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Chemical Composition and Insecticidal Potential of *Pulicaria incisa* (Lam) Essential Oil from Moroccan Plant Against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst.)

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Abstract: *Pulicaria incisa* (Lam) is a wild-growing plant in Morocco and has been traditionally used by farmers to control pests of stored grains. The present study was conducted to investigate the chemical composition and insecticidal effect of the essential oil of *P. incisa* against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst.) by different methods (contact, fumigation and ingestion). The aerial parts of the plant were subjected to hydrodistillation using a Clevenger-type apparatus. The essential oil composition was analyzed by gas chromatography (GC) and mass spectrophotometry (MS). Sixty-six compounds representing 89.4% of total oil were identified. The main components were α-Ocimene (15.17%), τ-Cadinol (6.79%), α-Cadinol (4.51%), Alloaromadendrene (4.45%) δ-Cadinene, (+) - (4.13%). The repellant toxicity test results revealed a higher repellency effect in *S. oryzae* than *T. castaneum*. Lethal concentration (LC50), varied between 15.49 - 1.73 µL/cm^2 and 20.89 - 2.29 µL/cm^2 respectively. In the fumigation test, adults of *S. oryzae* and *T. castaneum* were sensitive to the essential oil with LC50 values varying between 16.21 - 2.08 and 18.62 - 2.51 µL/L air. In addition, experiments have shown that the ingestion method is the most toxic towards both insects with LC50 values of the order of 12.59 - 1.51 µL/g for *S. oryzae* and 14.12 - 2.39 µL/g for *T. castaneum*. While the lethal time (LT50) values decreased with increasing essential oil concentration, and in all cases, the increased susceptibility of both insects was directly associated with oil concentration and exposure time. This study aims to valorize medicinal and aromatic plants of the Moroccan flora in order to find novel bio-insecticidal products. Furthermore, the study reports for the first time the insecticidal activity of *P. incisa* against adults *S. oryzae* and *T. castaneum*.

Keywords: essential oil; LC_{50}; LT_{50}; toxicity; insecticidal potential.

1. Introduction

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and the rust-red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), are among the most widespread and destructive stored product pests throughout the world. These two species can cause the total loss of an entire stock [1,2]. With over 60 percent of the African continent's population in rural areas and dependent on smallholder or family farming, the risk from the COVID-19 pandemic to food supply chains, market access, and nutrition is high [3]. In Morocco, the regular supply of the local market with cereals, particularly soft wheat, is a strategic issue for the country's food security. Therefore, the country to prioritize the reduction of post-harvest losses through the launch of a project in partnership with FAO, which aims to identify post-harvest losses and wastage of major agricultural products, to understand the underlying causes and factors better and guide to develop a national strategy and action plan to reduce these losses.

In the past two decades, the control of losses attributed to insects in stored food products relies heavily on the use of synthetic insecticides [4]. But some of them are banned, like phosphine (synthetic fumigant), which has been the method the most widespread and best known in Morocco and around the world [5,6]. Due to serious dangers to warm-blooded animals and the environment, as well as problems of persistent toxicity in cereals, the search for ecologically safe methods to control insect pests of stored food products is essential [7,8,9]. Therefore, plants offer an alternative source of insect control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment [10,11,12]. In several countries, essential oils (EOs) and powders from aromatic plants are traditionally used through fumigant or contact action to protect grains against storage pests [13,14,15]. They are characterized by a mixture of complex, volatile organic compounds produced by different plants from different families [16,17,18]. EOs of aromatic plants have been reported to possess a wide range of biological properties, including insecticidal activity [19,20]. Among these plants are the Asteraceae family, which are flowering plants growing in Saharan zones. These plants have a pleasant aromatic odor due to the presence of EO in the different parts of the plants. Numerous scientific works published in the literature have demonstrated various biological activities of certain Pulicaria species; among them, Mohammed *et al*. [21] report that the EO of extracted leafy stems *Pulicaria undulata* reveals an antioxidant antiproliferative and d-enzymatic inhibition. Zardi-Bergaoui *et al*. [22] show the antioxidant effect, antibacterial, anti-tyrosinase, and cytotoxic activities of EO of the Tunisian plant *Pulicaria vulgaris*. While, El-Sabagh *et al*. [23] studied the metabolite profiles of *Pulicaria crispa* and *Pulicaria incisa,* which revealed antioxidant and hepatoprotective effects only in relation to its bioactive metabolites. Likewise, El-Shahaby *et al*. [24] have demonstrated the antioxidant activity and the antimicrobial potential of extracts of *Pulicaria incisa*, which can be attributed to its richness in active metabolites. Other researchers have also reported insecticidal properties of the genus Pulicaria [25,26].

