

EVALUATION OF A BOTANICAL INSECTICIDE, LAVENDER (*LAVANDULA ANGUSTIFOLIA* (M.)) ESSENTIAL OIL AS TOXICANT, REPELLENT AND ANTIFEEDANT AGAINST LESSER GRAIN BORER (*RHYZOPERTHA DOMINICA* (F.))

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Abstract. In this study, the chemical composition of *Lavandula angustifolia* (Miller.1768) have been determined by gas chromatography-mass spectrometry. Then, we have evaluated the fumigant toxicity, the repellent and antifeedant properties, and also the effects on some biomarkers of essential oil extracted from *L. angustifolia* on adult of *Rhyzopertha dominica* (F. 1792) (Coleoptera: Bostrichidae). GC-MS analysis showed that this oil contains 56 compounds with linalool (20.42%) and linalyl acetate (13.24%) as the major components. This essential oil was found to exhibit insecticidal activity depending on the concentration and exposure period. In addition, the obtained results revealed an increase in the percent repellency. The enzymatic measurements showed a neurotoxic activity as evidenced by an inhibition of acetylcholinesterase (AChE). In addition, we observe a stimulation of the detoxification system as showed by an increase in glutathione-S-transferase (GST) activity and a decrease in glutathione (GSH) rate. Lastly, essential oil was investigated on nutritional indices. Results showed a decrease in the relative growth rate, relative consumption rate, efficiency of conversion of ingested food, and an increase in feeding deterrent index, accompanied by a decrease in digestive enzymes tested, α -amylase and protease in treated series when compared with control.

Keywords: biopesticide, fumigant toxicity, repellency, nutritional indices, biomarkers, digestive enzymes

Introduction

Stored grains are destroyed by insects, fungi, and vertebrates, but insect pests assume the greatest significance due to the favorable environmental conditions that promote their development (Aref and Valizadegan, 2015). According to the Food and Agriculture Organization (FAO), about 10-25% of the world's harvested food is destroyed by rodents and insect pests (Goergen et al., 2005). They induce qualitative and quantitative losses, such as grain weight loss, decrease in nutritional value and germination capacity of seeds, and product devaluation (Scheepens et al., 2011).

Producers rely on chemical insecticides to avoid losses (Ebadollahi and Sendi, 2015). Intensive use of synthetic insecticides, including phosphine, and sulfuryl fluoride, induce negative effects, such as residue threats, toxicity to non-target organisms, and outbreaks of secondary pests (Ngassoum et al., 2007). In Algeria, aluminium phosphide (Phostoxin^R) is commonly used to control infestations (Soltani-Mazouni et al., 2012). However, these insecticides possess strong secondary effects on the environment. In this context, there is an urgent need to develop eco-friendly materials and methods that only have slightly adverse effects on the consumers and the environment at large (Ebadollahi and Sendi, 2015).

Botanical insecticides are often effective alternatives to organophosphates or other neurotoxins for pest control (Gökçe et al., 2010). Plant materials, especially essential oils, which affects both behavioral and physiological aspects, have received elicited a great deal of attention as pest control substances (Isman et al., 2011). Many plant extracts can be used for stored product pest control. They act as bioactive chemicals, are selective, and have little or no harmful effects on the environment and non-target organisms (Regnault-Roger et al., 2012). They have been successfully exploited as insecticides (Tang et al., 2007), insect repellents (Islam et al., 2009) or insect anti-feedants (Gonzalez-Coloma et al., 2006) and may affect some biological parameters such as growth rate (Nathan et al., 2008), life span and reproduction (Isikber et al., 2006).

Fumigants from plant origins are said to have a greater potential in the future based on their efficacy, economic value, and use in large-scale storage (Lee et al., 2004). Among botanical insecticides, essential oils are natural products and can negatively affect the food consumption of insects; they are known as feeding deterrents or antifeedants (Wawrzyniak, 1996). *Lavandula angustifolia* Miller or true lavender is a perennial shrub of the family of Lamiaceae (Lis-Balchin, 2002), recognized for their antimicrobial, antioxidant, antifungal, and insecticidal activities (Yazdani et al., 2013; Costa et al., 2014; Duda et al., 2015; Badreddine et al., 2015). The oil profile of different sources of lavender might be different, so it is necessary to analyze the chemical composition of this oil by GC-MS.

Rhyzopertha dominica (F.), lesser grain borer, is regarded as a major stored product pest (Phillips et al., 2010), due to its high potential and wide host range of products. Both larvae and adults consume the germ and endosperm of wheat and rice during their development in grain, causing extensive damage (Dowdy and Mc Gaughey, 1992). A recent literature review was carried out to provide information on several subjects like biology, ecology, and control of *R. dominica* (Edde, 2012). The adults include sturdy fliers, which fly from warehouse to warehouse, causing severe infestation and convert the stored grains to mere frass (Negbenebor and Nura, 2020).

Chintala and Virani (2018) found that the total developmental period on the wheat variety under laboratory conditions at an average temperature of 30 ± 10 °C, and $70 \pm 5\%$ relative humidity was varied, with an average of 52.20 ± 5.66 days for males, whereas it was 95.85 ± 9.19 days for females.

In the present study, we determined the chemical composition of *L. angustifolia* EO and found that this oil is toxic to adults of *R. dominica*. In addition, we investigated the effects of this plant on *R. dominica* adults such as fumigant toxicity, repellent activity, feeding by measurement of some indices (relative growth rate, relative consumption rate, the efficacy of conversion of ingested food, and feeding deterrence index), and digestive enzymes. Finally, biomarkers of neurotoxicity and detoxification were also examined to give additional information on its mode of action. Our results indicate that the EO tested has the potential for controlling lesser grain borer.

Materials and methods

Insect rearing

The insect species used in this study i.e. lesser grain beetle borer, *R. dominica* was procured from a farmer (Youkous-Hammamet –Tebessa-Algeria; Latitude: $36^{\circ} 23'59''$ North, Longitude: $10^{\circ} 37'00''$ East, Altitude above sea level: 13 m). In cube containers, 1 kg wheat was used for insect rearing. For ventilation purposes, the containers (volume

2 L) were covered by mesh cloth. The incubators (SNIJDERS SCIENTIFIC-France) with 27 ± 1 °C and relative humidity of $65 \pm 5\%$ were used for insect rearing and experiments, as described by Aref and Valizadegan (2015). Experiments were carried out between May and July 2016, and in all experiments 7- to 14- old adult insects were used.

