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Thème :

Étude de l’effet des huiles essentielles d’une espéce endémique a l’egard des larves de trogoderma granarium.

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INTRODUCTION

I. INTRODUCTION

Agricultural and animal stored products are subjected not only to physico-chemical aggressions but also to biotic attacks by more than 20,000 field insects including six hundred species of beetles, more than 70 moth species and around 355 species of mites, resulting in quantitative and qualitative losses worldwide (Bhumi et al. 2017; Nagpal and Kumar 2012; Rajendran 2005). This unfortunate situation not only poses health hazards to humans, leading to malnutrition but also imposes a significant annual financial burden of millions of dollars on the national exchequer (Nagpal and Kumar 2012).

Globally, the infestation of stored grain insects results in grain losses ranging from 10% to 20% (Pedigo and Rice 2014; Rajendran and Sriranjini 2008). The khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) is a key stored product insect pest that has been categorized as a quarantine organism by European and Mediterranean Plant Protection Organization (EPPO 2011). It has been considered as one of the most hundred hazardous invaders in the world (Lowe et al., 2000). The ability of *T. granarium* to spread and proliferate is highly determined by the fact of its capability to feed on a wide range of commodities such as stored cereals, amylaceous products, and various non-grain commodities (Degri and Zainab, 2013; Athanassiou et al., 2016; Kavallieratos et al., 2019). Furthermore, its larvae may tolerate the insecticidal treatments, develop a survival ability in worse conditions and fall under facultative diapause for several years, which makes them a global threat to food security (Dwivedi and Shekhawat 2004; Edde et al., 2012; Myers and Hagstrum, 2012; Athanassiou et al., 2015).

Management of agricultural insect pests over the past half century has been largely depending on the use of chemical pesticides and fumigants (methyl bromide, phosphine) for field and post-harvest protection of crops. These chemicals pose adverse effects on human health and surrounding ecosystems (Damalas and Eleftherohorinos 2011; Islam and Ahmed 2016; Islam et al. 2016a). In addition, it was observed that repeated use of these techniques has resulted in the development of insect resistance and the scenario has become challenging for entomology researchers (Ahmedani et al. 2007). These issues have prompted scientists to search for new classes of safer pest control agents that would be inexpensive, biodegradable, non-toxic to humans, and environmentally friendly. Several studies have shown insecticidal and physiological effects of essential oils of aromatic plants and their constituents (Oftadeh et al., 2020; Dutra et al., 2020; Gong & Ren 2020; Guettal et al., 2021a, b; Tine-Djebbar et al., 2023). They show a broad spectrum of activity against pest insects including fumigants (Jayakumar et al. 2017), contact insecticides (Aryani and Auamcharoen, 2016), repellents (Ebrahimifar et al. 2021), antifeedants (Ali et al. 2017; Sayada et al., 2021a) and can also affect certain biological parameters such as growth rate (Senthil-Nathan et al. 2008), developmental duration and reproduction (Boughdad et al. 2011).

Before using these substances to combat pests, it is crucial to have a thorough understanding of plant essential oils (EOs), or their active metabolites, and how they interact with the physiology of insect pests (Shahriari et al. 2019). Plant essential oils are typically composed of complex mixtures of mono-and sesquiterpenoids (El-Saadony et al., 2022). They have various modes of action targeting different systems such as nervous and neuromuscular systems (pyrethrum, sabadilla, nicotine and riania), mitochondria (rotenone), and hormonal system (azadirachtin). They interact with behavioral activity, metabolic processes, anatomy, biochemical activities, and certain physiological functions (Ahmed et al. 2021; Karabörklü & Ayvaz, 2023).

In the view of above-mentioned facts, present study was undertaken to investigate the effects of three bioactive molecules named: carvone, linalool, and limonene on *T. granarium* larvae such as fumigant and contact toxicity, repellent activity, and feeding by measurement of some digestive enzymes (α-amylase, protease, chitinase and lipase). Finally, enzymes of intermediary metabolism (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and esterase) and energy reserves were also examined to give additional information on their mode of action.

