

Cellular and Molecular Biology

E-ISSN: 1165-158X / P-ISSN: 0145-5680



www.cellmolbiol.org

Insecticidal effects of the *Olea europaea* subsp. *laperrinei* extracts on the flour Pyralid *Ephestia kuehniella*

Souad Lahcene¹, Faiza Taibi², Nadjet Mestar¹, Samira Ali Ahmed¹, Mahieddine Boumendjel², Saida Ouafi³, Karim Houali^{1*}

¹ Laboratory of analytic biochemistry research and biotechnology (LABAB). Department of Biochemistry and Microbiology, Faculty of Biological Sciences and Agronomic Sciences.Mouloud Mammeri University, Tizi-.Ouzou, Algeria

²Research Laboratory on the Biochemistry and the Environmental Toxicology. Department of biochemistry. Science faculty. Badji Mokhtar Annaba University. Algeria

³ Research laboratory of arid zones (LRZA). Department of Population and Organism Biology. Faculty of Biologics Sciences. Sciences and Technologies Houari Boumediene University Bab Ezzouar (USTHB) Algieria

Correspondence to: houalitizi@yahoo.fr

Received May 14, 2018; Accepted August 2, 2018; Published August 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.11.2

Copyright: $\ensuremath{\mathbb{C}}$ 2018 by the C.M.B. Association. All rights reserved.

Abstract: In the present study, the aerial parts of the Laperrine olive (*Olea europaea subsp. Laperrinei*) are subjected to acid extraction and the chemical composition of the extracts is determined by HPLC-DAD. The main compounds found in all of extracts are: hydroxytyrosol (30.45%), tyrosol (0.69%), oleuropein (32.76%), ferrulic acid (17.77%), quercetin (31.57%) and hesperetin (6.90%). The extracts obtained from the leafy stems of Laperrine olive tree are tested on the moth *Ephestia kuehniella* flour. Their administration by inhalation of newly exuviated chrysalises extends the duration of nymphalid development and disturbs the exuviated adults reproduction, by reducing the period in which the eggs are being laid. Thus, compared to the control insects, the number of eggs laid by treated females is significantly reduced after the treatment by extracts. Besides, the administration of different extracts of adult butterflies has a premature mortality effect.

Key words: Olea europaea subsp. Laperrinei; Polyphenols; Bio-insecticides; Ephestia kuehniella; HPLC.

Introduction

The Laperrine olive-tree or southern olive tree is under-species of the olive-tree belonging to the Oleaceae's family. According to Besnard et al. (1), this endemic species occurs in sub-arid to arid habitas and is observed in Algeria only in Central Sahara (Ahaggar, Taessa, Abeleheg, Hoggar, Tefedest, Tassili and Mouyedir), essentially in mountainous regions which altitude reaches 2800 m, with low precipitation under 100 mm (2). This olive-tree is now known as an under-species of the complex taxonomic *Olea europaea* (3). The Laperrine olive-tree which real scientific name is *Olea europaea* subsp. *laperrinei* (Batt. and Trab.) is now a taxon close to the Mediterranean rim olive-tree.

This is an important genetic resource for its drought resistance quality which can be used as a rootstock of different olive-tree varieties cultivated in arid regions (2).

Many storage systems rely on the use of synthetic insecticides and fumigants, such as methyl bromide and phosphine to control pests of stored products (4). Chemical pesticides are economical and effective, but have the disadvantage of causing resistance in treated insects and are harmful to the environment and to humans (5, 6). Plants produce many molecules against parasites. One of the research strategies is to study the plant and its extracts in order to identify some compounds that can be used as natural insecticides (7). Many studies have shown that plant extracts are an alternative method of controlling insect pests of stored commodities because they are a source of bioactive natural compounds (8). These natural insecticides, called plant insecticides, have a great advantage over synthetic compounds because they are rapidly biodegradable (9).

The Laperrine olive-tree was not yet studied to evaluate its potential bio-insecticide activity; the literature lacks the quantitative or qualitative study of its phenolic compounds. Moreover, no evaluation study of insecticidal effect of olive leaves against *Ephestia kuehniella* was found. The present work is a preliminary study, which is a contribution to a better understanding of Laperrine olive tree and gives a way to value this plant as a bio-insecticide. Therefore, our aim in this study is to investigate the polyphenol content of Laperrine olivetree leaves extract and the insecticidal effects against to *Ephestia kuehniella*.

The flour moth *Ephestia kuehniella* Zeller is a food moth which damages are exclusively caused by caterpillars. Its larvae mainly attack stocked food such as flour, cereals grains (rice, corn and wheat), semolina, pastas, and rarely dried fruit such as raisins, figs and apricots. They reduce the quality of product by their presence and cause direct damages in food because of chemicals produced by mandible glands (10).