In this regard, the present study aims to evaluate the insecticidal effect of *P. incisa* EO in *vitro* toxicity of EO of this Moroccan aromatic plant was tested against adults of *S. oryzae* and *T. castaneum*, to find the most effective method (repellency/fumigation/ingestion) to achieve the highest insecticidal activity against both insects. This study reports for the first time

the insecticidal activity of *P. incisa* from Morocco, which farmers traditionally use to fight against pests of stored grains.

2. Materials and Methods

2.1. Plant material.

Aerial parts of *Pulicaria incisa* were collected in May and June 2016 in the Beni Mellal-Khenifra region, Morocco. The plant was identified at the Natural Substances and Biodiversity Laboratory of the Ibn Tofail University of Kenitra, Morocco, based on the document "Practical Flora of Morocco Volume 1, 2 and 3". The plant material was prepared and then dried in the shade at room temperature for four weeks.

2.2. Extraction of essential oil.

Pulicaria incisa was subjected to hydrodistillation for three hours (Clevenger). Plant material *P. incisa* was distilled with an additional phase briefly. The plant is cut and immersed in water and heated to boiling, after which the EO was evaporated with water vapor and finally collected after decantation. The distillate was isolated and dried in a Rota vapor to giving brownish-yellow oil. The extracted oil was stored away from air and light at a temperature of 4°C

2.3. Gas chromatography analysis.

EO was characterized by gas chromatography (GC) (Agilent 7890A Series GC) coupled to mass spectrometry (MS) equipped with split/splitless injector, 123-BD11 column (15 m, 0.32 mm, 0.10 µm) and electron impact ionization. One µL of 1% (v:v) oil solubilized in chloroform was injected into the column by 2:1 split mode using helium as carrier gas at 4 mL/min. The ion source and quadruple temperatures were 230 °C and 150 °C, respectively. The oven temperature program was started at 30 °C and maintained 2 min, increased at 10 °C/min until 80 °C and maintained 1 min, then increased until 200 °C by 4 °C/min and maintained 1 min, then increased until 330 °C at 25 °C/min and finally kept constant for 1 min. The composition was determined from the peak areas. The identification was performed using NIST 2014 MS Library.

2.4. Biological tests.

Bioassays were performed to study the insecticidal effect of *P. incisa* OE on adults of *S. oryzae* and *T. castaneum*. Three EO toxicity tests were performed using three different methods: repellency, fumigation, and ingestion. The effectiveness of EO was compared to positive controls using powdered malathion (2% Malathion) and aluminum phosphide fumigant, at a temperature of 27 ± 1 °C, relative humidity of 70 ± 5%.

2.4.1. Repellency toxicity.

The repellency test was conducted based on McDonald *et al.* [28] in a glass Petri dish (9 cm diameter, Whatman No.1 and 1cm high), which contained a 9 cm filter paper. The EO was diluted in acetone to prepare different concentrations: 2, 5, 10 and 20 µL/mL of acetone, corresponding to concentrations of 0.031, 0.079, 0.157 and 0.314 µL/cm² respectively. One mL of each concentration was spread uniformly with a micropipette over a filter paper disc. The

control only was treated with acetone. After completing the evaporation of the solvent (5 minutes), the treated filter papers were carefully placed in Petri dishes. Three replications were performed for each concentration of EO. Ten insects each of *S. oryzae* and *T. castaneum* species were introduced into each Petri dish, which was then closed immediately. Dead insects were counted daily for a period of 7 days.

2.4.2. Fumigant toxicity.

Fumigation with the EO of *P. incisa* was carried out in transparent, hermetic 1L plastic boxes as exposure chambers to test the toxicity of the EO against adults of *S. oryzae* and *T. castaneum*. The EO was spread over a 9 cm Whatman type filter paper, which was immediately placed inside the exposure chamber containing five Petri dishes (ensuring five replications). The following doses were applied: 2, 5, 10, and 20 µL [29]. Ten insects were introduced to each Petri dish and one untreated chamber served as the control. Dead insects were counted daily for a period of 7 days.

