International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 14S, 2025
https://www.theaspd.com/ijes.php

Chemical Structure Of Pinus nigra Cone Essential Oil and its Toxicity Assessment Against the Pest Beetle, Callosobruchus maculatus sp.

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ABSTRACT.
Plant extracts and essential oils were used as bioinsecticides products and are exhibited a toxicity to insect pests with a low effects on non target animals and environment. So these are showing a promising alternative sources, instead of conventional pesticides, for pest and vector insect control agents. With their insecticidal qualities, EOs are produced by a number of plant families. Pines are represented with more than 100 species generally distributed in the Northern hemisphere part and considered as one of the major trees of temperate forests and the pine species, *Pinus nigra* Arn. is a the main tree widely distributed. In Algeria, the unique natural population of the investigated subspecies *Pinus nigra* Arn. sp. *mauritanica* is restricted in the nature reserve of national park of Djurdjura. According to the interest in developing plant bioinsecticides, the present study was carried out in order to make a chemical analysis and to evaluate the adulticidal activities and the repellent effects of the Eo of the pine cones of *P. nigra* sp. *mauretanica* against the stored products adults of Cowpea seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera, Bruchidae). Chemical analyses of the EO extracted from the cones of *Pinus nigra* sp. *mauritanica* revealed the presence of 39 compounds belonging mainly to α-pinene (58.1%), (E)-β-caryophyllene (4.2%), caryophyllene oxide (3.3%), and abiatatriene (2.3%) that were the major compounds in the EOs of *P. nigra* cones. The bioassays showed that the cone of *P. nigra* EO exhibited a toxic effect against the treated beetles. So, this EO will be recommended to be used as an alternative bioinsecticide agents for pest control.
Keywords: Toxicology, plant extract, pests, insect, beetles, Pinus.

1. INTRODUCTION

Insects are the more diverse group of animals on Earth, and only 0.5% are considered pests, they destroy every year one fifth of the world's crop production (Bendjedid et al., 2024). Indeed, Insects in stored can contaminate foods, bran and grains directly via their surfaces and fragments of old cuticle parts and also indirectly by saprophytic microorganisms and all of these factors can affect quality products (Baş & Ersoy, 2020). The need, for the prevention and control of insect pests, is a crucial issue facing crop protection. To date, the most common strategy for controlling these pests has depended on the use of conventional pesticides, most of them are synthetic pesticides (Fernández-Grandon et al., 2020). Vector insects are playing a crucial role in the spread of various pathogens, viruses or bacteria (Amraoui, 2019), that can infect human, animal or plant hosts, thereby contributing to the dissemination of serious diseases. Culicidae and Phlebotominae are among the most distributed insect families found in Algeria (Zeroual et al, 2019; Aroussi et al., 2021). Each year, more than 700,000 deaths worldwide are attributed to vector-borne diseases (WHO, 2017).

Insects, pests or vectors, are commonly controlled by chemical insecticides. Although effective, their repeated use has disrupted natural biological control systems and led to resurgence of these insects, resulted in the development of resistance and had undesirable effects on environment, non-target organisms and human health concern (Jones et al., 2008; Adeleke et al., 2010, Ariyani et al., 2023). The increasing concern; over the level of chemical residues in the different environmental sites and especially in food, has encouraged researches to find new alternatives of conventional pesticides (Boudjelida et al., 2005). These synthetic pesticides are expensive and have in many cases only produced moderate results along with major ecological damage (Dhadialla et al., 2005; Aïssaoui & Boudjelida, 2014, Aïssaoui et al., 2022). Botanical extracts are receiving more attention and become a viable substitute for chemical pesticides in the management of insect pests, since the synthetic pesticides provide undesirable effects to human health, environment and resistance development of new pest genera (Isman, 2020). These components have several advantages over traditional pest control agents; such as specificity, biodegradability and low mammalian toxicity (Bendjedid et al., 2021). Hence, plant extracts and essential oils are used as bioinsecticides that exhibited a toxicity to insect pests with a low effects on non target animals and environment (Ariyani et al., 2023; Wang, et al., 2024). So these are showing a promising alternative sources, instead of conventional pesticides, for pest and vector insect control agents. Also, they are used in the culinary, cosmetic and pharmaceutical industries, because they are non-toxic to vertebrates when present in low quantities (De Sousa et al., 2023) and they present a quick degradation, poor residual capacity and different action mechanisms responsible for these attitudes (Yezli et al., 2024).

With their insecticidal qualities, EOs are produced by a number of plant families, including the Myrtaceae, Lamiaceae, Asteraceae, Apiaceae, and Rutaceae. These indicate their industrial values against a range of harmful insects, including Lepidoptera, Coleoptera, Diptera, Isoptera and Hemiptera (Chintalchere et al, 2020). Many plant species were tested against range of insects, such as the use of species of the genus *Cassia*, as protectants of stored legumes (Kestenholz et al., 2007). It is traditionally used as powder obtained by pounding the dried leaves and mixing with the stored commodity (Babu et al., 1999). The genus *Thymus* as one of the Lamiaceae family genera with the greatest biological interest that grows in the Mediterranean region (Kebi et al., 2020). Among them, *Thymus munbyanus* species which exhibits a variety of biological properties, including insecticidal (Bendjedid, 2021), antioxidant (Sadou et al., 2020) and anti-microbial effects (Tefiani et al., 2015).