Plant material and essential oil extraction

L. angustifolia (Miller.1768) were harvested during May-July 2016 from the Tebessa area (Northeast Algeria) and identified with the help of plant taxonomist Dr. Hioune Soraya (Department of Biology, Larbi Tebessi University, Tébessa). Then, the plant parts were washed with tap water, to eliminate soil and other surface contaminants. After the dryness, at laboratory temperature and obscurity, the plant material was cut into small pieces. Subsequently, 50 g of the air-dried aerial parts of the species were subjected to hydro distillation for 3 hours to 500 ml distilled water using a Clevenger-type apparatus according to the method recommended in the British Pharmacopoeia (1988). The oil obtained was collected and dried over anhydrous sodium sulfate and then stored in screw-capped glass vials in a refrigerator at 4 °C before the analysis. Thereafter, the yield was calculated based on the dried weight of the samples (Costa et al., 2014).

Gas chromatography-mass spectrometry analysis

The essential oil of *L. angustifolia* was subjected to GC and GC-MS analysis. Gas chromatography-Mass spectrometry (GC-MS) analysis was performed with an HP Agilent 6890 plus gas chromatograph (GC) equipped with an HP-5MS column (a length of 30 m \times internal diameter of 0.25 mm, and 0.25 mm film thickness). The column oven temperature was set at 60 °C for 8 min and then increased to 250 °C at the rate of 2 °C/min. The injector and detector temperatures were kept at 250 and 270 °C, respectively. The carrier gas was helium, the flow through the column was 0.5 ml/min, and the split ratio was set to 50:1 with an injection of 0.2 μ l of oil sample. The GC/MS analysis was performed with a Quadruple mass spectrometer that operated at 70eV. Constituent's identification was based on a comparison of retention times with those of corresponding reference standards using the NIST and WILEY libraries (Adams, 2001). Percentage compositions of essential oils were calculated in accordance with the area of the chromatographic peaks.

Fumigant toxicity assay

The fumigant toxicity of essential oil on *R. dominica* was tested in 60 ml glass vials. In each of them, 20 adults (both sexes, male or female, 7-14 days old) were released. No.2 Whatman filter paper disks were cut to 2.5 cm in diameter and attached to the undersurface of glass vial screw caps. Filter papers were impregnated with series of pure concentrations of essential oil: 25, 50, 100, 150, and 200 μ l/l air. The choice of applied concentrations was established after a preliminary screening using a range of concentrations. Control insects were kept under the same conditions without essential oil. Each dose was replicated six times. After 24, 48, and 72 hours, from the beginning of exposure, numbers of dead and alive insects were counted. In these experiments, those insects incapable of moving their heads, antennae, and body were considered dead. This was followed by a correction in the mortality percentage. Sublethal and lethal concentrations (LC₁₀, LC₂₅, and LC₅₀) and 95% confidence limits (95% FL) were determined.

Repellent bioassay

The repellent effect of the essential oil against adults of *R. dominica* was evaluated using the method of the preferred area on filter papers as described by Mc Donald et al. (1970). Thus, the filter paper discs of 9 cm in diameter used for this purpose have been cut into two equal parts. Four concentrations were prepared (1, 2, 4, and 8 µl/ml) and diluted with acetone. Then, 0.5 ml of each solution prepared was spread evenly over one half of the disc. After 15 min, the time required for completing evaporation of the solvent dilution, the two halves of the discs were glued together using adhesive tape. The filter paper disc was restored and placed in a box before kneading a batch of 10 adult insects was placed in the center of each disk. Each treatment was replicated six times and the percentages of insects present on treated (**G**) and control (**P**) areas were recorded after 30 min, 3 h, 6 h, and 24 h. The percentage of repulsion (RP) was calculated using the following formula (Mc Donald et al., 1970):

$$PR = [(P-G) / (P+G)] \times 100 \quad (\text{Eq.1})$$

The average values were then categorized in accordance with the following scale: [Class 0 (RP < 0.1%), class I (RP = 0.1%–20.0%), class II (RP = 20.1%–40.0%), class III (RP = 40.1%–60.0%), class IV (RP = 60.1%–80.0%) and class V (RP = 80.1%–100%)].

Determination of nutritional indices

To examine the impact of EO on feeding efficiency, some nutritional indices were determined. The assay was conducted as previously described by Bahrami et al. (2016). The experiment was carried out in Petri dishes (diameter 9 cm). The adults of *R. dominica* were starved for 3 h before the experiment to exude gut contents. The solutions were prepared from the EO by dilution in ethanol to produce two concentrations (1 and 4%, W/W) applied with a micropipette in 5 g of wheat, as elucidated by Gökçe et al. (2010). After evaporation for 15 min at room temperature, 10 individuals were introduced into each box. Wheat, to which only the solvent had been applied, was used as the control. The adults were incubated for 72 h at 25 ± 2 °C and on a 16:8 (L: D). The weight of the adult and wheat before and after each experiment was also determined. Weight loss of diet caused by water evaporation was quantified by establishing two positive control treatments of 5 g of diet treated with a plant extract or solvent. The nutritional indices were calculated for adults according to the formula specified by Huang et al. (2000):

$$\text{Relative Growth Rate (RGR)} = (A - B)/(B \times \text{day}) \quad (\text{Eq.2})$$

where,

A: weight of live insect after the experiment (mg to each insect)

B: weight of insect before the experiment (mg to each insect)

$$\text{Relative Consumption Rate (RCR)} = D/(B \times \text{day}) \quad (\text{Eq.3})$$

where,

D: dried weight of food consumed by insects (mg)

$$\text{Efficacy of Conversion of Ingested Food (ECI)} = \text{RGR}/\text{RCR} \times 100\% \quad (\text{Eq.4})$$

$$\text{Feeding Deterrence Index (FDI)} = [(C - T)/C] \times 100\% \quad (\text{Eq.5})$$

where,

C: food consumed in control (mg)

T: food consumed in treatment (mg).

Digestive enzyme assay

Enzyme extracts adults were sampled from controls and treated series (LC₂₅ and LC₅₀) by fumigation. The whole body was homogenized in 1 ml of universal buffer (pH 7) and centrifuged (13,000 g for 15 min) as previously described by Valizadeh et al. (2013). The supernatant was used as the enzyme source. Of the 10 insects, three replicates each were used for each dose. The protein concentrations in each sample were determined in parallel according to Bradford (1976) and used to calculate the specific activity. The α -amylase activity was measured in line with what was described (Kilani-Morakchi et al., 2017) using dinitrosalicylic acid (DNS) as the reagent and 1% soluble starch as substrate. 20 μ l of the enzyme were incubated for 30 min at 35 °C with 100 μ l universal buffer (pH 7) and 40 μ l soluble starch. To stop the reaction, 100 μ l dinitrosalicylic acid (DNS) was added and heated in boiling water for 10 min. Thereafter, absorbance was read at 540 nm after cooling. One unit of α -amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 35 °C.