Materials and Methods

Laperrine olive collection

The leaves and stems of *O. europaea* subsp *laperrinei* were gathered from Ouled Hanghassi in Tamanrasset's region (23°14'50.1"N, 5°29'13.7"E) on May 2015. Identification of the species was carried out according to the keys of the Ozenda flora manual (11). Authentication of the species is confirmed by Mr. Abdellah Salhi, Chief of the National Institute of Forestry Research Tamanrasset (INRF), Algeria. A reference specimen has been deposited at Mouloud Mammeri University (OLE1-2015 Tam, FSBSA / TO).The collected leaved stems are selected and dried in the open air, in the shade and protected from humidity.

Insect material

The moths were brought from Seybousse Mills in Annaba city (North-East of Algeria). The breeding is brought to the laboratory in a sterilizer under optimum development conditions, characterized by a temperature of 27 °C, a relative humidity nearing 70% and darkness (12, 13). The adults are laid down in glass jars with a volume of 1.5 l, 20 cm high and 10 cm diameter, containing flour and covered by a piece of tulle fabric fixed by an elastic band. A piece of cotton soaked with sugared water is laid on each jar in order to activate the growth of the pest insect. A daily follow-up allows to sex and to remove the male and female larvae in Petri dishes containing flour and pleated paper allowing larvae to enter the pupal stage (14). The distinction of the sexes is quite easy at the larval stage, where the male larvae have two brown spots on their posterior part corresponding to the testicles. Their dating is expressed in days after the nymph exuviations (15).

Plant extraction and bio-insecticide evaluation

The dried leaves and stems are grounded in an electro mechanical grinder. The achieved powders undergo an acid hydrolysis following the analytical protocol conceived by Lebreton et al. (16) and Jay et al. (17).

To two grams of vegetable powder we add 160 ml of 2N hydrochloric acid. The mixture is heated in a water bath at 40 $^{\circ}$ C. for 40 minutes, with air being blown up every 10 minutes. The aqueous phase is extracted three times with diethyl ether (60 ml - 60 ml and 40 ml).

This extraction makes it possible to collect the aglycones in the ethereal epiphase. The ether extracts are combined for spontaneous evaporation to dryness, under ventilated hood. The dry residue is recovered in 5 ml of methanol and stored at 4 °C.

The residual aqueous phase is taken up for the extraction of the C-glycosides with n-butanol (40 ml nbutanol: 40 ml). The butanol extract is evaporated to dryness, under a ventilated hood. The dry residue is recovered in 5 ml of methanol and stored at 4 °C. The raw extracts resulting from the hydrolysis are represented by the ether layer stemming from the leaves (LE) or from the stems (SE) and from a butanol phase obtained from the leaves (LB) or from the stems (SB). They are tested on the flour moth.

Dosages

Determination of flavones-aglycones content

The ethereal dry extract is recovered by methanol and adjusted in graduated flasks of 10 cm³. The differential dosage of total flavones-aglycones makes the Al⁺⁺ ions chelating properties infer on flavonoids. The height of the differential peak, proportionally to the concentration of flavones-flavonols which are present in the extract is determined by spectrometry on wavelength 430 nm. The total content of flavones-aglycones is expressed in mg of an equivalence quercetin/g of vegetable powder (mg EQ/g) using the following formula, Lebreton et al. (16):

T flavones- aglycones = $(\Delta A/\epsilon) \times M \times V \times (d/w) =$ 1. 3 x 10⁻² x $\Delta A \times V \times (d/w)$, where ΔA is the differential absorbance peak; ϵ is the molar absorption coefficient of quercetin in aluminium chloride (=23000); M is the quercetin molar mass (= 302); V is the volume of flavones-aglycones methanol solution; d is the dilution factor and w is the dry weight of hydrolyzed plant material.

Determination of Anthocyanins content

The water-soluble anthocyanins is dosed by spectrophotometry at anthocyanins maximal wavelength between 500 and 560 nm. According to Lebreton et al. (16), the content of total proanthocyanins is expressed in mg of an equivalent procyanidin/g of vegetable powder (mg EPC/g), using the following formula: T anthocyanins = $\eta x (A/\epsilon) x M x V x (d/w) = 5.2 x 10^{-2} x A x$ V x (d/w). γ is correction factor of oxidation yield of pro-anthocyanins into anthocyanins (estimated to 17%) ; A is the absorbance at the maximum absorption wavelength; ϵ is the molar absorption coefficient of cyanidin (=34 700); M is cyanidin Molar Mass (=306); V is the volume of butanol phase ; d is the dilution factor; w is the weight of dry matter of the hydrolysed vegetable material.