Pines are represented with more than 100 species generally distributed in the Northern hemisphere part (Enescu et al., 2016) and considered as one of the major trees of temperate forests (Duquesnoy et al., 2006; Farjon, 2018). The pine species, *Pinus nigra* Arn. (Pinaceae), is a main tree widely distributed in the Balkans and Asia (Enescu et al., 2016) and its largest extents are found in Turkey with 51% of productive forests (Atalay & Efe, 2012). Therefore, its fragmented reduced areas, in south Mediterranean countries, such as Algeria, have led to morphological variations (Rubio-Moraga et al., 2012), which became genetically distinguished after warm periods, due to the climate changes (Afzal-Rafii & Dodd, 2007). In Algeria, the unique natural population of the investigated subspecies *Pinus nigra* Arn. sp. *mauretanica* is restricted in the nature reserve of national park (Quezel & Medail, 2003) and other introduced species (Treep, 1976). It is not well mentioned that the intraspecies taxonomy, classification and chorology of this species are still unclear (Liber et al., 2003). The most widespread species, Aleppo pine with more than 35% of the forest area, the biological activities of its EO have been tested (Abi-Ayad et al., 2011; Fekih et al., 2014; Sadou et al., 2015; Djerrad et al., 2017; Ramdani et al., 2020). The resin of this tree is used for treating asthma, in traditional medicine (Meddour and Meddour-Sahar, 2015) and some kidney diseases (Berroukche et al., 2014).

According to the interest in developing plant bioinsecticides, the present study was carried out in order to make a chemical analysis and to evaluate the adulticidal activities and the repellent effects of the EO of the pine cones of *P. nigra* sp. *mauretanica* against the stored products adults of Cowpea Weevil, *Callosobruchus maculatus* (F.) (Coleoptera, Bruchidae). This species of beetles known commonly as the cowpea weevil or cowpea seed beetle (Tran & Credland,1995; Gupta et al., 2023). It is a member of the leaf beetle family, Chrysomelidae, and not a true weevil. It is often mistaken for *Callosobruchus chinensis*, another bean beetle species with a similar lifestyle. This common pest of stored legumes has a cosmopolitan distribution, occurring in all continent except Antarctica. The beetle most likely originated in West Africa and moved around the globe with the trade of legumes and other crops (Tran & Credland,1995).

2. MATERIALS AND METHODS

2.1. Plant material and essential oil extraction:

The mature cones of *Pinus nigra* sp. *mauretanica* were collected from the Tigounatine forest (Figure 1), in the National Park of Djurdjura at altitude of 1521m (latitude 36° 27' 09" N, longitude 004° 06' 26" E), northern Algeria, in order to complete the previous work done on fresh needles and twigs of the pine (Adjaoud et al., 2020). Then, the plant pine cones were dried for about ten days, in a ventilated area at room temperature. Finally, roughly cut for the oil extraction operation. Hydrodistillation was carried out using a Clevenger-type apparatus, where 300 g of plant material are introduced with a sufficient quantity of distilled water into a 2 L flask. The total extraction time was estimated at 3 h. The obtained distilled product was then dried with anhydrous sodium sulfate (Na_2SO_4) and recovered and stored in brown bottles, hermetically sealed and stored in a refrigerator (4°C) far away from light. EO yields were estimated on a dry weight (w/w).