Protease activity was assayed using casein (1%) as substrate following the procedure of Garcia-Carreno and Haard (1993). Briefly, 200 μ l of 1% casein solution was added to 100 μ l enzyme and 100 μ l universal buffer (pH 7), and the mixture was incubated at 37 °C for 60 min. Proteolysis was stopped by the addition of 800 μ l of 5% trichloroacetic acid (TCA). The mixture was centrifuged at 8000 g for 15 min and the absorbance was read at 280 nm. The activity was calculated from a curve using tyrosine (Sigma, Italy) as a standard. Six replicates were used for each concentration. Data were expressed in μ mol/min/mg protein.

Biomarker assay

The lethal concentrations (LC₂₅ and LC₅₀) were applied on *R. dominica* adult. Their effects were examined on AChE and GST activities at various times (24, 48, and 72 hours) following treatment. The AChE activity was determined using acetylthiocholine as a substrate according to the method of Ellman et al. (1961) as previously described (Dris et al., 2017). Succinctly put, adult heads were homogenized in the detergent solution D [38.03 mg EGTA (ethylene glycol tetra-acetic acid, 1 ml Triton X-100, 5.845 g NaCl, and 80 ml Tris bufer (10 mM, pH 7)]. The AChE activity was determined from the absorbance changes at 412 nm for 20 min. The activity was expressed as nM/ min/mg proteins.

The assay of GST was carried out according to Habig et al. (1974) with the use of GSH (5 mM). The adult decapitated body was homogenized in 1ml phosphate buffer (0.1 M, pH 6). The homogenate was centrifuged (14000 rpm for 30 min). 200 μ l of the resulting supernatant was added to 1.2 ml of the mixture GSH-CDNB in phosphate buffer (0.1, pH 7). Changes in absorbance were measured at 340 nm every minute for 5 min. The activity was expressed as nM/min/ mg proteins.

The rate of GSH was then determined following the method of Weckberker and Cory (1988). Adult bodies were homogenized in 1 ml of EDTA (0.02 M, pH 6). The homogenate was subjected to a deproteinisation with sulfosalysilic acid (SSA, 0.25%)

(W/V). The optical density was measured at 412 nm. The amount was expressed as nM/mg proteins.

Statistical analysis

The number of individuals tested in each series is given with the results. Data are presented as the mean \pm standard errors (SE). Data of corrected mortality and the significance between different series were subjected to one-way analysis of variance (ANOVA) followed by a post-hoc HSD Turkey test. All statistical analyses were performed using Prism 7 (GraphPad Software Inc., www.graphpad with a significant level $p < 0.05$).

Results

Extraction yield and chemical analysis

The results of the steam distillation show that the yield of extraction of *L. angustifolia* essential oil was $3.2 \pm 0.15\%$ (dry matter of the plant).

Gas chromatography-mass spectrometer analysis of *L. angustifolia* essential oil led to the identification of 56 components. The percentages and the retention times of the identified compounds of the essential oil of *L. angustifolia* are listed in *Table 1*. The oil profile is characterized by linalool (20.48%), camphor (13.15%), linalyl acetate (13.24%), 1,8 Cineole (12.96%), Boreneol (10%), α - Cadinol (4.25%) and α - Terpineol (4.07%) (*Fig. 1*).

Fumigant toxicity assay

Figure 2 shows the percent mortality of *R. dominica* after exposure to different concentrations of the tested essential oils. The highest percentage of mortality was seen at 200 $\mu\text{l/liter}$ air concentrations of *L. angustifolia*. Since 100% mortality was achieved at 72 h after exposure at the highest concentration (200 $\mu\text{l L}^{-1}$ air) of the tested oils, we calculated LC_{10} , LC_{25} , and LC_{50} values of the essential oil along with their fiducial limits (*Table 2*).

Repellency bioassay

In this study, this test was applied to *R. dominica* adult. The percent repellency of *R. dominica* adult against 1, 2, 4, and 8 $\mu\text{l ml}^{-1}$ concentrations of *L. angustifolia* essential oil at different periods after treatment are presented in *Table 3*. The obtained results showed an increased repellency percentage depending on the exposure period and concentration. The maximum repellency rate of 86.96% was recorded with a dose of 8 $\mu\text{l ml}^{-1}$ at 24 hours.

Effects on biomarkers

To obtain information on the mode of action of *L. angustifolia* EO, activities of AChE and GST and GSH amounts were determined following the treatment of *R. dominica* adult. The results are shown in *Table 4*. A significant decrease ($p < 0.001$) of AChE activities was observed when essential oil was used at their LC_{25} and LC_{50} at 24, 48, and 72 h.

Table 1. Chemical composition of *L. angustifolia* essential oil: retention time (RT) and concentration (%) of different constituents

N°	RT	Compound	Area
1	7.921	α - Pinene	0.51
2	8.693	Camphene	0.62
3	10.264	β -Pinene	0.62
4	11.030	3-Octanone	0.26
5	11.276	β -Myrcene	0.73
6	12.293	Δ . 3-Carene	0.11
7	12.813	Acetic acide, hexyl ester	0.35
8	14.004	1,8 Cineole (Eucalyptol)	12.96
9	14.418	Cis-Ocimene	0.44
10	15.073	β -Ocimene	0.51
11	15.652	γ -Terpinene	0.14
12	16.707	Linalool oxide Cis	0.75
13	17.657	α - Terpinolene	0.28
14	17.825	Furfuryl Alcohol	0.48
15	19.638	Linalool	20.48
16	19.975	Octen-1-ol, acetate	0.46
17	20.597	Trimethyl cyclo pentadiene	0.09
18	22.124	Camphor	13.15
19	22.322	Propanoic acid	0.29
20	22.635	Neroloxide	0.21
21	22.987	Pinocarvone	0.08
22	23.936	Borcenol	10
23	24.380	Terpinene-4-ol	1.00
24	24.804	Cryptone	0.22
25	25.590	α- Terpeneol	4.07
26	25.773	2- Pinen-10-ol	0.10
27	26.322	Berbenone	0.24
28	26.968	2,6- Dimethyl-3,5,7-Octatriene-2-ol,E	0.20
29	27.460	Borneol	0.36
30	28.168	Cis-Geraniol	0.84
31	28.399	Butyl 2-Methyl butanonoate	0.32
32	28.746	Carvone	0.36
33	30.115	Linalyl Acetate	13.24
34	30.804	β - Citral	0.16
35	31.537	1 α - Bornyl acetate	0.31
36	32.144	Lavandulyl acetate	0.77
37	33.797	Cuminol	0.11
38	33.797	Thymol	0.11
39	34.679	Hexyl-Tiglate	0.31
40	36.959	Neryl acetate	1.39
41	38.246	Geranyl acetate	1.65
42	39.918	Caryophyllene	0.62
43	41.041	α - Bergamotene	0.08
44	42.516	Trans- β Farnesene	0.11
45	44.636	Bicyclogermacrene	0.08
46	45.561	β -Bisabolene	0.09
47	45.764	Naphtalene	0.36
48	46.241	L-Calamenene	0.12
49	47.913	Caryophyllene oxide	0.12
50	49.798	1-Methylene-2-vinylcyclopentane	1.66
51	51.663	Carotol	0.31
52	53.451	α- Cadinol	4.25
53	53.957	Bisabolol oxide	0.38
54	55.012	Azunol	0.31
55	55.798	α - Bisabolol	2.14
56	58.675	Naphthalenone	0.15