2.4.3. Ingestion toxicity.

The soft wheat seeds used in this test were not treated chemically and were stored in controlled conditions (T: 25°C, H: 75%). For each test, 1 mL of each concentration 2, 5, 10 and 20 µL EO/mL of acetone was applied to 20 g of seeds, corresponding to the following concentrations: 0.1, 0.25, 0, 5 and 1 µL/g respectively. These treated seeds were placed in Petri dishes and thoroughly mixed. The trials were repeated three times for each dose. After 5 minutes, the time necessary to allow the solvent to evaporate, ten adult insects of each species were placed in all the Petri dishes. The blank obtained only 1 mL of acetone. Dead insects were counted daily for a period of 7days.

2.5. Statistical analysis.

The number of dead insects was counted every 24 hours. Mortalities in the treated boxes (MB) were expressed according to Abbott's [30]. The formula for corrected mortality (Mc), taking into account the natural mortality observed in the control boxes (Mt) according to the following formula:

$$Mc = (Mo-Mt)/(100-Mt) *100$$

To estimate the LC_{50} and LT_{50} using EO (The concentration and time of treatments necessary for the EO to kill 50% of the insects tested), regression lines were constructed by plotting the corrected mortality rate (given in Probits) as a function of the treatment concentration (taken as a log) [31]. The results obtained were the subject of an analysis of variance (ANOVA) to detect whether or not there were differences between the treatments at the 5% threshold.

3. Results

3.1. Characterization of the essential oil of the aerial parts.

The hydrodistillation of the dried aerials parts of *P. incisa* gave a brownish-yellow oil with an aromatic, fragrant odor. The oil yield was 0.40%. The GC-MS analysis is based on the Nist-2014 library. Sixty-six compounds were identified, representing 89.4% of the total oil

(Table 1). The main components were α-ocimene (15.17%), τ-cadinol (6.79%), α-cadinol (4.51%), alloaromadendrene (4.45%) δ- cadinene, (+) - (4.13%) (Figure 1).

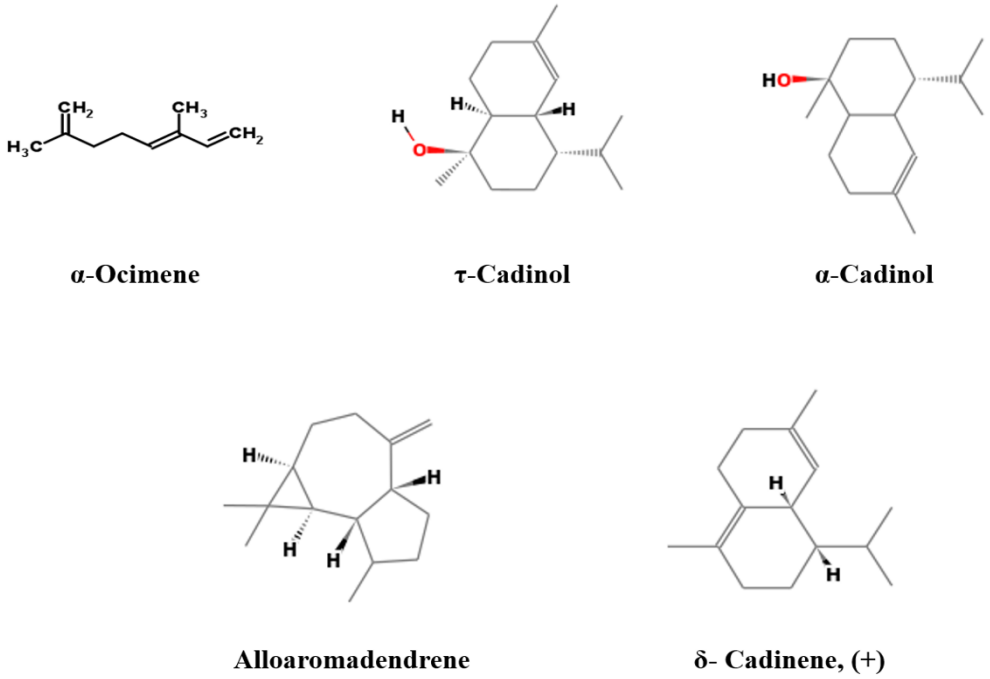

Figure 1. Chemical structure of selected major compounds identified in *Pulicaria incisa* essential oil.