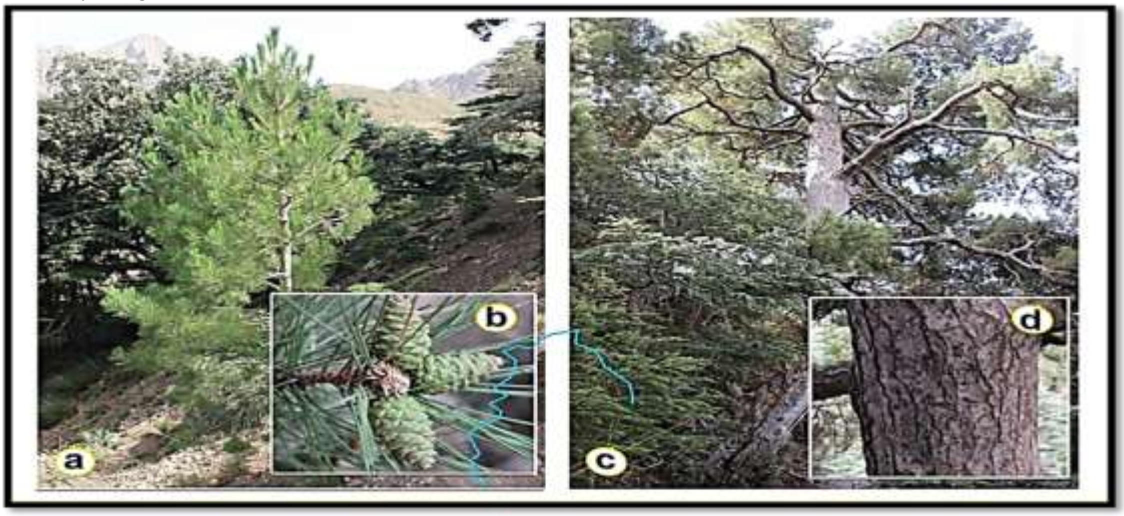

Figure 1. *Pinus nigra* sp. *mauretanica*. (a) Young tree, (b) immature female cones, (c) Old tree, (d) and trunk detail. (Personal Photo, 2020).

2.2. Gas chromatography - mass spectrometry (GC-MS):

GC analyses were performed on a Perkin Elmer Clarus 500 gas chromatograph equipped with a flame ionization detector (FID) and two fused silica gel capillary columns: BP-1 (polydimethylsiloxane, 50m x 0.22mm i.d., film thickness: 0.25 µm) and BP-20 (polyethylene glycol, 50 m x 0.22 mm i.d.). The oven temperature was programmed from 60 to 220 °C at a rate of 2 °C/min, then held isothermally for 20 minutes at 220 °C, with an injector temperature of 250 °C, a detector temperature of 250 °C, a carrier gas: hydrogen (0.8 mL/min), and a split ratio of 1:60. The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalization, without using correction factors. The RIs were determined relative to the retention times of a series of n-alkanes with linear interpolation ("Target Compounds" software, Perkin Elmer).

The EOs were analyzed using a Perkin Elmer TurboMass quadrupole detector directly coupled to a Perkin Elmer Autosystem XL, equipped with a fused silica gel capillary column (BP-1 polydimethylsiloxane, 50 m, 0.22 mm id, 0.25 µm film thickness). Carrier gas: helium (at 1.0 ml/min); split ratio: 1:80; injection volume: 0.2 µl; injector temperature: 250 °C; oven temperature programmed from 60 to 230 °C at a rate of 2 °C/min, then held isothermally for 20 minutes at 220 °C; ion source temperature: 250 °C; ionization energy of 70 eV.

^{13}C-NMR spectra were recorded on a Bruker AVANCE 400 Fourier transform spectrometer operating at 100.63 MHz for 13C, equipped with a 5 mm probe, in $CDCl_3$, with all shifts referenced to the internal standard TMS. The following parameters were used: pulse width = 4 µs (flip angle 45°); acquisition time = 2.7 s for a 128 K data table with a spectral width of 25000 Hz (250 ppm); decoupling in CPD mode; numerical resolution = 0.183 Hz/pt. The number of accumulated scans was 3000 for each sample (40 mg of essential oil in 0.5 mL of $CDCl_3$).

The identification of individual components was performed by: (i) Comparison of their GC retention indices (RI) on polar and non-polar columns with those of reference compounds compiled in a laboratory-built library and with literature data (Konig et al., 2001); computer comparison with commercial mass spectral libraries (NIST, 1999; Konig et al., 2001; Adams, 2007) and comparison of signals in the 13C-NMR spectra of the samples with those of reference spectra compiled in the laboratory spectral library, using laboratory-built software (Tomi et al., 1995; Tomi & Casanova, 2006; Bazzali et al., 2016).

2.3. Toxicity evaluation of *Pinus nigra* EO:

2.3.1. *Insects collection and rearing*: *Callosobruchus maculatus* (F.) was collected from a cultivated variety of cowpea (*Vigna unguiculata* (L.) Walp.) in farmers' storage granaries in selected locations in Bejaia. Cowpea beetles were reared in the laboratory on healthy cowpea seeds in an incubator set at 30 ± 1°C, a 12-hour photoperiod (technical modification by adding a lamp) and a relative humidity of 75% (a hygrometer was placed inside the room). The effectiveness insecticidal essays of *P. nigra* EO were conducted on non-sexual adults of beetles. Three additional tests on the insecticidal efficacy of *P. nigra* EO in controlling stored-product pests were conducted under laboratory conditions. These tests were: contact toxicity on seeds, fumigation (inhalation) and repellency.

-***Contact bioassay***: The test was conducted according to the method described previously (Tapondjou et al., 2003), using replications of twenty adult insects to assess the effect of EOs on beetle mortality. After a preliminary screening of the EO toxicity, the different concentrations were prepared, using an appropriate quantity of the EO was diluted in defined acetone quantity to obtain each test concentration. 1 ml aliquot of the test solution containing 2, 4, 8, 16, and 0 (control) µl/ml was poured onto a Petri dish (9 cm in diameter) containing 40 g of sterilized cowpea seeds (heated to 60°C for 2 hours). The treated Petri dishes were kept in an incubator at 30 ± 1°C and 75% RH (relative humidity). The mortality rate was checked daily for 4 days. 4 replicates were set up for each EO concentration and for the control. The insecticidal activity of the oil was expressed as the percentage of mean mortality.