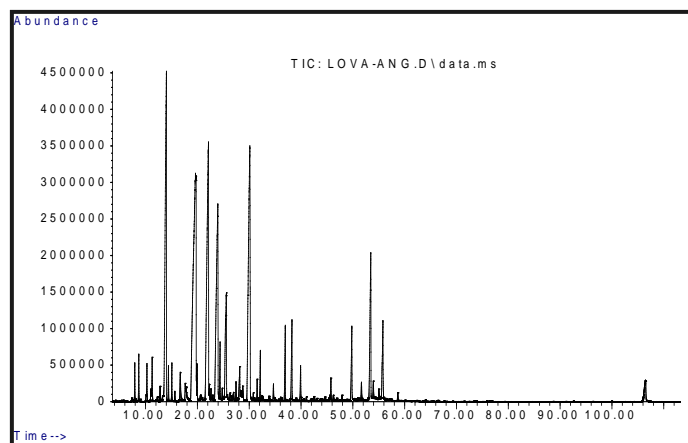


Figure 1. GC-MS chromatogram for essential oil obtained from *L. angustifolia*

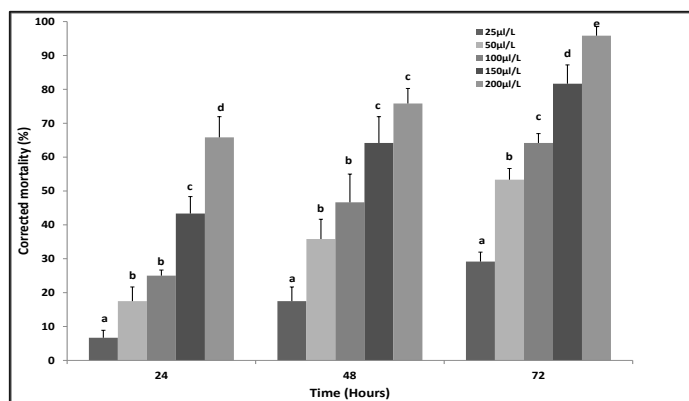


Figure 2. Efficacy of essential oil of *L. angustifolia* applied on adult of *R. dominica*: corrected mortality (mean \pm SE, $n = 6$ replicates each containing 20 adults)

Table 2. Lethal concentrations ($\mu\text{l/liter air}$, FL= 95%) of *L. angustifolia* essential oil against adult of *R. dominica*

Time (Hours)	Hill Slope	R ²	Concentrations ($\mu\text{l/liter air}$)		
			LC ₁₀ (95% FL)	LC ₂₅ (95% FL)	LC ₅₀ (95% FL)
24	1.77	0.94	46.23 (20.23-105.68)	85.90 (55.46-133.04)	159.22 (118.30-214.28)
48	1.20	0.97	14.75 (6.71-32.35)	36.72 (23.28-57.94)	91.62 (71.12-118.03)
72	1.36	0.94	9.79 (3.07-31.18)	21.92 (10.54-45.49)	48.97 (32.21-74.47)

Concerning GST activities, the treatment caused a significant induction at 24 h ($p < 0.01$; $p < 0.01$) and at 48 h ($p < 0.05$; $p < 0.01$) with LC₂₅ and LC₅₀, respectively, as well as at 72 h only with the highest concentration ($p < 0.001$). The amounts of GSH showed a significant decrease in the treated series at 24 h ($p < 0.01$) with LC₅₀ at 48 h ($p < 0.05$; $p < 0.01$) and at 72 h ($p < 0.01$; $p < 0.001$) with the two tested concentrations LC₂₅ and LC₅₀, respectively.

Table 3. Repellent activity (%) of essential oil of *L. angustifolia* against *R. dominica* adults at different exposure times

Concentrations	Time after treatment	RP (%)	Class
1 µl/ml	30min	26.66	II
	3hours	40.00	II
	6hours	43.33	III
	24hours	50.00	III
2 µl/ml	30min	32.66	II
	3hours	50.00	III
	6hours	53.33	III
	24hours	60.00	III
4 µl/ml	30min	50.00	III
	3hours	60.00	III
	6hours	63.33	IV
	24hours	73.33	IV
8µl/ml	30min	65.00	IV
	3hours	73.33	IV
	6hours	83.33	V
	24hours	86.96	V

Table 4. Effect of *L. angustifolia* essential oil applied at two concentrations (LC_{25} and LC_{50}) on the AChE, and GST activity (nM/min/mg of proteins) and GSH rate (nM/mg of proteins) in *R. dominica* adults (mean \pm SE, n= 3 pools each containing 20 individuals)

Time (hours)		Control	LC_{25}	LC_{50}
24	AChE	23.77 \pm 0.50 a	13.03 \pm 0.09 b	10.31 \pm 0.48 c
	GST	27.64 \pm 0.98 a	42.78 \pm 0.32 b	43.25 \pm 4.68 b
	GSH	14.78 \pm 0.43 a	12.84 \pm 1.10 a	9.63 \pm 0.76 b
48	AChE	22.84 \pm 0.45 a	14.36 \pm 0.13 b	10.24 \pm 0.75 b
	GST	28.99 \pm 1.24 a	38.24 \pm 0.35 b	42.15 \pm 3.60 b
	GSH	17.94 \pm 0.74 a	13.28 \pm 1.30 b	13.03 \pm 0.83 b
72	AChE	22.96 \pm 0.29 a	10.24 \pm 0.75 b	8.84 \pm 0.54 b
	GST	27.73 \pm 0.32 a	35.00 \pm 2.48 a	46.36 \pm 2.99 b
	GSH	18.51 \pm 0.31 a	14.83 \pm 0.76 b	11.65 \pm 0.38 c

For the same biomarker, the different letters indicate significant differences based on Tukey's HSD test (p < 0.05)

Effects on nutritional indices

Nutritional analyses revealed that the EO influenced all nutritional indices used when ingested by *R. dominica* adults (Table 5). When fed with a diet containing 1 and 4% (w/v) of EO, an increase in FDI (p < 0.01) of adults was observed for the two tested concentrations. However, a decrease in ECI (p < 0.05) was recorded for the higher concentration (4%). In addition, the RGR index was inversely proportional to the EO concentrations and it was significantly reduced (p < 0.001) with increased concentrations of EO (Table 5). This has shown that the highest concentration forces *R. dominica* to use less food and to have reduced growth. It was found that the essential oil of *L. angustifolia* has no effect (p > 0.05) on the RCR index.