3.2. Repellent activity tests.

Repellency test results are shown in Figure 2. The EO of *P. incisa* showed significant pest repellent activity to *S. oryzae* and *T. castaneum*. Oil was repellent even at low concentrations. Repellent action was highly dependent upon oil concentration and exposure time. The maximum activity (100% repellency) was observed at the highest concentration (0.157 µL/cm²) and (0.314 µL/cm²) after 4 days for *S. oryzae* and 5 days for *T. castaneum*. The mortality rate varied considerably depending on the time of exposure and the concentrations of the EO tested. Malathion insecticide also showed significant results. It caused 100% mortality in *S. oryzae* and 90% in *T. castaneum*.

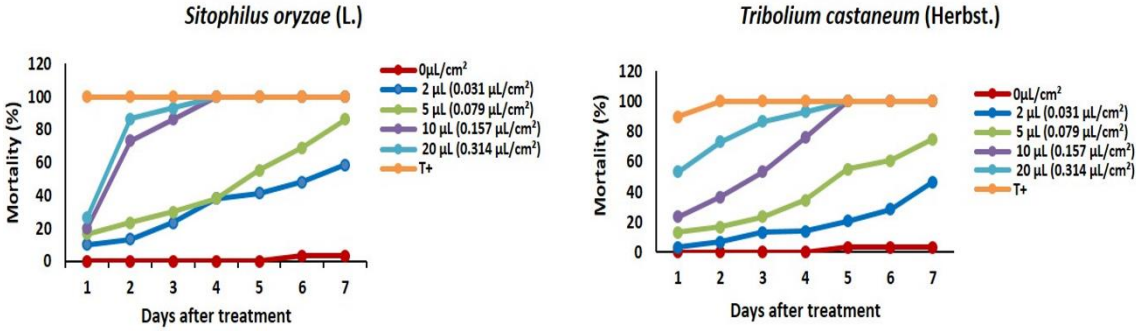

Figure 2. Mortality of *S. oryzae* and *T. castaneum* exposed to different concentrations of *P. incisa* essential oil by repellency test.

Table 1. Chemical composition of *P. incisa* aerial parts essential oil.

Peak No.	Compounds	$t_{R\ (min)}$	*P .incisa* (%)
1	Furfural	0.655	0.33
2	o-Xylene	0.773	3.41
3	Benzene, 1-ethyl-2-methyl-	1.428	0.32
4	Hemimellitene	1.503	0.89

Peak No.	Compounds	$t_{R\ (min)}$	*P .incisa* (%)
5	Mesitylene	2.190	0.11
6	Benzeneacetaldehyde	2.577	0.68
7	Benzene, 2-ethyl-1,4-dimethyl-	2.856	0.33
8	Linalool	3.650	0.55
9	Naphthalene	4.488	0.17
10	α-Terpineol	4.842	0.42
11	5-Octadecene, (E)-	5.067	0.50
12	8,9-Dehydrothymol	5.218	0.47
13	Coumaran	5.626	0.38
14	dl-Perillaldehyde	5.905	2.62
15	Anethole	6.141	0.16
16	Indole	6.238	0.14
17	o-Isopropylanisole	6.527	1.24
18	1, 1, 5-Trimethyl-1, 2-dihydronaphthalene	6.860	0.19
19	2H-1-Benzopyran-2-one, 3,4-dihydro-6-methyl-	6.935	0.35
20	Eugenol	7.107	2.02
21	α-Copaene	7.225	0.24
22	β-damascenone	7.419	0.35
23	Methyl perillate	7.612	0.75
24	1-Tetradecene	7.837	2.12
25	Methyleugenol	7.987	0.51
26	Coumarin, 3,4-dihydro-4,5,7-trimethyl	8.234	0.35
27	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	8.406	0.31
28	cis-Muurola-4(15),5-diene	8.632	0.16
29	Valencene	8.868	0.19
30	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	9.029	1.03
31	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	9.136	0.26
32	γ-Selinene	9.308	0.26
33	Thymyl isobutyrate	9.480	1.44
34	α-Muurolene	9.555	0.89
35	γ-Cadinene	9.737	1.59
36	Longifolene	9.855	1.42
37	δ-Cadinene, (+)-	10.070	4.13
38	(+)-Calarene	10.253	0.89
39	3,5-Heptadienal, 2-ethylidene-6-methyl-	10.564	1.22
40	Germacrene D-4-ol	11.015	3.32
41	β-Caryophyllene	11.230	0.92
42	Cyclooctene, 4-methylene-6-(1-propenylidene)-	11.348	0.35
43	2-Dodecen-4-yne, (E)-	11.723	1.22
44	Cetene	12.185	2.42
45	bicyclo 5.2.0 nonane 2-methylene-4 8 8-trimethyl-4-vinyl-	12.400	3.30
46	τ-Cadinol	12.625	6.79
47	α-Cadinol	12.904	4.51
48	Alloaromadendrene	13.484	4.45
49	1-[3-(2,6,6-Trimethyl-cyclohex-2-enyl)-4,5-dihydro-3H-pyrazol-4-yl]-ethanone	14.354	2.53
50	Oplopanone	14.665	0.82
51	Cyperotundone	14.901	0.55
52	Octanal, 2-(phenylmethylene)-	15.223	0.57
53	Isoaromadendrene epoxide	15.610	1.80
54	Z-3-Hexadecen-7-yne	15.749	2.15
55	Caryophyllene Oxide	16.232	0.36
56	α-Ocimene	17.080	15.17
57	2-Pentadecanone, 6,10,14-trimethyl	18.014	1.07