-***Inhalation Test***: The inhalation assay of *P. nigra* EO, for each test 20 adults *C. maculatus*, were placed in 200 ml glass jars containing a cotton imbibed with the used EO concentrations and four replicates were used for each test. Each dose of the EO were applied to a piece of cotton which is attached to the underside of the jar lid (Toudert-Taleb et al., 2014). The EO used concentrations were 2, 4, 8, 16, and 0 (control) µl/ml. The glass jars were maintained at 30 ± 1 °C and 75% RH. The insect mortality was recorded at 24, 48, 72 and 96 h after treatment.

- ***Repellency Test***: Repellency was tested using the method described by McDonald et al. (1970). Filter paper discs (Whatman No. 1, 11 cm diameter) were cut into two equal parts. One half of the disc was uniformly treated with 0.5 ml aliquots of EO solutions in acetone at doses of 2, 4, 8, and 16 µl/ml. The second half of the filter paper was treated with acetone as a control and all half-discs were air-dried for 15 minutes. Twenty non-sexual adults of *C. maculatus* were introduced into the center of each Petri dish, which was sealed with parafilm. Four replicates were performed for each of the tested concentrations. After 1 hour, the beetles present on both half-filters were counted. The repellency percentage (RP) values were calculated as follows: $\mathbf{RP(\%)} = \frac{\mathbf{Nc-Nt}}{\mathbf{Nc+Nt}}\ \mathbf{x\ 100}$

Nc is the number of insects on the negative control half-filter paper (with 0.5 ml of acetone) and Nt is that of the oppositely treated half-paper (with 0.5 ml of EO solutions in acetone). The average repellency value was calculated and assigned to repellency classes from 0 to V according to (McDonald et al., 1970): class 0 (PR = 0.1%), class I (PR = 0.1- 20%), class II (RP = 20.1- 40%), class III (RP = 40.1- 60%), class IV (RP = 60.1- 80%) and class V (RP = 80.1-100%).

2.3.2. Staistical analyzes: Mortalities of these treated insects, were recorded during different accorded periods for each test. The observed mortalities were corrected according to the formula of Abbott (1925). Since the mortality is lower than 4% in control series, the observed mortalities were considered and submitted to statistic and probit analyzes, where the lethal concentrations (LC_{10}, LC_{25}, LC_{50} & LC_{90}) and their confidence limits (95 % CL) were calculated with GRAPH PAD PRISM 6 software. All data was subjected to analysis of variance (ANOVA), and means were compared by least significant difference (LSD) using statistical software SPSS (version 27). Differences at $p < 0.05$ were considered to be significant**.**

3. RESULTS

Chemical analyses of the EO extracted from the cones of *Pinus nigra* sp *mauretanica* revealed the presence of 39 compounds with a content more than or equal to 0.01% (Table 1). In general and excluding unidentified compounds, chemical composition analysis showed that α-pinene (58.1%), (E)-β-caryophyllene (4.2%), caryophyllene oxide (3.3%), and abiatatriene (2.3%) were the major compounds in the EOs of *P. nigra* cones (Figure 1). It was noted the presence of various minor components; β-pinene, α-campholenal, trans-pinocarveol, trans-verbenol, cis-verbenol, pinocarvone, borneol, terpinen-4-ol, myrtenal, α-terpineol and myrtenol. These 39 compounds had a cumulative area corresponding to 84.9% of the cumulative areas of all the constituents of the EO. This was composed entirely of terpene derivatives. Nine (09) monoterpene hydrocarbons (63.2%) were identified, with the most important component was α-pinene (58.1%). While the oxygenates ones (alcohols, aldehydes, ketones and esters) were represented with 4.9 % of all the constituents of the EO. Hydrocarbon sesquiterpenes constituted 5.7% of the EO; the most important was (E)-β-caryophyllene (5.7%) and the oxygenates were represented by the epoxide functional group hold a combined value of 4.2 %. Among the diterpenes, two (02) hydrocarbons (4%) were identified, the abiatatriene (2.3%) and abiatadiene (1.7%). The oxygenated fraction of this class (2.6%) was dominated by aldehydes, which the most important compound was dehydro abietal (1.3%) (Figure 2).

Table 1: Chemical composition of the EO extracted from the cones of *Pinus nigra* sp. *mauretanica*. Chemical group: MH = Monoterpenes Hydrocarbons; MO = Monoterpenes Oxygenated; SH = Sesquiterpenes Hydrocarbons; SO = Sesquiterpene Oxygenated; DH = Diterpenes Hydrocarbons; DO = Diterpenes Oxygenated.