Determination of C. Glycosides content

The butanol dry extract is recovered in 10 cm³ of methanol and dosed by spectrophotometry at a wavelength of 340nm. The content in total C-glucosides is expressed in mg equivalent orientin/g of vegetable powder (mg EO/g) with the following formula:

T C. glycosides = $(A/ \varepsilon x M xV x (d/w) = 2.5 x 10^{-2} x A x V x (d/w)$. A is the absorbance at the maximum absorption wavelength; ε is the molar absorption coefficient of orientin (=18850); M is the orientin molar mass (= 448); V is the volume of the methanol solution; d is the dilution factor and w is the dry weight of hydrolyzed plant material.

Chemical composition

Chemical analysis of different extracts is obtained by DAD liquid chromatography (Agilent Serial 1100) under the following conditions: the mobile phase corresponds to acidified water at 0.2% with acetic acid at pH 3.1 and with acetonitrile by linear elution gradient during 30 minutes at 1.5ml/min, starting with 95% of water and with 100% of acetonitrile. The injected volume corresponds to 5µl of extract diluted in methanol. The identification of different compounds is made by comparing different time standards under the same conditions (18).

Treatment by inhalation

In order to estimate the insecticidal effect of the olive-tree raw extracts, they are administered to chrysalises and to newly exuviated adults by inhalation (saturation of their environment) (14). The different extracts are sprayed on filter paper and deposited into Petri dish or into the insect tubes.

As for the chrysalises, in a Petri dish, the raw extract is sprayed on a pleated paper on which are laid four newly exuviated female pupae immediately after the nymph exuviations which represents the early development of lepidopterous ovaries (13). This administration at the stage of the life cycle is a test for the fertility of females and the percentage of eggs lay. Seven repetitions are carried out for ach treatment; the control plates are treated with solubilisation solvent, represented by methanol.

In the case of adults, from their exuviation, a couple is displayed in a tube containing 30 g of flour. The crude extract is sprayed on a pleated paper introduced in the tube. Seven repetitions are handled by treatment, the control boxes are treated with methanol.

The effect of raw extracts on *Ephestia kuehniella* reproduction is assessed based on the following parameters:

• The nymph development duration which corresponds to the time expressed in days, which separates the nymph exuviations from the adult exuviations;

• The pre-oviposition period represented by the number of days separating the adult emergence from the beginning of the egg laying;

• The period of oviposition is estimated by the number of egg laying days;

• The female fertility, which corresponds to the number of eggs, lay during the whole oviposition duration.

Then, ten newly exuviated male and female adults are introduced in a tube of 15 cm length and 3 cm diameter, containing 30 g of flour. The treatment is done by a raw extract atomization of pleated paper which is in the tube. The action is repeated thrice. The control tubes are treated with methanol. The toxicity tests of the various extracts are made on the daily count of the dead treated insects over a period of 21 days, corresponding to a 100% control mortality rate (7). W e aim to estimate the insects longevity submitted to the olive-tree raw extracts. The longevity is estimated through the mortality rate of the treated control insects which is calculated by the following formula:

Observed mortality = (Number of dead individuals/ Number of total individuals) x100. The experiment lasts 21 days.

Statistical analysis

The results are expressed under an average form affected by its standard deviation. The t-Student test is used to analyze the level of statistical significance between the series of averages treated by pairs in the case of the conducted dosages.

The influence of the different extracts on the moth reproduction parameters is subject to an ANOVA to a factor. The normality of the data is specified at first by the Pearson test. The statistical analysis is realized with



Figure 1. Flavone compounds content of different extracts obtained from the leaves powder (LP) and from the stems powder (SP) of the Laperrine olive-tree.

the software program Statbox 6. In the case of a significant difference, we use the Newman-Keuls test to define the homogeneous groups.

Results

Dosages

Figure 1 recaps the calculation results of phenolic compounds contents extracted from the Laperrine olivetree leaved stems. The data statistical analysis shows a significant difference (P < 0.001), of the flavones-flavonols and anthocyanins absolute content. Indeed, we can notice in the case of leaves powder, an average content of 0.389 ± 0.004 mg equivalent quercetin /g of vegetable powder and 1.573 \pm 0.012 mg equivalent procyanidin /g of vegetable powder, whereas in the case of stems powder, we can record 0.21 ± 0.06 mg equivalent quercetin /g of vegetable powder and 0.803 ± 0.006 mg equivalent procyanidin /g of vegetable powder. As for the C-glycosides the Student test enhances a significant difference with an average of 1.167 ± 0.002 mg equivalent orientin/g of vegetable powder for LP (Leaves Powder) and 1.161 ± 0.001 mg equivalent orientin /g of vegetable powder for SP (Stems Powder).