Table 5. Effects of essential oil (% (w/v) of *L. angustifolia* on nutritional indices of *R. dominica* adults (mean \pm SE, n= 3 pools each containing 10 individuals)

Concentrations	RGR	RCR	ECI	FDI
	(mg/mg/h)		(%)	
0 %	0.17 \pm 0.01 ^a	2.23 \pm 0.46 ^a	8.39 \pm 2.09 ^a	-
1%	0.07 \pm 0.01 ^b	1.73 \pm 0.31 ^a	4.51 \pm 1.41 ^a	34.11 \pm 10.08 ^b
4%	0.02 \pm 0.00 ^c	1.60 \pm 0.05 ^a	1.55 \pm 0.14 ^b	54.39 \pm 5.80 ^a
<i>P</i>	0.0002	0.4120	0.0438	0.0035
<i>F</i>	45.79	1.032	5.513	16.76
<i>df</i>	2	2	2	1

RGR: relative growth rate; **RCR:** relative consumption rate; **ECI:** efficiency of conversion of ingested food; **FDI:** Feeding Deterrence Index; Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Tukey's Multiple Range Test

Effects on digestive enzyme activities

α -amylase is an enzyme hydrolyzing starch to maltose and glycogen to glucose. Adults EO-treated with LC₅₀ showed a significantly lower α -amylase activity in comparison to controls ($F_{2,6} = 11.88$; $p < 0.01$) (Fig. 3). The mean values recorded were 5.63 ± 0.68 $\mu\text{mol}/\text{min}/\text{mg}$ proteins for controls, 4.56 ± 0.39 $\mu\text{mol}/\text{min}/\text{mg}$ proteins for the LC₂₅, and 3.17 ± 0.16 $\mu\text{mol}/\text{min}/\text{mg}$ proteins for the LC₅₀. Statistical analysis revealed a significant difference between the control series and the highest tested concentration series ($p < 0.01$).

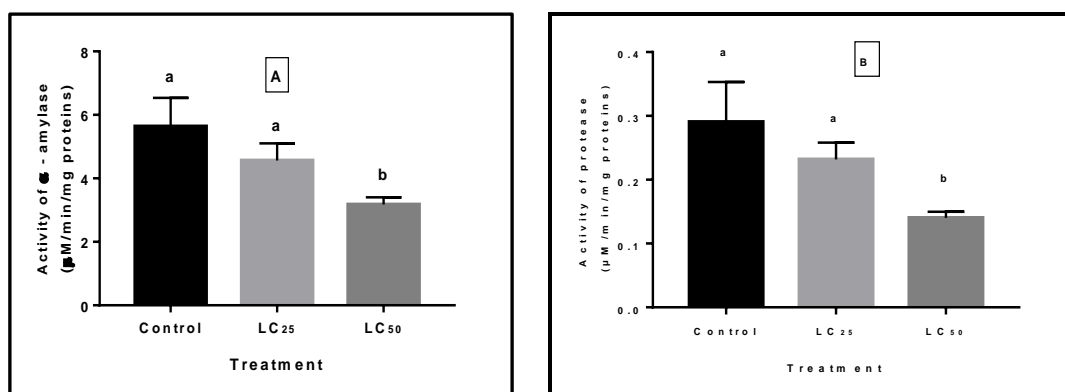


Figure 3. Effect of *L. angustifolia* EO, applied on adults of *R. dominica* on activity of α -amylase (A) and protease (B), ($m \pm SE$; n= 3 replicates each of 10 insects). Different small letters indicate a significant difference between control and treated series ($p < 0.01$)

General protease activity was lower in EO-treated adults with LC₅₀ as compared to the controls ($F_{2,6} = 10.96$; $p < 0.01$) (Fig. 3). In control series, the mean values recorded were 0.29 ± 0.004 $\mu\text{mol}/\text{min}/\text{mg}$. In treated series, the mean values recorded were 0.23 ± 0.01 $\mu\text{mol}/\text{min}/\text{mg}$ proteins for the lowest concentration (LC₂₅) and 0.14 ± 0.00 $\mu\text{mol}/\text{min}/\text{mg}$ proteins for the highest concentration (LC₅₀). According to statistical analysis, there was a significant difference between the control series and the highest tested concentration series ($p < 0.01$).

Discussion

Essential oil yield and composition

The qualitative and quantitative composition of lavender essential oil depends on genotype, growing location, climatic conditions, and morphological features (Prusinowska and Śmigielski, 2014). The quality of this oil depends on the high content of these major compounds and their mutual proportions (higher than 1) (Prusinowska and Śmigielski, 2014). Lavender oil primarily consists of linalyl acetate, linalool, lavandulol, 1,8-cineole, lavandulyl acetate, and camphor (Prashar et al., 2004). However, the relative level of each of these constituents varies in different species (Cavanagh and Wilkinson, 2002; Woronuk et al., 2011). The various lavenders have similar ethnobotanical properties and major chemical constituents (Cavanagh and Wilkinson, 2002).

In this study, the major components of *Lavandula angustifolia* (Lamiaceae) oil were linalool (20.48%) and linalyl acetate (13.24%) with a total yield of 3.2% relative to the dry matter. The EO of *L. angustifolia* from Iran contains 1,8-cineole (65.40%), borneol (11.50%), and camphor (9.50%) as the most abundant compounds (Hajhashemi et al., 2003). Whereas, 1,8-cineole is the primary compound (38.40%), followed by *cis*-verbenol (4.30%) and cymene-8-ol (3.80%) in the EO of *L. angustifolia* from the Cherrhell region (North Algeria) (Dob et al., 2005). These variations could either be attributed to differences in elevation, the genetic makeup of the plant, or due to an adaptive process to particular ecological conditions (Verma et al., 2010). Moreover, compositional variations can be observed in oils from different organs of the same species (Salleem et al., 2012). Distillation may also influence the composition of the oil, because isomerization, saponification, and other reaction may occur under distillation conditions (Zheljazkov et al., 2013). The results obtained by Zheljazkov et al. (2013) revealed the influence of the duration of distillation on the lavender oil yield and its composition. The maximum efficiency of the distillation process (2%) is achieved after 2 h and the minimal oil yield (1%) is obtained after 40 min of distillation (Wesołowska et al., 2010).