N°	Components	Chemical group	(%)	N°	Components	Chemical Family	(%)
01	Tricyclene	MH	0.1	21	□-Terpineol	MO	0.2
02	□-Pinene	MH	**58.1**	22	Myrtenol	MO	0.3
03	Camphene	MH	1.3	23	Verbenone	MO	0.3
04	Thuja-2,4(10)-diene	MH	0.7	24	*trans*-Carveol	MO	0.1
05	□-Pinene	MH	tr	25	Bornyl acetate	MO	0.3
06	Myrcene	MH	1.5	26	□-copaene	SH	0.2
07	*p*-Cymene	MH	0.3	27	(*E*)-*β*-Caryophyllene	SH	**4.2**
08	Limonene*	MH	1.1	28	(*E*)-□-Farnesene	SH	0.4
09	□-Phellandrene*	MH	0.1	29	□-Humulene	SH	0.7
10	*p*-Cymenene	MH	0.2	30	4-épi-cubebol	SO	0.1
11	□-Campholenal	MO	0.8	31	□-Cadinene	SH	0.2
12	*trans*-Pinocarveol	MO	1.0	32	Caryophyllene oxide	SO	**3.3**
13	*trans*-Verbenol	MO	0.6	33	Humulene epoxide II	SO	0.5
14	*cis*-verbenol	MO	0.3	34	Manoyl oxyde	SO	0.3
15	Pinocarvone	MO	0.2	35	Abietatriene	DH	**2.3**
16	*p*-Mentha-1,5-dien-8-ol	MO	0.4	36	Abietadiene	DH	1.7
17	Borneol	MO	0.1	37	Labda-8(17)-13(Z)-Dien-15-al	DO	0.6
18	Terpinen-4-ol	MO	tr	38	Labda-8(17)-13(E)-Dien-15-al	DO	0.7
19	*p*-Cymen-8-ol	MO	0.1	39	Dehydroabietal	DO	1.7
20	Myrtenal	MO	0.5				

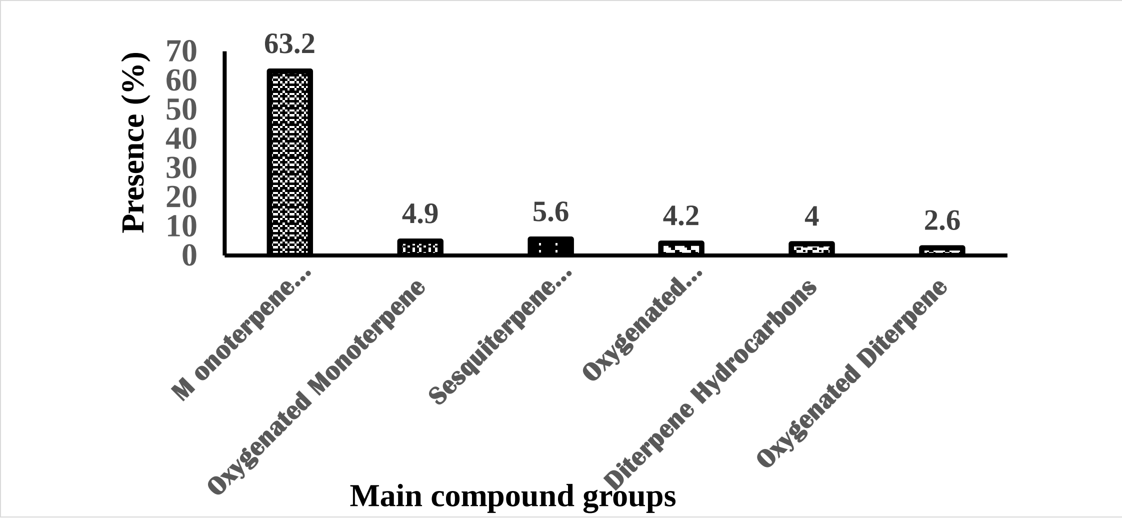

Figure 2. The main compound groups of the chemical structure of the EO extracted from the cones of *Pinus nigra* sp *mauretanica*.

Adulticidal bioassay of *P. nigra* EO against *C. maculatus*: The results of the contact effect of the *P. nigra* EO against beetles of *Callosobruchus maculatus*, species, by the treatment of the cowpea seeds revealed that the corrected mortality values increased with a concentrations response relationship (Figure 3). The accumulated mortality, observed during the contact test period of 4 days varies from 34 % for the 2 µl/ml reached 71 % for the highest concentration of 16 µl/ml area. Therefore, the recorded mortality of the inhalation test was 25 % for the lowest concentration and reached the 93 % for the highest concentration of 16 µl/ml (Figure 3). The results of the statistical analyzes reveal a highly significant effect between concentrations ($p < 0.001$). Also, the statistical analysis for the comparison of the mortalities of the two used tests, showed a significant effect between the different tests.