MATERIALS AND METHODS

II. MATERIALS AND METHODS

2.1. Insects rearing

Trogoderma granarium Everts (Coleoptera: Dermestidae) is an economic insect pest of stored products and has the status of quarantine species in several countries (Papanikolaou et al., 2019). Considered as an "alien invasive-species" in different parts of the world, it is a voracious primary feeder of sound cereal grains, broken food kernels, and their processed commodities with the ability to cause 30-70% losses in a short period of time (Kavallieratos et al., 2019). This beetle is one of the most notorious pests affecting stored cereals in numerous tropical and subtropical regions worldwide (Ahmedani et al., 2011). The larvae are spindle-shaped, with a brownish-yellow color, and covered in abundant tufts of hair (Fig. 1). They exhibit remarkable resilience, being capable of enduring adverse environmental conditions for extended periods (Appert, 1985). They also create silken webbing and fecal pellets, further degrading the quality of the stored food. The adult beetles are dark brown to black in color and have a characteristic appearance due to their humped thorax. They possess well-developed antennae and chewing mouthparts.

The collection of this pest was carried out at the Algerian interprofessional cereals office of Tebessa (OAIC). Its mass rearing was conducted at the Water and Environment Laboratory at the University of Tebessa. Individuals were kept in plastic jars, containing healthy, uninfested, and untreated wheat obtained from the cooperative for cereals and legumes as a food substrate. The rearing was maintained at 27 ± 3°C and 65% relative humidity for many generations in order to obtain healthy and pure individuals. The systematic position of this species is as follows:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Family: Dermestidae
Genus: Trogoderma
Species: *Trogoderma granarium* (Everts, 1898)

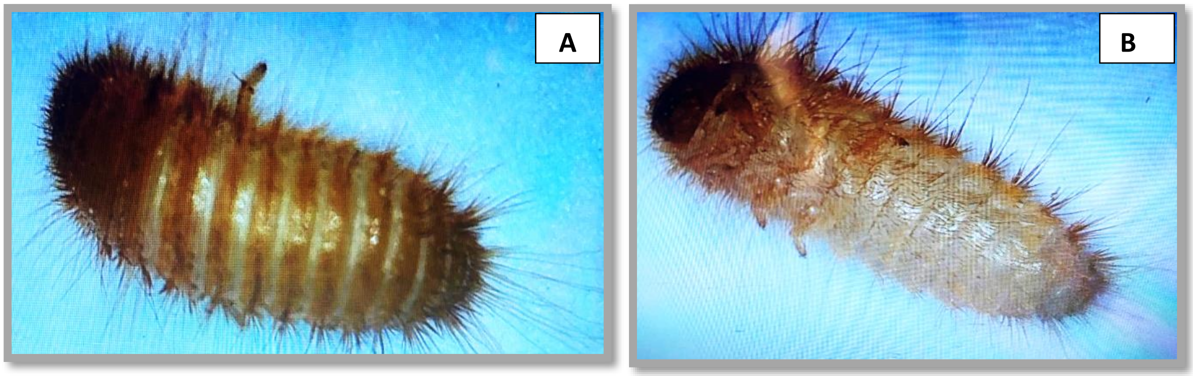


Figure 1. Trogoderma granarium: dorsal (A) and ventral (B) face (Personal photos).

2.2. Chemicals

2.2.1. Carvone

Carvone is a monoterpene ketone (2-methyl-5-(1-méthylethenyl)-2-cyclohexen-1-one) ($C_{10}H_{14}O$) with a boiling point of 230 °C, which has an asymmetric carbon. Chemically, carvone exists in two forms (enantiomers (+)-carvone and (−)-carvone) with the same chemical and physical properties and which differ only in their rotatory power (Fig. 2). This monoterpene is present in the essential oils of some plant species, including *Mentha spp.*, *Origanum spp.*, *Rosmarinus spp.*, *Thymus spp.*, and many others (Bouyahya et al .,2021; Bouyahya et al ., 2020a ;Bouyahya et al., 2020b ;Bouyahya et al., 2020c ;bouyahya et al., 2019 ;Bouyahya et al., 2017a ;Bouyahya et al., 2017b ;Bouyahya et al., 2017c).

