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CHEMICAL STUDY AND INSECTICIDAL ACTIVITY OF TWO SPECIES OF MOROCCAN PINUS: *PINUS HALEPENSIS MILL.* AND *PINUS PINASTER SOL.*

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Abstract

This work around the chemical composition and the insecticidal activity of the essential oils of two species of Moroccan pine, *Pinus halepensis Mill.* and *Pinus pinaster Sol*., from the region of Khenifra (Morocco), extracted from the dried aerial parts. The chemical composition of the essential oils extracted by hydrodistillation were studied by gas chromatography coupled with mass spectrometry (CPG and CPG / MS). Seven (7) constituents, representing (93.65%) of the essential oil have been identified for *Pinus halepensis Mill.* and thirteen (13) constituents, representing (94.27%) of the essential oil have been identified for *Pinus pinaster Sol.* The major compounds of *Pinus halepensis Mill.,* is α-pinene with a toner of (88.62%) although the major compounds of *Pinus pinaster Sol.* are: α- pinene (76.91%) Camphene (2.01%) β- pinene (6.35%) Limonene (2.74%) Caryophyllene (2.31%) with a toner of (90.32%). The insecticidal property has been effected against a species of small beetle insects *Bruchus signaticornis*, in other words the lentil beetle. This preliminary study showed that the essential oils of *Pinus halepensis Mill.* and *Pinus pinaster Sol.* have a high toxicity against this insect. The results of the tests showed a very important activity during 24 hours of the treatments confirmed by the values of LD_{50}, this activity was probably due to the main constituents. After contact for ten days at a dose of 2.4×10^{-2} µl / cm^3, *Bruchus signaticornis* is completely destroyed in the presence of the two essential oils from the sixth day of contact, this insecticidal activity of essential oils is probably to the kinetics of evaporation of the constituents.

Keywords: *Pinus halepensis Mill; Pinus pinaster Sol; Essential oil; insecticidal activity; Bruchus signaticornis.*

Introduction

Leguminous crops are primordial in Morocco and are mainly located in regions with favorable rainfall [1]. Over the period 2011-2015, they covered an average of 3% of the agricultural land area, i.e., 385,000 hectares (48% of beans, 19% of chickpeas, 12% of lentils, and 11% of peas). Over the period 2006-2017, the average production is estimated at 2.8 million quintals (i.e., a yield of 7.3 quintals per hectare) [2]. Lentil (*Lens culinaris*), one of the essential food legume crops grown in Morocco, plays an important nutritional role, as it provides essential proteins and calories. Unfortunately, various enemies attack the stored products [3]. These storage enemies fall into three main categories: molds, insects, and rodents (rats and mice). These pests can damage much of the stored product. Some improvements in storage systems can often better protect the stored product and therefore limit losses. There are many methods of protecting local produce in storage [4, 5]. In Morocco, traditional methods, such as using certain natural materials (plants, minerals, oil), are still very effective [6]. They have multiple pharmacological and medicinal properties such as anticonvulsant, antispasmodic, antimicrobial, antiparasitic, antimutagenic, neuroprotective, local anesthetic, analgesic, and anti-inflammatory [7]. The uses in perfumery, cosmetics, or phytosanitary are also traditional by associating the flavors and fragrances to the antibacterial character [8]. Those in the nutrition field or food (ice, flavored sugar, ...) are more recent. The development of aromatherapy leads to an increase in the use of these essential oils as a relaxing agent among others and gives these oils an aspect terroir and return to nature's much-used media [9-10].

Pine is a conifer of the genus Pinus, family Pinaceae. This genus, by far the most important of the conifers, comprises many species, many of which are important tree species. Of the 111 species of pines that exist on Earth, many can live for a very long time. A dozen species can reach 1000 years old. Pines are conifers with needle leaves grouped in bundles of 2, 3 or 5, the fruiting bodies of which are cones made up of scales in which the seeds are found [11-12].

In Morocco, four species and varieties of pine are spontaneous. These are *Pinus halepensis* (Aleppo pine), *Pinus pinaster subsp. hamiltoni var. magrebiana* and *var. Iberica* (maritime pine of the Iberian Peninsula), and *Pinus clusiana var. Mauretania* (black pine of North Africa). The last two species exist in Morocco only in the western Rif. Small stands in the Rif, Middle Atlas, and High Atlas by the other two species. These four species cover about 82 115 ha. On a surface of approximately 250 000 ha are added important artificial afforestation to these natural stands [13].