Peak No.	Compounds	$t_{R\ (min)}$	*P .incisa* (%)
58	Cedran-diol, (8S, 14)-	18.873	0.13
59	Longiverbenone	19.399	0.95
60	Heptadecane	20.784	0.31
61	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	24.788	0.17
62	9-Nonadecene	25.701	0.67
63	Eicosane	25.840	0.50
64	1-Nonadecene	29.608	0.42
65	Heneicosane	31.594	0.49
66	Tetracosane	35.094	0.25
	Monoterpenes hydrocarbons		15.17
	Oxygenated monoterpenes		6.75
	Sesquiterpenes hydrocarbons		19.64
	Oxygenated sesquiterpenes		16.31
	Others oxygenated compound		5.15
	Benzene derivative		9.61
	Coumarin derivative		0.7
	Norisoprenoids		0.35
	Others		15.72
	Total identified		**89. 4**

t_R: Retention Time.

3.3. Fumigant toxicity.

The results revealed that all EO concentrations induced significant mortality compared to the control (without treatment) for both insect species. Insect mortality was directly proportional to the concentration and duration of exposure (Figure 3). During the 7th day after the exposure period, all other concentrations tested (2, 5, 10, and 20 µL/L of air) recorded mortality of more than 50%. The concentration of 20 µL/L of air of *P. incisa* resulted in 100% mortality of *S. oryzae* and *T. castaneum* after 3 and 4 days of exposure, respectively. The mortality recorded in the positive control (fumigant of aluminum phosphide) was less effective after the entire duration of treatment for the two insects.

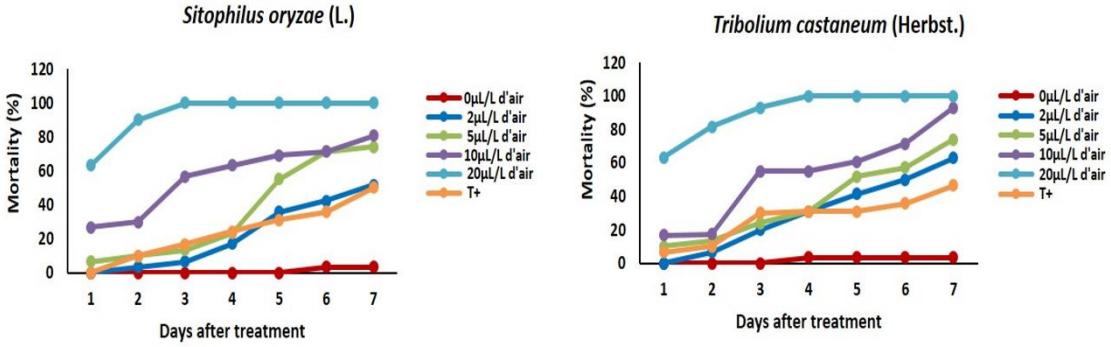

Figure 3. Mortality of *S. oryzae* and *T. castaneum* exposed to different concentrations of *P .incisa* essential oil by fumigant test.

3.4. Ingestion activity tests.

The results of toxicity by ingestion of *P. incisa* EO showed that all the tested concentrations induced significant mortality compared to the control for the two species of insect pests. Figure 4 shows the percentage of adult mortality of *S. oryzae* and *T. castaneum*, which varied with different EO concentrations and exposure time. The results indicate that the concentrations of 0.5 µL/g and 1 µL/g induce total destruction of both insects. Therefore, the

weevil *S. oryzae* was found to be more sensitive to oil exposure than *T. castaneum*. However, the mortality recorded in the positive control dishes treated with Malathion is more effective, inducing total mortality after 2 days of treatment for the two insects.