The results of dosages realized in both phases produced by the leaves powder and the stems powder show a very significant difference in favor of the leaves extracts.

Chemical composition

The chemical composition of different extracts is carried out by liquid chromatography. 21 compounds are identified "Table 1". Our results show the presence, in all extracts, of hydroxytyrosol, tyrosol and oleuropein specific to *Olea europaea* (19).

Laperrine olive-tree raw extracts effect on *Ephestia* kuehniella reproduction

Raw extracts effect on the nymphal development period

The raw extracts applied at a chrysalis stage of the *Ephestia kuehniella*, all extend the duration of pupal development compared to the control insects, for which we record a value of 7.14 ± 0.24 days.

The applied statistical test brings out a significant effect (P < 0.001), we obtain an average of 38.29 ± 0.41 days for LB. The pupae exuviation is done after 14.29

Table 1. Main chemical compounds identified in olive tree Laperrine extracts.

Chemical compounds	Ethereal Leaves extract		Ethereal Stems extract		Butanol Leaves extract		Butanol Stems extract	
	Retention time (min)	Area (%)	Retention time (min)	Area (%)	Retention time (min)	Area (%)	Retention time (min)	Area (%)
Hydroxytyrosol	4.838	30.45	4.853	18.78	4.830	5.32	4.850	8.47
Tyrosol	5.898	0.45	-	-	5.852	0.43	5.887	0.69
Caffein	-	-	6.467	0.49	6.453	0.75	4.483	0.71
Aesculein Acid	-	-	-	-	-	-	7.002	1.36
Caffeic Acid	7.062	0.61	-	-	7.046	0.84	-	-
Vanillin	8.673	2.12	8.656	1.06	-	-	-	-
Ferrulic acid	9.257	5.40	9.233	0.95	9.234	6.65	9.239	17.77
P coumarique acid	-	-	-	-	-	-	9.672	2.03
3 hydroxy 4 methoxycinamic Acid	-	-	-	-	9.773	0.79	9.799	0.79
Oleuropein	10.119	4.13	10.198	32.76	10.103	4.86	10.222	13.21
Naringenine 7 oglucoside	-	-	10.456	2.38	-	-	10.490	2.86
3,4,5 trimethoxybenzoic acid	-	-	10.817	4.99	-	-	10.814	4.25
m-anisic acid	-	-	-	-	11.938	1.75	-	-
Luteolin	12.491	1.31	-	-	-	-	-	-
Quercetin	12.865	29.18	12.901	11.43	12.787	22.31	12.866	31.57
cinamic acid	13.897	1.35	-	-	-	-	-	-
Apigenin	-	-	-	-	14.379	3.31	14.367	1.90
Hesperetin	15.028	6.90	15.056	5.63	15.061	2.54	15.006	4.28
Orientin	-	-	-	-	17.729	2.75	17.741	0.49
Vitexin	-	-	-	-	18.192	1.96	18.177	1.34
Isovitexin	-	-	-	-	18.495	1.24	18.468	1.07

- : Absence.

 \pm 0.41 days for SE, 11.00 \pm 0.57 days pour LE and 9.57 \pm 1.63 days in the case of SB.

Thus, the most interesting insecticide effect seems to be the effect corresponding to the butanol extract from the leaves 'powder for which the pupae exuviation is effective only from the 38th day and to the effect of ethereal extract obtained from the stems powder with a pupae development which lasts till the 14th day "Figure 2".

Laperrine olive-tree raw extracts effect on the pre-oviposition period

The comparative examination of the after-treatment results shows a significant difference (P < 0.01) between SB, corresponding to the homogeneous group a, for which the pre-oviposition period is on average of 1.57



Figure 2. The period of nymphal development related to the applied treatment (LE: Ethereal Leaves extract, LB: Butanol Leaves Extract, SE: Ethereal Stems extract, SB: Butanol Stems extract, C: Control and a, b, c, d, e : Homogeneous groups).

 \pm 0.49 days and the control which presents an average value of 1.00 \pm 0 days.

In comparison with the control, the treatments left do not present any significant difference "Table 2", as the statistical performed test class them all in the same homogeneous group. Group b which presents an average pre-oviposition period of 1.00 ± 0 days.

Laperrine olive-tree raw extracts effect on the oviposition period

One day after the mating, the *Ephestia kuehniella* control female starts its egg laying which lasts on average 4.29 ± 0.41 days.