Insecticidal activity

EOs are lipophilic in nature and interfere with basic metabolic, biochemical, physiological, and behavioral functions of insects (Brattsten, 1983). These physical properties, such as high boiling point, high molecular weight, and low vapour pressure, are barriers for application in large-scale fumigation (Daglish, 2006).

In Africa, EOs have traditionally been used by small farmers to protect stored grains from insect pests. Inspired by the traditional practices in Guinea, extracts of four West African plant species, *Tagetes minuta* (Asteraceae), *Hyptis suaveolens* (Lamiaceae), *Ocimum canum* (Lamiaceae), and *Ocimum basilicum* (Lamiaceae), were assayed against adults of the bruchid *Callosobruchus maculatus* (Chrysomelidae) as protectants for stored cowpeas (Keita et al., 2000). In Algeria, powders from dry leaves of four plant species: *Ficus carica* (Moraceae), *Eucalyptus globulus* (Myrtaceae), *Olea europaea* (Oleaceae), and *Citrus limon* (Rutaceae), were evaluated under controlled conditions against *C. maculatus* (Kellouche and Soltani, 2004). Meanwhile, in developing countries, aromatic plants were widely used for stored-product insects in traditional agricultural systems. Currently, there is a move to replace these plants with steam-distilled EOs (Regnault-Roger et al., 2012). Botanical insecticides affect various insects in different ways depending on the physiological characteristics of the insect species and the type of the insecticidal plant (Hikal et al., 2017). The components of various botanical

insecticidal can be classified into six groups: repellents (Isman, 2006), feeding deterrents/antifeedants, toxicants (Tripathi et al., 2001), growth retardants (Papchristos and Stamopoulos, 2002), and attractants on stored product insects (Rajashekar et al., 2012).

In our study, the essential oil of *L. angustifolia* applied on *Rhyzopertha dominica* by fumigation was evaluated. Mortality was found to increase with the applied concentration and the exposure time. Fumigation studies showed that the essential oil had a “knockdown effect” on the test insect.

Essential oils usually extracted from various parts of the plant are traditionally used through fumigant or contact action to protect grains against storage pests, in some Asian and African countries (Shaaya et al., 1991). The insecticidal activity of some essential oils from Lamiaceae and other plants has been evaluated against several stored product insects (Negahban et al., 2007; Ayvaz et al., 2009). Rozman et al. (2007) revealed significant toxicity of *L. angustifolia*, *Rosmarinus officinalis* (Lamiaceae), and *Thymus vulgaris* (Lamiaceae) against *T. castaneum* (Tenebrionidae), *S. oryzae* (Dryophthoridae), and *R. dominica* (Bostrichidae). Shaaya et al. (1997) found that oils extracted from a Lamiaceae species have fumigant toxicity against four major stored-grain insects, namely *R. dominica*. As per the findings, an increase in concentration led to an increase in mortality rates. In the study of Shokri-Habashi et al. (2011), the essential oil of *Carum copticum* L. (Apiaceae) against *R. dominica* was evaluated and the LC₅₀ values were equal to 19.01 and 15.12 µl/l air after 24 and 48 h exposure times, respectively.

A monoterpenoid, linalool, has been demonstrated to act on the nervous system, affecting ion transport. The release of acetylcholine (Barocci et al., 2000) and can inhibit acetylcholinesterase (Mazzonetto, 2002) which at least in part accounts for the insecticidal effects of lavender EO. The insecticidal activity varied with insect species, oil concentrations, and exposure time. It can be concluded that essential oil products are generally broad-spectrum, due to the presence of several active ingredients that operate via several modes of action (Chiasson et al., 2004). Toxicity of pure and mixed compounds depends on their physicochemical properties and is the final result of the different toxicokinetic and toxicodynamic steps (penetration, distribution, metabolism, and interaction with the site of action) (Mc Donald et al., 1970).

Ho et al. (1995) concluded that adult mortality might be attributed to the contact toxicity or to the abrasive effect on the pest cuticle (Mathur et al., 1985), which might also interfere with the respiratory mechanism of insects (Agarwal et al., 1988).

Repellency activity

The repellent activity is a physiological phenomenon that occurs in insects as a defense mechanism against toxins secreted by plants. A botanical pesticide has a repellent property, which keeps insect pests away and protects the crops (Isman, 2006) with minimal impact on the ecosystem, as they remove insect pests from the treated materials by stimulating olfactory or other receptors (Talukder, 2006). The effectiveness and duration of repellency of chemicals depend on the type of repellent (active ingredient and formulation), the mode of application, and the local condition (temperature, humidity, and wind) (Barnard, 2000).

The present study revealed the effective repellent activity of *L. angustifolia* EO against *R. dominica*. The results showed that all tested concentrations induced a repellent activity with concentration and period-response relationship. According to Mc Donald et al. (1970), this plant belongs to the repellent class V. The repellent activity is related to major

active compounds and other chemical constituents (Abdelaziz et al., 2014). Hori (2004) revealed that, as one of the principal components of shiso oil, linalool displays a repellent activity against *L. serricone* (Anobiidae) (Mauchline et al., 2008). We found that *L. angustifolia* EO contained high amounts of linalool, as other researchers have reported (Danh et al., 2013). Similar observations have been made after application of Citrus limonum EO which have revealed significant repellent effects on *Sitophilus granarius* (Guettal et al., 2020). Zhang et al. (2017) reported the repellent activities of six *Zanthoxylum* (Rutaceae) species essential oils against two storage pests including *T. castaneum* (Tenebrionidae) and *L. serricone* (Anobiidae) adults. Ghavani et al. (2017) found that *Ziziphora tenuiore* (Lamiaceae), *Myrtus communis* (Myrtaceae), *Achillea wilhelmsii* (Compositae) and *M. piperita* (Lamiaceae) essential oils have repellent activities against human fleas, *Pulex irritans* (Pulicidae). Rahdari and Hamzei (2017) demonstrated the efficacy of *M. piperita* (Lamiaceae), *R. officinalis* (Lamiaceae), and *Coriandrum sativum* (Apiaceae) oils for applying in organic food protection due to the repellent activity of essential oils on *T. confusum* (Tenebrionidae). The repellent activity depends on the anti-insect mechanism and non-persistent volatility of essential oil sample (Hikal et al., 2017). However, the effectiveness of the repellents is might be attributed to multiple factors including the type of active ingredients, formulation, mode of application, environmental factors (temperature, humidity, and wind), the sensitivity of the insects to repellents, and the biting density (Hikal et al., 2017).

Effects on nutritional indices

In a bioassay of no-choice tests, the parameters used to evaluate antifeedant activity were relative growth rate, relative consumption rate, efficiency on the conversion of ingested food, and feeding and deterrence indices.