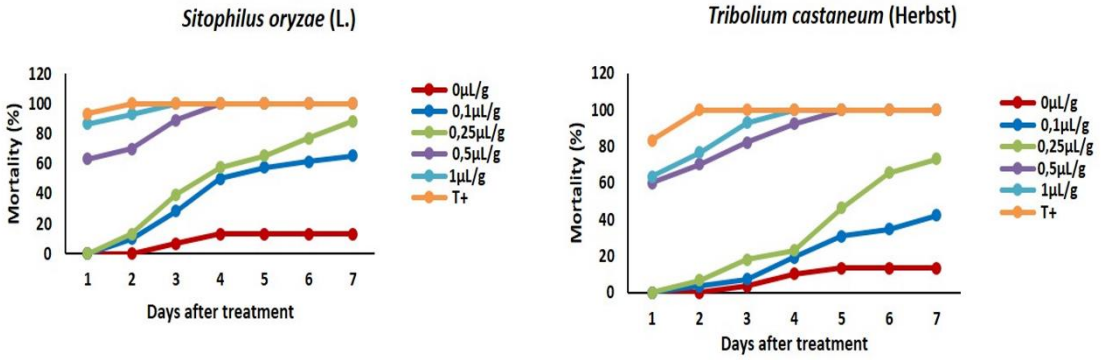

Figure 4. Mortality of *S. oryzae* and *T. castaneum* exposed to different concentrations of *P .incisa* essential oil by ingestion test.

4. Discussion

Pulicaria incisa is a wild-growing plant in Morocco. The yield of the EO extracted from the aerial parts of this plant was 0.50%. Our results agree with previous studies carried out on the same species previously studied for other biological activities. A similar yield of 0.50% was obtained by Chaib *et al*. [32] after hydrodistillation of aerial parts of the Algerian plant. The OEs of *Pulicaria incisa* sub. growing wild in Egypt has a pleasant aroma with an average yield of 0.66% [33]. Another study carried out on *Pulicaria vulgaris* growing in Tunisia gave essential yellow-colored oils and a yield of 0.05% [22]. Authors reported that the genus Pulicaria yields relatively moderate quantities of EO. To the best of our knowledge, this is the first report on the chemical composition of the EO produced from the aerial parts a of *Pulicaria incisa* grown in Morocco. We used gas chromatography-mass spectrometry (GC-MS) for the analysis of the EO. A total of sixty-six compounds have been identified. This oil was rich in sesquiterpenes hydrocarbons (19.64%), oxygenated sesquiterpenes (16.31%) and monoterpenes hydrocarbons (15.17%). Other similar studies on the genus Pulicaria revealed mainly the presence of oxygenated monoterpenes (64.0%) and aromatic derivatives (18.8%) [34], sesquiterpene lactones [35,36], and caryophyllene derivatives [37,38]. In addition, the EO of *Pulicaria incisa*, growing in Egypt, was characterized by the high content of carvotanacetone (66.01%) and chrysanthenone (13.26%) [33]. Our results and those previously reported clearly indicate variations in chemical composition, which depend on the plant origin of the EO. Moreover, most Pilucaria species are considered good sources of bioactive components having a broad spectrum of biological activities**.**

In the current research, the potential of repellency, fumigant and ingestion toxicity of *P. incisa* EO was examined. According to the doses, the results of the statistical analysis of EO reveal the existence of a significant difference between the percentage of mortality in the control batches and those treated with EO, which explains why these products show an insecticidal effect on the two pests studied. For repellent bioassay, both insects were susceptible to EO and both adults were repelled at all concentrations. The probit analysis, LC_{50} values (lethal concentration for 50% mortality) of *P. incisa* EO against *S. oryzae* and *T. castaneum*, varied between 15.49 - 1.73 µL/cm^2 and 20.89 - 2.29 µL/cm^2 respectively for different

observation (Table 2). Lethal time (LT_{50}), to kill 50% of *S. oryzae* adults, varied between 6.3 - 0.8 days and 11.5 - 1.2 days for *T. castaneum* for different oil concentrations tested (Table 3).