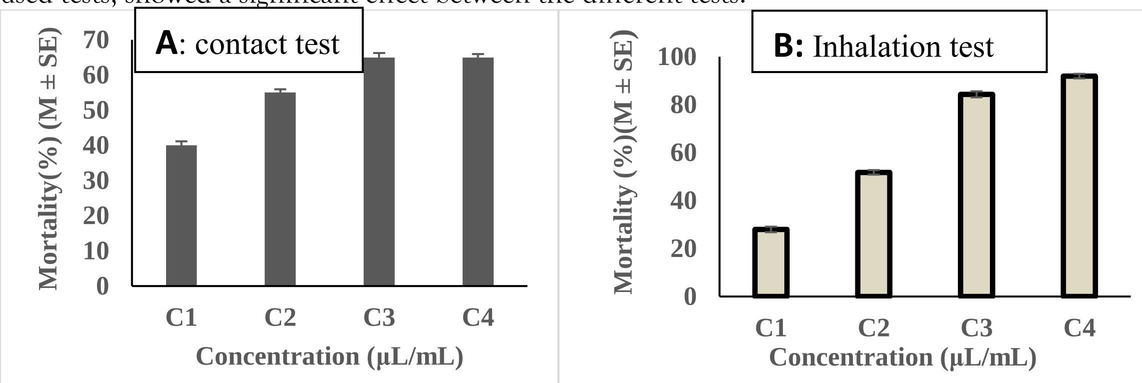

Figure 3. Toxic effect of *P. nigra* EO against *C. maculatus* adults treated at different concentrations (µl/ml). **A**: contact test. **B:** Inhalation test. Corrected Mortality (%): values are significantly different by LSD test at $P < 0.001$.

The regression curves were determined, after the transformation of the observed mortality of *C. maculatus* adults, to probit and the tested concentrations, of cone *P. nigra* EO, in to decimal logarithms (Figure 4). The coefficient of determination ($R^2 = 0.92$ & 0.76) reveals an equal positive relation between the probit of the mortality and the decimal logarithms of the tested concentrations (Figure 4). The curve showed a concentration response relationship, since the mortality increased with the increase of the concentrations.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 14S, 2025
https://www.theaspd.com/ijes.php

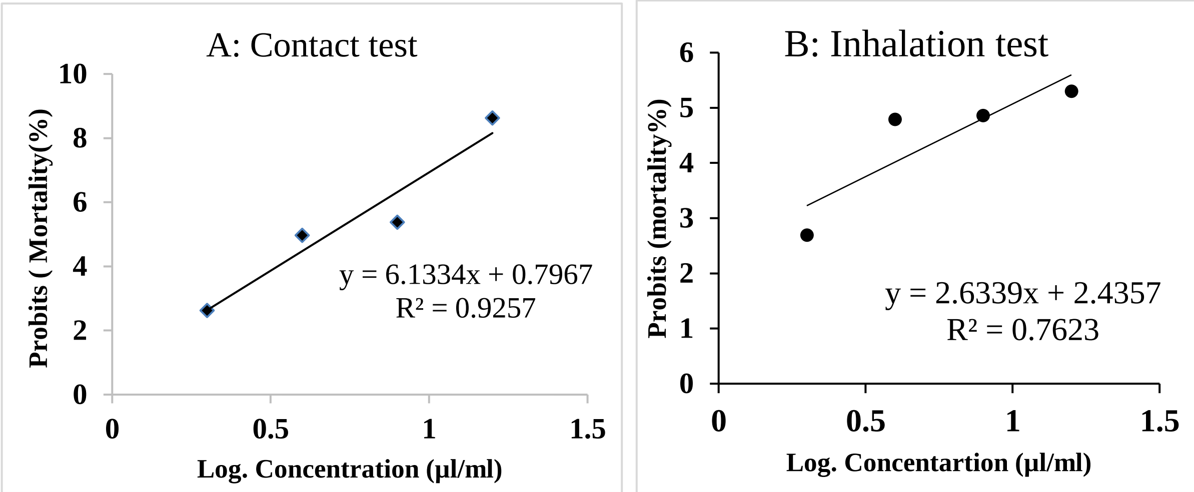

Figure 4. Concentration response relationship of ***P. nigra*** **EO against *Callosobruchus maculatus*** adults treated at different concentrations (µl/ml). **A:** contact test. **B:** Inhalation test. (R^2= coefficient of determination).

The lethal concentrations (LC), for both tests, were estimated from the linear regression (Figure 3), at the 95% confidence limits (LLC=Lower LC- UCL=Upper LC) (Table 2). For the concentrations tested, the highest effect activity was observed with the highest one of 16µl/ml the 2 used tests, with the LC_{50} and LC_{90} values of 5.330 & 19.491 µl/ml for the contact test and 4.39 & 23.01µg/ml for inhalation test (Table 2). The slope values were also calculated and presented in table 1. The use *P. nigra* cone EO against *Callosobruchus maculatus* showed a concentration-response relationship mortality. At higher concentrations, the adult showed restless movement, without mobility, for some times with abnormal wagging and then died.

