-7-en-3-one, 13á-methyl-13-vinyl-	1.07

Essential oils analysis

The essential oils from the needles and cones obtained by hydro-distillation were characterized visually by their transparent color and fresh pine odor. Table (8 and 9) shows the variations in the chemical constituents identified in the essential oils from the different parts (needles and cones) of *P. halepensis*. The chemical constituents of the essential oil from needles of *P. halepensis*. Are illustrated in Table (8) showed 37 compounds, of which 91.01% were identified which implied. The major constituents of oil extractives from *P. halepensis* needles were caryophyllene (40.78%), α-pinene) 10.30%) H-cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4-methylene-,[1aR-(1aà,4aà,7à, 7aá, 7bà)] (9.53), 3-carene(7.58%), 3- Methylene-bicyclo[3.2.1]oct-6-en-8-ol(4.07%), thunbergol (4.03%), butylphosphonic acid, di(2-phenylethyl) ester (3.48%), bicyclo [4.1.0]hept-2-ene, 3,7,7-trimethyl (3.08%), à-phellandrene (1.99%), caryophyllene oxide (1.79%) and 1,3,6,10-cyclotetradecatetraene, 3, 7,11-trimethyl-14-(1-methylethyl), [S(E,Z,E,E)] (1.30%).

Table(9) encompasses the chemical constituents of the essential oil from cones of *P. halepensis*. Which implied 32 compounds, compised 99.82% of the total weight compounds. The major constituents of oil extractives from *P. halepensis* cones were caryophyllene (38.26%),3-carene (31.78%), bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl) -(11.74%), 1,4-cyclohexadiene,1-methyl-4-(1-methylethyl)-(3.01%),1-phenanthrenecarboxylicacid,1,2,3,4,4a,4b,5,9,10,10 α-decahydro-1,4α-dimethyl-7-(1-methyl ethyl) -,methyl ester, [1R-(1à,4aá,4bà,10aà)]-(1.64%), naphthalene,1,2,4a, 5,8,8 α-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1à,4aá,8aà)-(ñ)-

(1.09%) and podocarp- 7-en-3-one and 13α-methyl-13-vinyl-(1.07%).

CONCLUSIONS

Upon the result obtained, it can be concluded that the essential oil extracted from needles and cones of *pinus halepensis*, which comprised antifungal and termiticidal compounds has highly potential against dry-wood termites *Kalotermes flavicollis* individuals and pathogenic damping-off fungi (*Fusarium solani* and *Rhizoctonia solani*). Future research should focus on analysis and identification of the active substances of extracted essential oils from *Pinus halepensis* to develop the green pesticides.

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الملخص العربى

التركيب الكيميائي للزيوت الأساسية المستخلصة من شجرة الصنوبر الحلبى وتأثيرها الإبادى للنمل الأبيض والفطريات

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أخذت عينات الأوراق الإبرية والمخاريط من أشجار الصنوبر الحلبى الموجودة في مزرعة مركز البحوث الزراعية– الإسكندرية وذلك في أكتوبر ٢٠١٤. تم استخلاص الزيوت بالتقطير المائي باستخدام جهاز Clevenger-type وفقا للدستور الصيدلي الأوربي(٢٠٠٧) تم تحديد التركيب الكيميائي لتلك الزيوت باستخدام GC-MS. اختبرت فاعلية الزيوت المستخلصة على النمل الأبيض *Kalotermes flavicollis* وذلك في معمل قسم الحشرات الاقتصادية كلية الزراعة– جامعة الإسكندرية. واختبرت فاعلية الزيوت المستخلصة على كل من فطريات الذبول الطري الفيوزاريوم سولانى والريزوكتونيا سولانى في معمل قسم الغابات والأشجار الخشبية–كلية الزراعة– جامعة الإسكندرية. اظهر التركيب الكيميائي للزيوت المستخلصة من الأوراق الإبرية إنها تحتوى ٣٣ مركب كيميائي وكان أهمها:

caryophyllene (40.78%), α-pinene (10.30%) and 3-carene (7.58%)

كما احتوت المخاريط على ٣٢ مركب كيميائي وكان أهمها:

caryophyllene (38.26%); 3-carene (31.78%)

أظهرت نتائج اختبار فاعلية الزيوت على حشرات النمل الأبيض أن الزيوت الأساسية المستخلصة من الأوراق الإبرية والمخاريط كانت فعاله وحققت نسبة موت قدرها ١٠٠% بعد ثلاثة أيام من بداية الاختبار.

كما أظهرت نتائج اختبار فاعلية الزيوت على الفطريات– أظهرت النتائج ان الزيوت الأساسية المستخلصة من الأوراق الإبرية والمخاريط كانت فعاله على الفطر ريزوكتونيا سولانى وثبطت النمو تماما وكانت أقل تأثيرا على فطر الفيوزاريوم سولانى.

أظهرت النتائج التأثير الفعال المبشر للزيوت المستخرجة من أوراق ومخاريط الصنوبر الحلبى لما تحتويه من مركبات مضادة للحشرات والفطريات مما يعد تطبيقا جيدا وآمنا لصحة الأنسان والحيوان والنبات.