The discovery of novel toxins and/or antifeedants from plant extracts has been recently emphasized as a potential method for the development of ecologically safe pesticides (Wheeler et al., 2001). A high antifeedant index normally indicates a decreased rate of feeding, thereby resulting in the starvation of an insect. According to Isman (2002), the concept of using insect antifeedant for crop protectants/insect control is intuitively attractive; he listed many potent insect antifeedants that are extracted from the plant along with their chemical composition. Botanical pesticides inhibit feeding or disrupt insect feeding by rendering the treated materials unattractive or unpalatable (Talukder, 2006; Rajashekar et al., 2012). The insects remain on the treated material indefinitely and eventually starve to death (Hikal et al., 2017). In this regard, Liao et al. (2017) demonstrated that the oil of *M. alternifolia* (Myrtaceae) and their constituents possessed obvious antifeedant activities against *Helicoverpa armigera* (Noctuidae). However, Rama Rao et al. (2005) reported antifeedant and growth inhibitory effects of seed extracts of custard apple in hexane, ethyl acetate, and methanol against *Trogoderma granarium* (Dermestidae). The various bioassay showed that crude seed extracts of *A. squamosa* (Annonaceae) have both toxic as well as antifeedant properties as reported by Leatemia and Isman (2002). *Piper nigrum* (Piperaceae) and *Jatropha curcas* (Euphorbiaceae) extracts showed an antifeedant action against *C. cephalonica* (Pyralidae) larvae which increased with increasing extract concentrations (Khani et al., 2012); these results are in line with the present findings where concentration-dependent antifeedant and insecticidal potency was observed against the adult of *R. dominica*.

Reduction of RCR, ECI, and ECD led to a delay in adult growth and formation of smaller adults which have a direct relationship with the fecundity and longevity of the

insect (Sogbesan and Ugwumba, 2008). The observed decrease in ECI indicates that more food is being metabolized for energy and less is being concerted to body substance (growth); ingested EO also exhibited some chronic toxicity. Similarly, this result is in consonance with previous findings' reporting on the antifeedant activity of *M. azedarach* such as those of Coria et al. (2008) for *Aedes aegypti* (Culicidae), Bullangpoti et al. (2012) for *S. frugiperda* (Noctuidae), and Aouadia et al. (2012) for *Drosophila melanogaster* (Drosophilidae). ECI is an overall measure of an insect's ability to utilize the food ingested for growth and development (Koul et al., 2004). The relative consumption rate is used for measurement exploitation of food by insects. This index shows the rate of feed connected weight in insects at a certain point in time. The RGR and RCR reduction may be an indication of damages caused by allelochemicals present in the essential oil to the peritrophic membrane or cell surfaces in the midgut (Nasr et al., 2017). The rate of feeding in insects depends on the water and physicochemical properties of food (Srinivasan and Uthamasamy, 2005). The aqueous and ethanolic extracts of *Melia azedarach* exhibited an antifeedant activity and reduced the food consumption of *S. littoralis* (Noctuidae) larvae according to the applied concentrations (Akacha et al., 2017). In the study carried out by Gökçe et al. (2010), *Humulus lupulus* (Cannabaceae) and *Arctium lappa* (Asteraceae) exhibited antifeedant activity in *Choristoneura rosaceana* (Tortricidae) larvae, in addition to contact toxicity, while *Bifora radians* (Apiaceae) was an antifeedant and exhibited toxic effects when ingested. These plants are also known to deter the feeding of *Leptinotarsa decemlineata* (Chrysomelidae) larvae (Gökçe et al., 2006). Correspondingly, the experiment of Taghizadeh et al. (2014) revealed a decrease of nutritional indexes, RGR, RCR, and ECI of *L. decemlineata* (Chrysomelidae) with increased concentrations of EOs of six tested plants. In fact, due to the tendency of insects to consume food, growth rate and food consumption decreased. Also, FDI increased with increased concentrations of all essential oils.

This indicated that the active compounds present in the plant inhibit the larval feeding behavior while others disrupt hormonal balance or make the food unpalatable. These active substances may directly act on the chemosensilla of the larvae resulting in feeding deterrence (Hikal et al., 2017).

Effect on digestive enzyme activities

Digestion refers to the process wherein ingested macromolecules by insects break down to smaller ones to be absorbable via epithelial cells of the midgut. Several enzymes based on food materials play critical roles in this process. Any disruption in their activity disables insects to provide their nutrients for biological requirements (Zibae, 2011). Digestive enzymes, such as amylases, lipases, and proteases, play an important role in the body of insects by converting complex food materials into smaller molecules necessary in order to provide energy and metabolites (Teimouri et al., 2015).

Our results showed a clear disruption of digestive enzyme's activities responsible for the broken down of dietary components before its absorption by the intestinal epithelium. Many of the natural plant compounds used in the control of insect pests are known to affect digestive enzymes.

α -amylase is a midgut and salivary enzyme involved in starch and other carbohydrate metabolism and its activity level depends on feeding diet (Shekari et al., 2008). This enzyme was reduced in the series treated with LC₂₅ and LC₅₀ compared to the control series. The reduction of this enzyme activity could be caused by a cytotoxic effect of

different extracts on epithelial cells of the midgut that synthesize α -amylase (Jbilou et al., 2008).

Shekari et al. (2008) demonstrated that α -amylase activity level in *Xanthogaleruca luteola* (Chrysomelidae) treated by *A. annua* (Asteraceae) extract decreased after 24 h and sharply increased after 48 h. Zibae and Bandani (2010a) showed that *Artemisia annua* (Asteraceae) extract caused a reduction of α -amylase activity as the function (as concentrations) of plant extract in *Eurygaster integriceps* (Scutelleridae). Merx-Jacques and Bede (2005) also demonstrated that increased activity of amylase in *Spodoptera exigua* (Noctuidae) larvae fed on artificial diets in comparison with the larvae fed on legume, *Medicago truncatula* L. (Fabaceae). Azadirachtin was reported to disrupt insect physiology and its ability to digest food (Senthil-Nathan, 2013; Shannag et al., 2015). It significantly reduced the activity of α -amylase of adults of *D. melanogaster* (Drosophilidae) surviving to azadirachtin-treated third instar larvae (LD₂₅, LD₅₀) (Kilani-Morakchi et al., 2017).