Pinus halepensis Mill., it is a Tree of height 10-15 m, with rather a tortuous trunk on the coast, straight in the mountains, with unevenly distributed spreading branches. The leaves are 5-10cm x 0,5-1 mm, flexible, glaucous green with acute apex and more or less revolute margins, united in pairs in a common basal sheath with 2-3 fibrous bracts of 5-8mm. The male cones oblong of 5-10mm gathered at the top of branches [14]. Female cones pendulous 7-10 x 4-5cm, pedunculate, solitary or fasciculated by 2-3, ovate-subsonic, persistent for several years, with woody scales with a shiny, shallowly keeled rhomboidal apical scape. Seeds of 5-7mm winged. About 3.5 million hectares in the Mediterranean basin are covered by this conifer [15]. The species extends from 9° West longitude (Morocco) to 36° East longitude (Jordan) and from 45° North latitude in France to 31°30' latitude in Palestine. Its origin in Morocco, Spain, and France is natural. For the reforestation of forests in Morocco, this species is often used. Its distribution is discontinuous from the Mediterranean, in the north, to the southwest of the High Atlas. Thus, it is distributed from zero meters in the north to 2600 meters of altitude, in the center of the High Atlas [16].

It is a medium-sized tree, 15-20m tall, with irregular habit with clear crown, more or less spreading in old subjects, the leaves are 10-20 cm x1,5-2mm, evergreen, rigid, with acute apex, with finely denticulated margins, joined in pairs in a 10-20mm sheathing. The conical yellow male cones are 5-10mm long, agglomerated at the top of the branches. [14]. Female cones light brown shiny 8-10 x 5.5cm, subsessile, solitary or grouped, subconical, with woody scales, rhomboidal apical rind strongly carinated and mucronate. The seeds are 7-8mm with

20-30mm wings. This species of the subhumid and humid Mediterranean bioclimates is also widely used for reforestation in the mountains of Morocco, particularly in the Rif and the Middle Atlas where it represents about 90% of the resinous reforestation [16].

Material and Methods

Plant material

Samples of *Pinus halepensis Mill* and *Pinus pinaster Sol* species were collected during the flowering period and verified by a botanist at the Khenifra Forest Research Center, Morocco.

Extraction of the essential oil (EO):

The samples were collected and dried for ten days in the shade and in the open air. The extraction was carried out by hydrodistillation in a Clevenger type apparatus for three hours, three distillations were carried out for each sample of 1000g with 1L of water in a flask surmounted by a column and a refrigerator. The gasolines less dense than water were collected by simple decantation and dried over anhydrous sodium sulfate (Na_2SO_4) before analysis [17-18]. The yield of essential oil (expressed as a percentage) is calculated as the ratio between the weight of the oil extracted and the weight of the plant material used. The essential oil yield was determined relative to the dry matter, evaluated from 100g oven-dried for 48 h at 60 ° C. The resulting essential oil is stored at a temperature of 4 ° C in the dark [19].

Gas chromatography/mass spectrometry (GC/MS)

Essential oils were analyzed by gas chromatography-mass spectrometry (GC/MS). The coupling was performed on a Hewlett-Packard model 5970 (quadrupole detection system), equipped with a fused silica capillary column of 2mm× 0.23mm type DB1; temperature programming from 50°C to 200°C, with a gradient of 5°C min-1. Retention indices were determined by gas chromatography on two fused silica capillary columns (25 m × 0.25 mm) type OV-101 and Cabowax 20 M, with the same temperature programming as used for coupling (Shimatzu GC-14A instrument equipped with a flame ionization detector and an integrator model C-R4A).