Figure 3 shows that when the LB extract is applied, the number of days of the female egg laying diminishes in a significant way (P < 0.001); Indeed, the oviposition period is reduced by half with an average value of 2.43 ± 0.49 days, we can count 3.00 ± 0.57 for SB, 3.29 ± 0.41 days for SE and 3.71 ± 0.41 days for LE.

Laperrine olive-tree raw extracts effect on the Ephestia kuehniella female fecundity

The application of raw extracts on the female para-**Table 2**. The Laperrine olive-tree raw extracts effect on the *Ephestia kuehniella* pre-oviposition period.

	Control	Leaves powder	Stems powder
Ether extract	1.00 ± 0	1.00 ± 0	1.00 ± 0
Butanol extract	1.00 ± 0	$1.14{\pm}~0.24$	$1.57{\pm}~0.49$



site reduces on average of 1/4 the number of laid eggs during the oviposition duration in comparison with the control, which counts an average of 190.71 ± 10.04 eggs. The ANOVA presents a significant effect (P < 0.001) with an average value of 40.57 ± 7.35 eggs for LE, 41.86 ± 13.02 eggs for SE and respectively 56.71 ± 10.04 eggs and 51.86 ± 21.31 eggs in the case of LB and of SB "Figure 4".

Thus, all the tested extracts seem to have the same effect on the *Ephestia kuehniella* female, as the Newman-Keuls test class them all in the same homogeneous group, the b group.

Laperrine olive-tree raw extracts effect on the Ephestia kuehniella adults mortality

The Laperrine olive-tree raw extracts are administered on the *Ephestia kuehniella* pest by inhalation, in order to estimate their insecticide activity on the adults mortality.

From the adult exuviation, many insects are placed in a tube containing pleated paper sprayed with the raw extract. A daily counting of dead adults is done until it reaches 100% mortality.

The illustrating results in Figure 5 show that the applied treatment has an efficient insecticide effect on the pest insects.

In the optimum development conditions, the control adults mortality significantly starts on day 5 of the breeding. Indeed, we can record an average value of 6.67 ± 4.44 %. After the application of the LE and SE extracts on adults, immediate effects arise. Those effects may result in an average mortality rate 24 h after the



Figure 4. Female fecundity according to the applied treatments (LE: Ethereal Leaves extract, LB: Butanol Leaves extract, SE: Ethereal Stems extract, SB: Butanol Stems extract, C: Control and a, b : Homogeneous groups).





beginning of the experiment, 3.33 ± 4.44 % for LE and 10.00 ± 6.67 % for SE.

As for the adults treated by LB and SB, we can notice a starting mortality in day 2 of the treatment with respective average values of 3.33 ± 4.44 % and 10.00 ± 6.67 %.

A lethality of 50% is recorded in day 4 of the treatment with SB (50 \pm 6.67%), and in day 5 for the LE treatments (50 \pm 6.67%) and SE (50 \pm 0%). That value is not reached until the 5th and 6th day of the LB treatment exposure, and only in day 17 in the case of the control insects (50 \pm 0%).

The mortality rate is significantly marked for each day of the experiment. The control adults insects live on average 20 days after which, we can count 100% mortality.

In relationship with the obtained results, it seems that the whole insects die after 10 days exposure to the SE and LB extracts, after an eleven-day-treatment with LE, and a fourteen-day-treatment with SB to have all the adult insects dead.

Discussion

The quantitative analysis by the UV-visible spectrophotometry method, of the phenolic extracts of *Olea europaea* subsp. *Laperrinei*, allowed us to assess their average amount of flavones-flavonols, which correspond to the average value of 0.389 ± 0.004 mg/g for the leaves powder and 0.221 ± 0.006 mg/g for the stems powder, in anthocyanin, we have 1.573 ± 0.012 mg/g in the case of leaves powder and 0.803 ± 0.006 mg/g for the stems powder. As for the C-glycosides, we calculate an average amount of 1.167 ± 0.002 mg/g, in the case of leaves powder and 1.161 ± 0.001 mg/g for the stems powder. Those amounts clearly reveal that the Laperrine olive-tree leaves contain more phenolic compounds than the stems.

The results of chemical analysis of various extracts show that the levels and types of identified compounds vary according to the nature of extract. Our results are relevant to the work of Nashwa et al. (20), who identifies quercitin, apigenin, oleuropein and hesperetin in leaf extracts *Olea europaea* L, according to Pereira et al. (21) and Dekanski et al. (22), who found the presence of caffeic acid, quercitin, luteolin, apigenin and oleuropein in the same extract.