Proteases are a group of enzymes that hydrolyze peptide bonds in proteins and convert them into their respective amino acids. Proteases have a crucial role in the food digestion of insects. Three subclasses of proteinases are involved in digestion classified according to their active site group: serine, cysteine, and aspartic proteinases. The oligopeptides resulting from proteinase action are attacked from the N-terminal end by aminopeptidases and from the C-terminal end by carboxypeptidases (Zibae, 2011). Studies by Johnson et al. (1990) and Senthil-Nathan et al. (2006) inferred those botanical insecticides may interfere with the production of certain types of proteases and disable them to digest ingested proteins. In the present study, compared with the controls, protease activity was significantly reduced in *R. dominica* after the treatment. The reduction of protease activity under botanical insecticide treatment was reported in several insects' species (Paranagama et al., 2001). Increased activity of proteases in *Ectomyelois ceratoniae* (Pyralidae) is probably caused by the need of insects for protein (Teimouri et al., 2015). Hemati et al. (2012) found significant differences in proteolytic activities in *Helicoverpa armigera* (Noctuidae) larvae reared on different host plants.

These results may reflect interference of *Lavandula* essential oil with the regulation of feeding and metabolism, which clearly supports the secondary antifeedant action of this oil that included a reduction in food consumption and digestive efficiency, thus reducing the access of nutrients for biological requirements. Perturbations of digestive enzymes cause a reduction in energy and metabolites and consequently affect normal growth.

Effects on biomarkers

Previous studies have shown that compounds extracted from diverse plants exhibited anti-insect properties by disturbing neuro-endocrine and growth regulatory processes (Xiao et al., 2012). Four types of detoxifying enzymes have been found to react against botanical insecticides including general esterases (EST), glutathione S-transferase (GST), and phosphatases (Zibae, 2011).

Acetylcholinesterase is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neuro-transmitter acetylcholine in the nervous system (Wang et al., 2010) and is an important target for insecticides (Van Leeuwen et al., 2005). Huignard et al. (2008) observed that the EO of *O. basilicum* (Lamiaceae) inhibited neuronal electrical activity by decreasing the amplitude of action potentials and reducing both the post-hyperpolarization phase as well as the firing frequency of action potentials. Several monoterpenes contained in EOs are neurotoxic to insects (Regnault-Roger et al., 2012).

Zibae and Bandani (2010b) showed that *Artemisia annua* (Asteraceae) extract induced inhibition of the AChE activity in higher doses in *Eurygaster integriceps* (Scutelleridae), which agreed with the findings of studies about the effect of botanical insecticides on AChE inhibition. The alteration of AChE was observed in the cockroach, *Periplaneta americana* (Blattidae) (Shafeek et al., 2004) and *Blatta orientalis* (Blattidae) treated with AZA (Tine et al., 2016). A number of monoterpenes also act on acetylcholinesterase. Terpinen-4-ol and 1,8-Cineole, found in EOs of *Eucalyptus globulus* (Myrtaceae), *Laurus nobilis* (Lauraceae), and *Origanum majorana* (Lamiaceae) (Regnault-Roger et al., 1993), inhibit acetylcholinesterase (Mills et al., 2004). Our results are similar to those obtained by Al-Sarar et al. (2014) showing that EOs of *Mentha longifolia* (Lamiaceae) and *Lavandula dentata* (Lamiaceae) inhibited the AChE activity in *C. maculatus* (Chrysomelidae) adults. The AChE was also inhibited in the treated mosquito larvae by *O. basilicum* (Lamiaceae) (Dris et al., 2017). The results of Acheuk et al. (2017) revealed that the exposure of *T. castaneum* (Tenebrionidae) adults to *Limoniastrum guyonianum* (Plumbaginaceae) extract significantly reduced AChE activity. This acetylcholinesterase inhibition could be a possible mechanism of action of *L. guyonianum*. Kabir et al. (2013) indicate that the extract of *Seseli diffusum* (Apiaceae) exhibited a potent larvicidal activity and induced strong neurobehavioral toxicity against the 4th instar larvae of *A. aegypti* (Culicidae). Inhibition of AChE causes accumulation of acetylcholine at the synapses, which will lead to paralysis and eventually, death of the insect. In a recent study, Gade et al. (2017) noted that stigmaterol and 1-hexacosanol, biological compounds from *Chromolaena odorata* (Asteraceae) were responsible for larvicidal activity against *Cx. quinquefasciatus* (Culicidae) via their neurotoxicity. At a molecular level, these compounds were found able to inhibit the acetylcholinesterase activity in *C. quinquefasciatus* and *A. aegypti* (Culicidae).

On the other hand, glutathione S-transferases (GST) are the mainly cytosolic enzymes that catalyze the conjugation of reduced glutathione (GSH) with a wide range of lipophilic toxicants bearing electrophilic sites (Habig et al., 1974). GSTs play an important role in insecticide resistance (Zibae et al., 2009) and participate in the primary detoxification, in phytophagous insects, of plant allelochemicals (Yu, 1987). Some plants defend allelochemicals against the GST activity induced by phytophagous insects (Yu, 1982; Vanhaelen et al., 2001). Vanhaelen et al. (2001) showed that Brassicacea secondary metabolites induced GST activity in *Myzus persicae* (Aphididae) and several Lepidopteran species such as *Heliothis virescens* (Noctuidae), *Trichoplusia ni* (Noctuidae) and *Anticarsia gemmatalis* (Erebidae). The influence of plant allelochemicals on GST activity is not limited to the herbivores and was observed in several predators (Francis et al., 2000). By applying *A. annua* (Erebidae) extracts on *E. integriceps* (Scutelleridae) adults, Zibae and Bandani (2010b) and Zibae (2011) reported that activity level of GST increased significantly at 24 h post-treatment. The induction of GST was observed in *B. orientalis* treated with AZA (Tine et al., 2016) and in *Cx. pipiens* (Culicidae) treated with *O. basilicum* (Lamiaceae) (Dris et al., 2017).

For GSH, this tripeptide is known as an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. It has been confirmed as a good indicator for oxidative stress; the amounts of GSH within cells are often used as a measure of cellular toxicity. In our experiments, it was evident that the increase of GST activity was also correlated with a decrease in GSH amounts after treatment with EO of *L. angustifolia*. Indeed, GSH is known as a non-enzymatic oxidative stress parameter; GSTs conjugate xenobiotics with the use of

reduced GSH. The reduction of GSH was observed in *Z. variegatus* (Pyrgomorphidae) exposed to pyrethroids (PYRs) and *O. gratissimum* (Lamiaceae) leaf extract, in *B. orientalis* (Blattidae) treated with AZA (Tine et al., 2016) and in *Cx. pipiens* (Culicidae) treated with *Thymus vulgaris* (Lamiaceae) (Bouguerra et al., 2018).

Conclusion

In recent years, the use of synthetic insecticides in the fight against agricultural pests has inflicted unintended damages on both human life and the environment. Plant materials with insecticidal properties have been traditionally used for generations in some parts of the world. These studies provide an interesting opportunity to develop bioinsecticides, repellents, and antifeedant formulations based on the extracts from plants. For all these reasons, we can infer that the essential oils of *L. angustifolia* could be considered a natural alternative in the control of stored grains insects such as *R. dominica*.

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