According to the results of the fumigation test, the adults of *S. oryzae* and *T. castaneum* were susceptible to the *P. incisa* EO with LC_{50} values varied between 16.21 - 2.08 and 18.62 - 2.51 µL/L air. The LT_{50} values for *S. oryzae* is ranged from 6.2 - 0.7 days and 7.4 - 0.9 days for *T. castaneum*. The susceptibility of *S. oryzae* and *T. castaneum* to plant EO was illustrated in the previous studies that support the results of the present study. For example, the contact and fumigation bioactivity of *Tagetes terniflora* EO against *T. castaneum* and *S. oryzae*, has been shown to be toxic by contact for *S. oryzae* but less toxic for *T. castaneum* [39]. On the other hand, strong toxicity was observed with *Foeniculum vulgar* Mill and *Pimpinella anisum* L. EOs against *T. castaneum*, which recorded LC_{50} of 91.28 and 43.75 µL/L of air after 24 hours respectively[40]. Similar results were obtained using *Mentha microphylla* oil by the fumigant test, which exhibited the highest insecticidal activity among Egyptian oils tested against *S. oryzae* and *T. castaneum* with LC_{50} values of 0.21 µL/L 4.51 µL/L respectively [41]. The EO of the Iranian wild plant *Perovskia abrotanoides* has a similar effect on these two insects, with the lowest concentration (32 mL/L of air) causing 100% mortality *S. oryzae* and *T. castaneum* After 15 and 8 hours of exposure [42].

The EOs of certain plants of Myrtaceae growing in Australia and *Artemisia sieberi* from Iran has been reported to possess fumigant toxicity against these two adult insects [43,44]. Another study showed the effect of *Echinacea purpurea* EO from the Asteraceae family by different methods against *S. granarius* and *T. castaneum*, this oil caused a 99.59% mortality of *S. granarius* after 72 hours and a strong repellent activity of 98% against *T. castaneum* at a dose of 1% [45]. Amini *et al*. [46] studied four Nepeta L. species against the rice weevil, They revealed that *Nepeta glomerulosa* exhibited the highest fumigant activity with LC_{50} of 124.318 µL/Lair, while *Nepeta binaloudensis* and *Nepeta cataria* showed an effect 100% repellent at a dose of 25 µL/30 cm^2 against *S. oryzae*.

The interaction between the two insects and the EO of *P. incisa* also lasted for seven days by ingestion bioassay. The LC_{50} calculated within *S. oryzae* and *T. castaneum* were of the order of 12.59 - 1.51µL/g and 14.12 - 2.39 µL/g, respectively. The LT_{50} values for *S. oryzae* is ranged from 4.7 - 0.8 days and 8.5 - 0.9 days for *T. castaneum*. Furthermore, as the EO concentration increased, the LT50 values decreased and in all cases, increased susceptibility of both insects was directly related to oil concentration and exposure time.

In the present study, *P incisa* EO was extremely toxic against the rice weevil and the red flour beetle. A possible explanation for these results is again linked to the richness of the genus Pulicaria inactive compounds. α-Ocimene has been found to be the main component of EO. But the toxicity may be due to the synergistic effect of different components. Our essential oil was composed of complex mixtures (sesquiterpene hydrocarbons, oxygenated sesquiterpenes and monoterpene hydrocarbons), which may be the origin of this insecticidal activity. These compounds are often present as major components of EOs in aromatic plants of the Asteraceae family. EOs can cause direct mortality and drastically reduce pest fertility, longevity, and vitality [47,48]. Based on these various facts, herbal medicines can play an important role as safe botanical insecticides against stock pests.

5. Conclusions

The data presented in this study demonstrate the insecticidal power of the *P. incisa* plant growing wild in the region of Beni Mellal-Khenifra, Morocco. Farmers use this plant to

preserve the quality of their seeds. Our results reveal the insecticidal effect of *P. incisa* against *S. oryzae* and *T. castaneum* by different methods (ingestion, repulsion and fumigation), determined by us is the first to be published. This work clearly justifies the interest in the effectiveness of Moroccan essential oils, which respond to global interest by the development of substances based on plant products as natural insecticides against pests of stored products. The use of botanical insecticides based on our selected EO can thus become an important alternative. However, further studies are needed to assess the biological efficacy in the field, the formulation feasibility of these products and the verification on a broad spectrum of insect pests.

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Conflicts of Interest

The authors declare that there was no conflict of interest.

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