Insecticide tests

Lentil bruchids (*Bruchus signaticornis*) is a species of small chrysomelid beetle insects whose apodous larvae feed on lentil seeds in the pods as they grow. In plastic boxes of one-liter capacity, transparent and screened, the insects were reared on the lentils. The whole set was placed in a chamber with a temperature of 30°C and relative humidity of 70%. In an experimental chamber containing ten insects, essential oils in increasing concentrations of 1.7×10^{-2}; 2.4×10^{-2}; 3.5×10^{-2}; 6.4×10^{-2} and 12×10^{-2} µl /cm^3 respectively on Whatman paper No. 1. The whole set is introduced in a fumigation chamber included in the experimental chamber (semi-aerated). Three repetitions were performed for each concentration. The number of dead insects is recorded after 1 day, subsequently, live insects are placed in 50 g of lentil grains and the count of dead adults is performed after 10 days (at a concentration of 2.4×10^{-2} µl /cm^3). The corrected mortality in treated insects is expressed according to Abbot's formula [20-21].

$$LC = \left(\frac{(L0\text{-}L1)}{(100\text{-}L1)} x100\right)$$

L_C= Mortalité Corrigée. L_0= Mortalité observée chez les insectes. L_1= Mortalité observée chez les témoins.

Results and discussions

Chemical composition

The species used in this study were harvested during their flowering period. The results obtained from the different samples are presented in Table 2.

The average essential oil yields of *Pinus halepensis Mill.* and *Pinus pinaster Sol.* were calculated based on the dry plant matter of the aboveground part of plants. The samples of *Pinus halepensis Mill.* and *Pinus pinaster Sol.* provided a rate of 0.63% and 0.55% respectively. According to Sadou et al. (2015) [22], the yield of essential oils from the needles of *Pinus halepensis Mill.* harvested in the spring at two Algerian sites, the forest of Lake Mellah in El Kala National Park, and the Zaarouria forest in Souk Ahras were respectively of the order of 0.81% and 0.3%. The same species collected in the region of Sidi Fredj in Algeria is of the order of 0.52% [23]. According to Amitouche & Rakem (2017) [24], the essential oil yield of *Pinus pinaster Sol.* of Algeria is around 0.85%.

So we can conclude that the influencing factors on the yield are numerous. This variation is probably due to the sensitivity of the essential oil to biotic and abiotic factors.

The analysis of the results of the chemical composition carried out by gas chromatography coupled with the mass spectrometry of essential oil of *Pinus halepensis Mill.* and *Pinus pinaster Sol.* studied are mentioned in Table 3. The analysis of the results showed the chemical composition of the essential oils of *Pinus halepensis Mill.* and *Pinus pinaster Sol.*, Seven (7) constituents, representing (93.65%) of the essential oil have been identified for *Pinus halepensis Mill.* and thirteen (13) constituents, representing (94.27%) of the essential oil were identified for *Pinus pinaster Sol.* The main essential oil compound of *Pinus halepensis Mill.*, Is α-pinene with a toner of (88.62%) although the main essential oil compounds of *Pinus pinaster Sol.* are: α- pinene (76.91%) Camphene (2.01%) β- pinene (6.35%) Limonene (2.74%) Caryophyllene (2.31%) with a toner of (90.32%) .

The results obtained by Dob et al. (2005) [23], show that the chemical composition of the essential oil of *Pinus halepensis Mill.* was dominated by β-caryophyllene, these results are in agreement with data obtained by Roussis et al. (1995) [25], who found that monoterpene (41.8%) was dominated in Greek pine oils with remarkable differences in the amounts of component over all of the caryophyllene (19.05%).

According to Vidrich et al. (1988) [26], the results obtained reported that β-caryophyllene (26.31%) plays an important role in Italian Pinus oil. Macchioni et al. (2003) [27], found that the main compounds of Aleppo pine needle oil cultivated in Italy were: myrcene (27.9%), α-pinene (18.1%) and β-caryophylene (16.4%), with (73.2%) of monoterpenes and (21.2%) of sesquiterpenes.

The results of the chemical composition of essential oils obtained from the leaves of *Pinus halepensis* collected in the region of Tessimsilt and Djelfa (Algeria) carried out by Tazerouti et al. (1993) [28], show that myrcene (8.65 %) and α-pinene (17.56%), respectively dominated in later sites.

Our results differ from those obtained by Hmamouchi et al. (2001) [29], who studied the oil composition of needles of the same sample species in Morocco, in which α-pinene (23.3%) s 'is found to be dominant and β-karyophylene (14.2%) present. Several reports on the composition of needle oils from other Pinus species revealed that monoterpene hydrocarbons were the main constituents of most of the oil, they often made up 50% or more of the oil [30-33]. The variability can be explained by the differences in environmental conditions: climate, altitude, geographic location, harvest season, the parts of the plant used and the distillation technique or extraction process. It should also be noted that the production of essential and aromatic oils from the plant results from a series of physiological, biochemical, metabolic and genetic regulations.