Table 2. Toxicity analysis of *P. nigra* EO applied by contact and inhalation against adult of *Callosobruchus maculatus* beetle. Determination of lethal concentrations (LC µl/ml) and their 95% Fiducial limits (LCL: Lower LC& UCL: upper LC).

Lethal Concentrations	LCL >LC> UCL) (µL/mL) Fiducial limits (95%)	
	Contact test.	*Inhalation test*
LC_{10}	0.029>0.299 >3.025	1.541>0.003 >0.689
LC_{25}	0.354>1.263>4.490	0.019>0.216>2.406
LC_{50}	3.035>5.330 >9.359	3.217>4.39 >13.31
LC_{90}	9.992>19.491>43.901	18.41>23.01>38.01
Slope	**0.763** (0.55-0.91)	**0.261** (0.075-0.447)

***Repellency Test*:** The bioassay for the repellency effect the same different concentrations of the cone *P. nigra* EO were tested against adult of *C. maculatus* beetle. The results revealed a different repellency percentage (RP = Mean ± SD), varied between 40 % and 65 % These were classed, according to McDonald et al. (1970) (Table 3). The two first concentrations (2 & 4 µl/ml) were in classes III and the remain ones (8 &16 µl/ml) were in class IV. The average of repellency of the all test is represented by 65 % and this suggest a that, the cone *P. nigra* EO have exhibited Moderately repellent effect towards adult of *C. maculatus* beetle (Table 3).

Table 3. Repellency effect of the *P. nigra* EO cone against adult of *C. maculatus* beetle. The repellency (RP= Mean ± SD) were classed according to Mc Donald et al. (1970).

Concentration (µl/ml)	Repellency (%)	Mc Donald's Class
2	40^{b} ± 1.15	class III
4	55^{ab} ± 0.95	class III

8	65[a] ± 1.25	class IV
16	65[a] ± 0.95	**class IV**
Test Mean	**56 %**	**class III: Moderately repellent**

4. DISCUSSION

4.1. Essential Oil Extract:

Due to its restricted distribution, ecological status and applied conservation strategies, research has been conducted on *P. nigra* sp. *mauretanica*, from Djurdjura, Algeria. This study described the presence and chemical composition of the EO of this species and to valorize their bioactivities against insects. The results showed that the yield of EOs varied depending on plant parts (Adjaoud, 2020). In total 39 chemical compounds, entirely of terpene derivatives were identified from *P. nigra* sp. *mauretanica* cone EOs. These were represented mainly by monoterpene hydrocarbons with the most important component was α-pinene (58.1%). These were followed by the Hydrocarbon sesquiterpenes which constituted 5.7% of the EO. Comparatively a total of 99 chemical compounds were identified from needles and twigs of *P. nigra sp*. mauritanica. 83 components representing 97% of the total EO, were identified in twigs while needles contained 93 components, accounting for 98% (Adjaoud, 2022). Indeed, a certain number of common constituents are found almost constantly in the EOs of the cones of the different subspecies of *P. nigra*. These are α-pinene, sabinene, β-pinene, β-caryophyllene, caryophyllene oxide, which are widely described in the literature (Bader et al., 2000; Macchioni et al., 2003; Tumen et al., 2010; Amri et al., 2022), but each essential oil is characterized by a specific constituent, often largely in the majority. On the other hand, other compounds such as β-phellandrene, limonene, bornyl acetate, germacrene D and abietadiene are identified either as minority compounds or found in traces in the cones, or are associated with other organs or other species of the genus *Pinus* (Yang et al., 2010b; Nam et al., 2014; Lis et al., 2019; Khouja et al., 2020; Khedhri et al., 2022; Amri et al., 2022).

Several authors reported that □-pinene, germacrene D and □-pinene were the dominant components present in EOs of Black pines (Kubeczka & Schultze 1987; Roussis et al. 1995; Ioannou et al. 2014, Adjaoud, 2020). In most cases, all patterns are built on □-pinene as a major compound where content rate reached the threshold of 60% in *P. nigra* var. *banatica* from Serbia (Sarac et al. 2014). The composition of the oil extracted from *P. nigra* cones, from samples collected at Italian sites, showed limited qualitative similarities with some quantitative differences (Vidrich et al. 1996). The predominant constituents of these oil were mainly α-pinene (68.6%) and β-caryophyllene (9.1%) (Bader et al., 2000). Other study showed from the same place (Macchionin et al., 2002) high content of α-pinene, with the presence of sandaracopimaradiene and the absence of (E)-β-caryophyllene, caryophyllene oxide and abiatatriene. The present work made the difference, which can be related to the pedoclimatic conditions of the two countries, the extraction conditions, the collection period and the stage of development of the plant. Similarly, in Turkey, the EO of *P. nigra* cones (Tumen et al., 2010) presented a chemical composition very close to that identified in the present work, with the exception of 18-norabieta-8, 11,13-triene, which was not identified. Moreover, the EO extracted from the cones collected at Tunisia (Amri et al., 2022), showed a difference in chemical composition than that obtained in this present analysis.