With its varied actions on subcellular, cellular, and molecular actions, carvone is a natural product widely used in the pharmaceutical industry, cosmetics and agriculture (De Carvalho & Da Fonseca, 2006; Morcia et al., 2016). Furthermore, it has such effects as antioxidant (Zhao & Du, 2020), insecticide (Fang et al., 2010) and repellent (Yoon et al., 2007).

Figure 2. Carvone; a bioactive molecule, in liquid form (Personal photo).

2.2.2. Limonene

Chemically, limonene is 1-methyl-4-(1-methyl phenyl) cyclohexene consisting of 2 isoprene units having molecular formula as $C_{10}H_{16}$ (Burdock & Fenaroli, 2004). Limonene is a monocyclic terpene produced by more than 300 plants across the world (Burdock & Fenaroli, 1995), it is the most relevant compound in essential oils from the peels of citrus fruits, such as orange. Limonene is a colorless liquid and it exists as two optical isomers, named R- or L-limonene, and as a racemic mixture (Zuliakha & al .2015). In the Code of Federal Regulations, limonene is recognized as a safe substance (Thomas & Bessiére, 1989; Mira, 1999). Therefore, it is widely used in several industries, in edibles as a flavor additive in foods, and perfumeries and in cleaning products as a fragrance (Filipsson et al., 1998). Besides, this compound also has insecticidal properties (Ibrahim et al., 2001; Aciole et al., 2011; Ruiz et al., 2011).

Figure 3. Limonene; a bioactive molecule, in liquid form (Personal photo).

2.2.3. Linalool

Linalool exists in two enantiomeric forms, S-(+)- and R-(−)-linalool, which differ in their olfactory and physiological properties (Peana et al 2002). Many plants produce linalool, with members of the Lamiaceae (mints), Lauraceae (laurels, cinnamon, rosewood) and Rutaceae (citrus), often accumulating linalool in abundance (SIDS Initial Assessment Report. 2002) . Linalool is an unsaturated monoterpene alcohol with the specific odor description; “light and refreshing, floral-woody, with a faint citrusy note” (Arctander .1994). It is also the principal component of many essential oils known to exhibit several biological activities such as antibacterial and antiplasmodial effects (Van zyl et al.2006). Traditionally, it has been (and still is) used in the form of natural products such as dried herbs, as a fumigant for the storage of cereals and as a deterrent against pests (Kamatou & Viljoen, 2008). The repellent properties of this molecule together with negligible toxicity to humans and low tendency to bioaccumulation, justifies further research to investigate its feasibility as a phytobiocide.

Figure 4. Linalool; a bioactive molecule, in liquid form (Personal photo).

Results

Ⅲ.Results :

3.1Insecticidal activity:

3.1.1 Fumigation toxicity:

After a screening test, different concentrations of carvone, limonene and linalool were applied against *T. granarium* larvae through fumigation. Parallel control groups were also set up. No mortality was observed in the control series.

The corrected mortalities recorded during the fumigation toxicity tested by carvone ranged from 15 % at 24 h to 41.67 % at 72 h for the lowest dose (66.66 µl/ml), and from 58.33% at 24 h to 96.67% at 72 h for the highest dose (266.66 µl/ml). For limonene, the corrected mortalities ranged from 8 % at 24 h to 28 % at 72 h for the lowest dose (500 µl/ml), and from 34% at 24 h to 96 % at 72 h for the highest dose (1000µl/ml). Finally, for linalool corrected mortalities ranged from 0 % at 24 h to 24 % at 72 h for the lowest dose (133.33 µl/ml), and from 26% at 24 h to 98 % at 72 h for the highest dose (1066.66µl/ml).