Insecticidal activities

The results of insecticidal tests of the essential oil of *Pinus halepensis Mill.* and *Pinus pinaster Sol.* against *Bruchus signaticornis* are shown in Tables 4 and 5 respectively as percentages of the means, and in Figure 1. And to clarify the reading, Table 6 shows the results as the 50% lethal doses (LD_{50}). The study of the insecticidal activity of the essential oil of *Pinus halepensis Mill.* and *Pinus pinaster Sol.* confirmed that the insecticidal activity is high and varies widely depending on the dose used. In addition, this activity is probably due to the main constituents. Based on the lethal dose of 50% (LD_{50}), it is concluded that the two essential oils exhibit a significant and remarkable insecticidal activity and effective in more precise conditions. After contact for ten days at a dose of 2.4×10^{-2} µl / cm^3 (Figure 2), bruchus signaticornis is completely destroyed in the presence of the two essential oils Pinus halepensis Mill. and Pinus pinaster Sol. From the sixth day of contact, this insecticidal activity of essential oils is probably due to the kinetics of evaporation of the constituents.

Conclusion

In this work we have studied the chemical composition and the insecticidal activity of the essential oils of *Pinus halepensis Mill.* and *Pinus pinaster Sol.* The characterization obtained by chromatography shows that the constituents of the

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essential oil of *Pinus halepensis Mill.* are weak compared with the essential oil of Pinus pinaster Sol. Seven constituents, representing (93.65%) of the essential oil of *Pinus halepensis Mill*. and thirteen constituents, representing (94.27%) of the essential oil of *Pinus pinaster Sol*. The main essential oil compound of Pinus halepensis Mill., Is α-pinene with a toner of (88.62%) although the main essential oil compounds of Pinus pinaster Sol. are: α- pinene (76.91%) Camphene (2.01%) β- pinene (6.35%) Limonene (2.74%) Caryophyllene (2.31%) with a toner of (90.32%). this difference can be explained by biological and environmental differences such as the age of the plants, the nature of the soil and climate, and the harvest period. The preliminary results of insecticidal activity are very important, and can be relied on in future research as an alternative to syntectic compounds, and to introduce them into insecticidal formulations because they are durable and biodegradable.

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Table 1. Region of harvest of Pinus species.

Botanical name of the plant	Harvest area
Pinus halepensis Mill.	Khenifra (Morocco)
Pinus pinaster Sol.	Khenifra (Morocco)

Table 2. Yield of essential oil of different species studied.

Species	Yield (%)
Pinus halepensis Mill.	0.63
Pinus pinaster Sol.	0.55

Table 3. Chemical composition of the essential oil of *Pinus halepensis Mill.*, and *Pinus pinaster Sol.*

Peak	KI	Component	Pinus halepensis mill.	Pinus pinaster sol.
1	939	α- pinène	88.62	76.91
2	953	Camphène	0.94	2.01
3	980	β- pinène	1.08	6.35
4	991	Myrcène	1.16	0.9
5	1005	α- phéllandrène	0.01	0.17
6	1011	Δ-3-carène	0.42	0.01
7	1018	α- terpinène	-	0.23
8	1031	Limonène	-	2.74
9	1062	δ- terpinène	-	0.11
10	1088	Terpinolène	-	1.08
11	1163	Trans-β-terpineol	-	0.32
12	1189	α- terpineol	1.43	1.14
13	1418	Caryophyllene	-	2.31
Total			**93.65**	**94.27**

- : absence, KI: Kovat Index.