Great deals of studies have shown that flavonoids

are implied in the mechanisms of plants defense against insects attacks. That property seems to apply *in vitro* conditions, as according to Teixeira da Silva (23) and Golawska et al. (24), flavonoids have an insecticide effect. This observation supports our experiment. Our study reveals an insecticide effect of flavone extracts obtained from the leaves powder and from the stems powder of the Laperrine olive-tree on *Ephestia kuehniella*. The inhalation of raw extracts by the chrysalis extends their pupal development duration and disturbs the adults reproduction. Out of the four tested extracts, only SB seems to extend the pre-oviposition period in a significant way.

We can notice that after the mating the oviposition period is significantly reduced after the females exposure to the tested raw extracts; nevertheless, the LB fraction mostly disturbs the adults reproduction, as it reduces the egg-laying period to 2.43 days in comparison to 4.29 days, observed in control in the optimum conditions of the moth reproduction.

All the obtained fractions from the Laperrine olivetree's powder disturb the adults fecundation by reducing on average of 2/3 the number of laid eggs.

The results obtained suggest that Laperrine olive extracts have an insecticidal effect against *E. kuehniella*. This effect is probably due to the presence of oleuropein, quercetin and apigenin which can play a fundamental role in bio-insecticidal activity (24, 25).

The different articles consulted show the effects of essential oils on the fecundity of stored food pests and supports our results obtained on *Ephestia kuehniella*. Thus, work on the essential oil extracted from the *Ar*-temisia herba alba white shows a disturbance of the reproduction of the pyral by lengthening the duration of the pre-ovipotion and of the nymphal development and reducing the period of laying (14). Even if the female is able to lay eggs, the number of eggs is reduced because of the reduction of oviposition period.

Similar results have been obtained with *Tagetes* minuta, *Hyptis suaveolaen*, *Ocinum canum*, *Ocimum basilicum* and *Piper guineense* (26). Kellouche et al. (27) have recorded no egg-laying with the *Calloso-bruchus maculatus* females after the administration of *Mentha x piperita* (Peppermint) essential oil with a dose of 20 μ l/50 g. *Olea europaea* leaf powder has a limited effect on the number of eggs laid by the cowpea weevil.

The raw extracts interfere with a double mechanism on the insects reproduction and on their longevity. When administered to adults, the Laperrine olive-tree raw extracts provoke a significant mortality rate in comparison to the control insects.

Indeed, we observe to start mortality from 24 hours after the experiment (3.33% - 10.00%), between the 4th and the 6th day of the experiment, 50% of the adult insects die whereas the whole pests die between the 10th and the 14th day after the administration of our extracts.

Delimi et al. (14), have observed that the white wormwood essential oil is efficient against the pest *E.kuehniella*. The adults lifetime progressively diminishes with the increasing of the applied dose.

We can also notice that the Goosefoot (*Chenopodi-um*) and the Gum-trees (*Eucalyptus*) essential oils and the *Chenopodium ambrosioides* powder have a great insecticide activity.

They have been tested on six stored food pests *Callosobruchus maculatus*, *C. chinensis* (Cowpea weevil), *Acanthoscelides obtectus*, *Sitophilus granarius*, *S. zeamais* and *Prostephanus truncatus*. A concentration of 0.4% provoke a mortality of 60% of the Cowpea weevil after a two-day-treatment (28, 29).

According to Kellouche et al. (30), leaf powder of Olea europaea significantly reduces adult longevity of cowpea weevil. The acetone extract of leaf powder of Olea europaea variety Chamlali causes a 100% mortality in the adults of Myzus percicae and reduces the penetration of the *Phthorimaea operculella* larvae in potato tubers at 71.7% and the percentage of spawning of the female at 93.3% (25). In addition, fed on olive-leaf meal, adults of the Desert Locust (Schistocerca gregaria), remain brick red color and smooth, character indicating a persistent of juvenile state, which makes the olive tree a potential tool for preventive fight against desert locust (31). The work of Mohammed (32), on the ethanolic extract of the olive tree shows that it induces a mortality of 98.7% of the larvae and 80.9% of adults of Tribolium confusum. The treatment of twigs infected with psylla (Euphyllura olivina costa), with phenolic compounds extracted from the olive tree causes in adults an average mortality ranging from 63.5% to 71.7% depending on the variety infected (33).

The plants resources constitute a great bioactive molecules reservoir which can constitute solutions to the environmental issues of sustainable development. Those molecules naturally synthesized by plants play a role in retro-regulation of insects population by plants, a kind of natural regulator of the stored food products pests. Those molecules could be used as a substitute for synthetic chemicals which bad effects on ecosystems and on human health are clearly evident.