According to the results of the chemical composition analyzes of the of the Eos of the different species, it can be said that each species presented a very specific composition and chemotype. The majority of these studies confirmed the richness of the EOs of these species in α-pinene. The variation in the chemical composition observed between the EOs of the *P. nigra sp.* is probably linked to the differential regulation of the various constituents of the essential oil by abiotic and biotic factors, also many ecological factors such as temperature, relative humidity, sunlight and soil.

4.2. Toxicity Bioassay:

Among the widely used products in control pests are chemical neurotoxic synthetic pestticides (WHO, 2017). However, the extensive use of these chemicals in urban and semi urban environments, has resulted in the development of insect resistance to these insecticides. These have encouraged searchers to propose new biopesticides, such as plant extracts and their essential oils. Generally, the active toxic ingredients of plant are secondary metabolites that are evolved to protect them from herbivores. The insects feed on these secondary metabolites potentially encountering toxic substances with relatively non-specific effects on a wide range of molecular targets. These targets range from proteins (enzymes, receptors, signaling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other cellular components (Rattan, 2010). This in turn, affects insect physiology in many different ways and at various receptor sites, the principal of which is abnormality in the nervous system.

The present study indicated that the tested *P. nigra* EO cone exhibit a toxic effects with a concentrations-response relationship mortality; against the adult of *C. maculatus* beetle pest. The toxicity of the *P. nigra* EO cone, expressed by the mortality, increased significantly following the increase in time of exposure and the concentrations. It is documented that some Eo plants, such as *Chenopodium ambrosioides* EO, have an insecticidal effect on *C. maculatus* and other stored grain pests (Tapondjou et al., 2003; Duquesnoy et al., 2006; Chauhan et al., 2022). Also, *Eucalyptus saligna, Cupressus sempervirens* EOs used against *S. zeamais* and *Tribolium confusum* (Tapondjou et al., 2005; Boukraa et al., 2020) and The *Callistemon viminalis* (Myrtaceae) EO, against *A. obtecus,* have shown a high insecticidal effect (Ndomo et al., 2009). Similar result, on biological activities, were reported when the conifer EO, from Algeria and Tunisia, was used against *C. maculatus*. This affected very significantly the longevity, fertility and emergence of the species (Kassemi, 2014; Hedjal-Chehheb et al., 2013; Taleb-Toudert, 2015). Toxicological study using the *Pseudocytisus integrifolius* and *Nepeta nepetella* EOs against five stored food pests (*Callosobruchus maculatus, Acanthoscelides obtectus, Sitophilus granarius, Rhyzopertha dominica* and *Tribolium castaneum*) to assess some biological parameters such as mortality, fecundity and adult emergence. *N. nepetella* EOs exerted more or less significant toxicity towards these insects. These EOs reduced female egg-laying and adult emergence of all pests. In addition, a larvicidal effect was observed on *T. castanum*, and an adulticidal effect on the other four insects (Taleb-Toudert, 2015). Furthermore, the EOs of *Xylopia aethiopica*, rich in hydrocarbon monoterpenes and sesquiterpenes, caused high mortality in the bruchidea beetle, *C. maculatus* (Thiam et al., 2021). Previous work reported a strong biocidal activity of EOs from *Origanum campactum* and *Rosmarinus officinalis* EOs, because of these EOs, are containing mainly carvacrol and 1,8-cineole (Baghouz et al., 2022). Studies, have also highlighted the inhalation efficacy of essential oils from the needles of *Pinus caribaea var. hondurensis* (Kirima & Omara, 2022). Recent studies have highlighted the efficacy of EOs from several plants against *C. maculatus* adults. EOs from *Juniperus oxycedrus* and *Pelargonium graveolens*, containing mainly α-pinene and citronellol/geraniol respectively, demonstrated high toxicity. However, *Artemisia herba-alba* EO, rich in chrysanthenone and champhor, was less active (Kherroubi et al., 2021). Therefore, the degree of the effectiveness depends on the part of the used plant, which is generally attributed to their chemical composition. *The P. nigra* EO is particularly rich in terpene compounds. Hence its biocidal activity could be linked this group and potentially influenced by the other chemical functional groups present in the EO (Ngamo et al., 2007; Nyamador, 2009). These oils are reported to be able to penetrate rapidly the insect and disrupt its physiological functions (Abdelgaleil et al., 2009; Abd-Elhady, 2012). In addition, several other factors can influence the biocidal efficacy of essential oils, including exposure time, dose, and synergistic and antagonistic interactions between components of an oil. Therefore, a deeper understanding of these factors is essential to optimize the use of essential oils as biopesticides.

In Algeria, pests and vector insects control programs are based mainly on chemical pesticide. From the present study, it was concluded that the cone of *P. nigra* EO is recommended to be used as an alternative bioinsecticide agents for pest control, since are showing a significant toxic effects.

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