Table 4. Insecticidal activity of the essential oil of *Pinus halepensis Mill.* on *Bruchus signaticornis.*

	witness	1.7×10^{-2} µl/cm^3	2.4×10^{-2} µl/cm^3	3.5×10^{-2} µl/cm^3	6.4×10^{-2} µl/cm^3	12×10^{-2} µl/cm^3
E1	0	10	40	60	80	100
E2	0	20	40	50	80	100
E3	0	20	40	70	90	100
Average	0	16.66	40	60	83.33	100

Table 5. Insecticidal activity of the essential oil *Pinus pinaster Sol.* on *Bruchus signaticornis*.

	witness	1.7×10^{-2} µl/cm^3	2.4×10^{-2} µl/cm^3	3.5×10^{-2} µl/cm^3	6.4×10^{-2} µl/cm^3	12×10^{-2} µl/cm^3
E1	0	20	50	60	90	100
E2	0	20	50	60	80	100
E3	0	20	40	70	90	100
Average	0	20	46.66	63.33	86.66	100

Table 6. LD_{50} of insecticidal activities of *Pinus halepensis Mill.* and *Pinus pinaster Sol.*

Species	DL_{50} µl/cm^3
Pinus halepensis Mill.	0.0258
Pinus pinaster Sol.	0.0242

Figure 1. Insecticidal activity of the essential oils of *Pinus halepensis Mill.* and *Pinus pinaster Sol.* on *Bruchus signaticornis* for a period of one day in different concentrations.

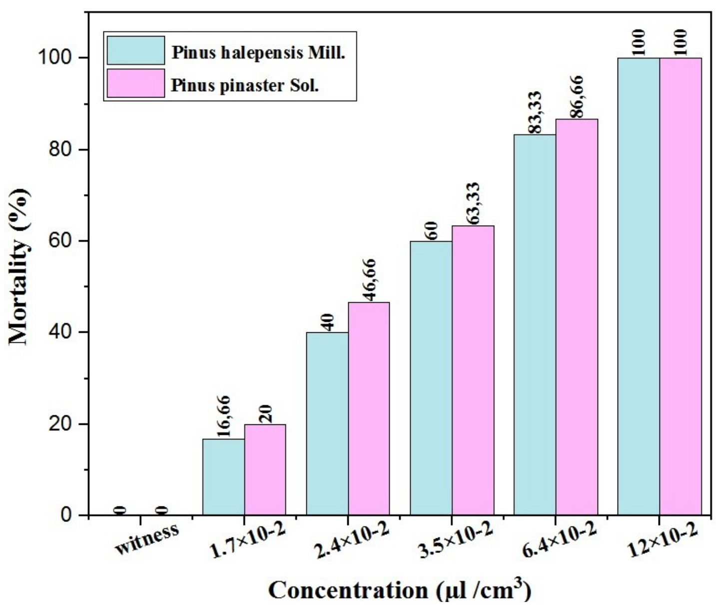

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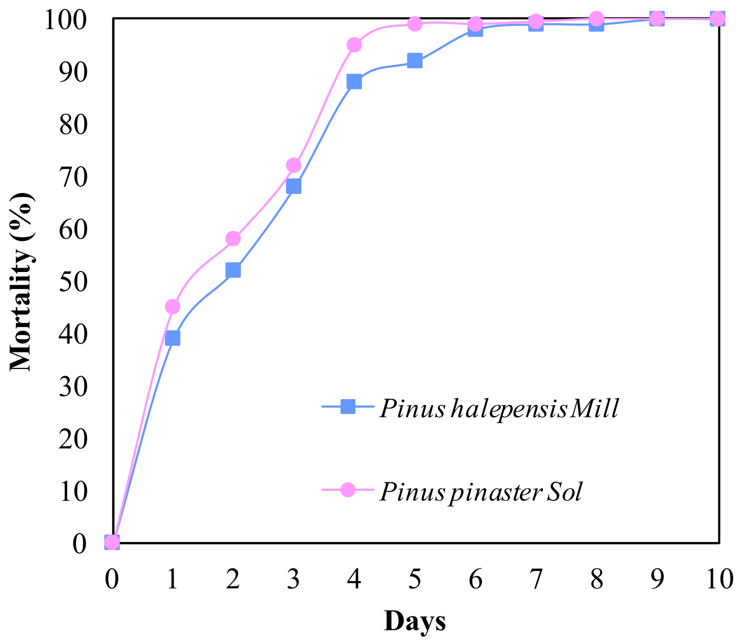
Figure 2. Evaluation of the mortality of essential oils of *Pinus halepensis Mill.* and *Pinus pinaster Sol*. against *Bruchus signaticornis* for a period of ten days at the concentration C = 2.4 × 10^{-2} µl / cm^3.



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