The Laperrine olive-tree plant extracts, an underspecies of Algerian Central Sahara have shown an insecticide double effect on the *Ephestia kuehniella* pest by extending in a significant way the pupal development duration and by affecting in a limited way the pre-oviposition period and reducing the oviposition period and the number of eggs laid by the female. A significant mortality rate is also observed depending on the administered extracts to the flour moth. Those results constitute a new run to explore in order to understand the mechanisms of molecular action of those extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We express our gratitude to Pr. Yasmina Benazzoug (USTHB) for her stimulating discussions and to Pr. Nadia Bouguedoura for the acid hydrolysis at the LRZA (USTHB).

We present our special thanks to Dr. Zhor Bettache and Dr. Tahar Benakmoun of the central laboratory of the scientific police for the HPLC analysis.

References

1. Besnard G, Anthelme F, Baali-Cherif D. The Laperrine's olive tree (Oleaceae): a wild genetic resource of the cultivated olive and a model-species for studying the biogeography of the Saharan Moun-

tains. Acta Bot Gallica. 2012; 159(3):319-328.

2. Baali-Cherif D, Besnard G. High genetic diversity and clonal growth in relict populations of *Olea europaea subsp. laperrinei* (Oleaceae) from Hoggar, Ageria Ann Bot. 2005; 96 (5): 823-830.

3. Green PS. A revision of *Olea L*. (Oleaceae). Kew Bull. 2002; 57: 91-140.

4. Kheirkhah M, Ghasemi V, Yazdi AK, Rahban S. Chemical composition and insecticidal activity of essential oil from *Ziziphora clinopodioides* Lam. used against the Mediterranean flour moth, *Ephestia kuehniella* Zeller. J Plant Prot Res. 2015, 55(3): 260-265.

5. Nayak MK, Collins PJ, Kopittke RA. Residual toxicities and persistence of organophosphorus

insecticides mixed with carbaryl as structural treatments against three liposcelidid psocid species (Psocoptera: Liposcelididae) infesting stored grain. J Stored Prod Res. 2003; 39: 343-353.

6. Bilal H, Akram W, Ali Hassan S, Zia A, Bhatti AR, Mastoi MI, Aslam S. Insecticidal and Repellent Potential of Citrus Essential Oils Against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Pakistan J. Zool. 2015; 47(4): 997-1002.

7.Chaib I. Research on insecticidal plants in Tunisia: Review and discussion of methodological approaches. Tunisian J Plant Prot. 2011; 6: 109-125.

8. Park IK, Lee SG, Choi DH, Park JD, Ahn YJ. Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). J Stored Prod Res. 2003; 39: 375–384.

9. El idrissi M, Elhourri M, Amechrouq A, Boughdad A. Étude de l'activité insecticide de l'huile essentielle de *Dysphania ambrosioides* L. (Chenopodiaceae) sur *Sitophilus oryzae* (Coleoptera: Curculionidae) [Study of the insecticidal activity of the essential oil of *Dysphania ambrosioïdes* L. (Chenopodiaceae) on *Sitophilus oryzae* (Coleoptera: Curculionidae)]. J. Mater. Environ. Sci. 2014; 5 (4) :989-994.

10. Johnson JA, Valero KA, Hnnal MM. Effect of low temperature storage on survival and reproduction of india meal moth (Lipidoptea : Pyralidae). Crop Prot. 1997; 16:519-523.

11. Ozenda P. Flore et végétation du Sahara. 3ème ed. CNRS, Paris, 2004.

12. Soltani-Mazouzi N, Hami M, Gramdi H. Sublethal effects of methoxyfenozideon reproduction of the mediterranean flour moth, *Ephestia kuehniella*. Invertebr Reprod Dev. 2012; 56(2):157-163.

13. Delimi A, Taibi F, Bouchelaghem S, Boumendjel ME, Hennouni-Siakhène N, Chefrour A. Chemical composition and insecticidal activity of essential oil of *Artemisia herba alba* (Asteraceae) against *Ephestia kuehniella* (Lepidoptera: Pyralidae). Int J Biosci. 2017 ; 10(2) :130-137.

14. Delimi A, Taibi F, Fissah A, Gherib S, Bouhkari M, Cheffrour A. Bio-activité des huiles essentielles de l'Armoise blanche *Artemesia herba alba* : effet sur la reproduction et la mortalité des adultes d'un ravageur des denrées stockées *Ephestia kuehniella* (Lepidoptera). Afr Sci. 2013; 9(3): 82-90.

15. Taibi F, Smagghe G, Amrani L, Soltani-Mazouni N. Effect of ecdysone agonist RH-0345 on reproduction of mealworm, Tenebrio molitor. Comp Biochem Physiol. 2003; 135(3):257-267.

16. Lebreton Ph, Jay M, Voirin B, Bouchez MP. Sur l'analyse qualitative et quantitative des flavonoïdes. Chimie analytique. 1967 ; 49(7):375-383.

17. Jay M, Gonnet JF, Wollenwebere F, Voirin B. Sur l'analyse qua-

litative et quantitative des aglycones flavoniques dans une optique chimiotaxinomique. Phytochem. 1975; 14 (7): 605-1612.

18. Ouafi S, Bounaga N. Phenolic compounds as markers for Algerian cultivars of the date palm *Phoenix dactylifera* L. Acta Hortic. 2010; 882:321-327.

Ortega-Garcia F, Peragon J. HPLC analysis of oleuropein, hydroxytyrosol and thyrosol in stems and roots of *Olea europaea* L. cv Picual during ripening. J sci food Agric. 2010; 20 (13): 2295-2300.
 Nashwa, Morsy FS, Abdel-Aziz ME. Efficienty of olive (*Olea europaea* L.) leaf extract as antioxidant and anticancer agents. J Agroaliment Processes Technol. 2014; 20(1): 46-53.

21. Pereira AP, Ferreira ICFR, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. Molecules. 2007; 12: 1153-1162.

22. Dekanski D, Janicijevic-Hudomal S, Tadic V, Markovic G, Arsic I, Mitrovic DM . Phytochemical analysis and gastroprotective activity of an olive leaf extract. J Serb Chem Soc. 2009; 74 (4):367-377.
23. Teixeira da Silva JA. Mining the essential oils of the Anthemidae. Afr J Biothechnol. 2004; 3(12): 706-720.

24. Golawska S, Kapusta I, Lukasik I, Wojcicka A. Effect of phenolics on the pea aphid, *Acrythosiphon pisum* (Harris) population *Pisum sativum* L. (Fabaceae). Pestycydy/ Pesticides.2008; 3-4: 71-77.

25. Ben Hamouda A, Boussadia O, Khaoula B, Laarif A, Braham M. Studies on insecticidal and deterrent effect of olive leaf on *Muzus persicae* and *Phthorimaea operculella*. J Entomol Zool Studies. 2015; 3(6):294-297.

26. Keita SM, Vincent C, Schmit JP, Ramaswamy S, Bélanger A. Effect of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J Stored Prod Res. 2000; 36 (4):355-364.

27. Kellouche A, Ait-Aider F, Labdaoui K, Moula D, Ouendi K, Hamadi N, Ouramdane A, Frerot B, Mellouk M. Biological activity of ten essential oils against cowpea beetle, *Callosobruchus maculatus Fabricius* (Coleoptera : Bruchidae). Int J Integ Biol. 2010; 10(2): 86-89.

28. Tapondjou LA, Adle C, Bouda H, Fontem DA. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post harvest grain protectant against six-stored product beetles. J Stored Prod Res. 2002; 38: 395-402.

29. Kosma P, Roméo B, Bouba D, Bouba-Armand A, Goudoum A. Bioefficacity of the powder of *Melia azedarach* seeds and leaves against *Callosobruchus maculatus*, on cowpea seeds (*Vigna unguiculata*) in storage. E3. J Agric Res Dev. 2014 ; 5 (4) : 072-078.

30. Kellouche A, Soltani N. Activité biologique des poudres de cinq plantes et de l'huile essentielle d'une d'entre elles sur *Callosobruchus maculatus* (F.). Int J Tropical insect sci. 2004; 24 (1):184-191. 31. Ben Hamouda A, Ammar M, Ben Hamouda MH. Effect of *Olea europaea* and *Cestrum parquii* leaves on the cuticule and brain of the desert locust, *Schistocerca gregaria* Forsk. (Orthoptera : Acrididae). Pest Technol. 2011; 5(1):55-58.

32. Mohammed HH. Repellency of ethalonic extract of some indigenous plants against *Tribolium confusum* (Coleoptera :Tenebrionidae). J Agric Vet Sci. 2013; 2(6): 27-31.

33. Ouguas Y, Hilal A, Elhadram I. Effet biocide des extraits phénoliques oléicoles sur les adultes du psylle de l'olivier *Euphyllura olivina* costa (Homoptera : Psyllidae) sur deux variétés d'olivier Menara et Arbequine au Maroc. Rev. Ezzaitouna.2010; 11(1):1-15.