Mortalities increase significantly according to the applied doses of and the time after treatment in *T.granarium* larvae treated by fumigation. For carvone, at 24 h ($F_{3,20}$= 91; $p<0.0001$), 48 h ($F_{3,20}$=115.7; $p<0.0001$), and 72 h ($F_{3,20}$=95.52; $p<0.0001$). For limonene, at 24 h ($F_{3,16}$= 29.04; $p<0.0001$), 48 h ($F_{3,16}$=91.50; $p<0.0001$), and 72 h ($F_{3,16}$=115.1; $p<0.0001$). Finally, for linalool, at 24 h ($F_{3,16}$= 28.07; $p<0.0001$), 48 h ($F_{3,16}$=62.93 ; $p<0.0001$), and 72 h ($F_{3,16}$=159.6; $p<0.0001$).

The results indicate that the three bioactive components applied through fumigation exhibit insecticidal activity with a dose-response relationship towards *T. granarium*. The ranking of doses using the Tukey's HSD test reveals the existence of 3 groups for carvone in the 3 periods. The treatment with limonene reveals the existence of 3 groups in 24, 48h, and 4 groups in 72h. Finally, for linalool, we have 3 groups in 24h and 4 groups in 48h and 72h after treatment.

The dose-response curve expressing the percentage of mortality as a function of the logarithm of the applied doses (Fig. 12) allowed for the estimation of lethal concentrations (LC) along with their confidence intervals and HillSlope (Table 5).

Table 4. Toxicity of carvone (A) limonene (B) linalool (C) applied through fumigation (µl/l of air) on *T. granarium* larvae at different periods: Corrected mortality (%) (mean ± SEM, n = 5 repetitions of 10 individuals each): Tukey's HSD test.

A

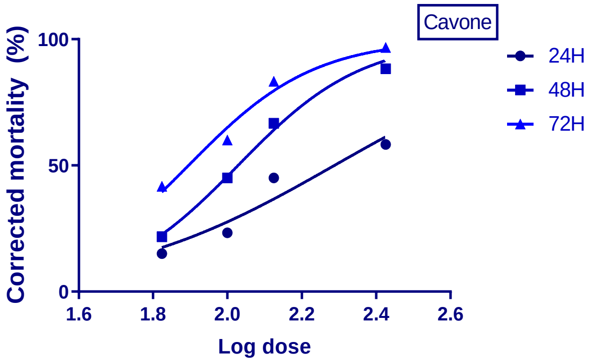
dose time	66.66	100	133.33	266.66
24 h	15.00±5.00 a	23.00±4.44 b	45.00±5.00 c	58.33±2.77 d
48 h	21.66±2.77 a	45.00±6.66 b	66.66±4.44 c	88.33±5.55 d
72 h	41.67±5.56 a	60.00±3.33 b	83.33±4.44 c	96.67±4.44 d

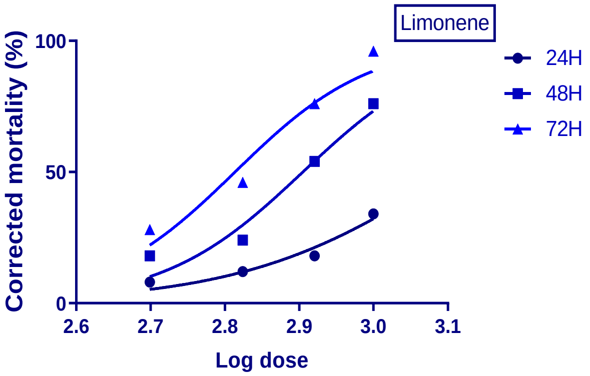
B

dose time	500	666.66	833.33	1000
24 h	8.00±3.20 a	12.00±3.20 a	18.00±3.20 b a	34.00±4.80 c
48 h	18.00±3.20 a	24.00±4.80 a	54.00±7.20 b	76.00±4.80 c
72 h	28.00±6.40 a	46.00±4.80 b	76.00±4.80 c	96.00±4.80 d

C

dose time	133.33	266.66	533.33	1066.66
24 h	0.00±0.00 a	6.00±4.80 a	14.00±4.80 b a	26.00±4.80 c
48 h	18.00±6.40 a	38.00±3.20 b	52.00±6.40 c	82.00±6.40 d
72 h	24.00±4.80 a	54.00±4.80 b	80.00±4.00 c	98.00±3.80 d





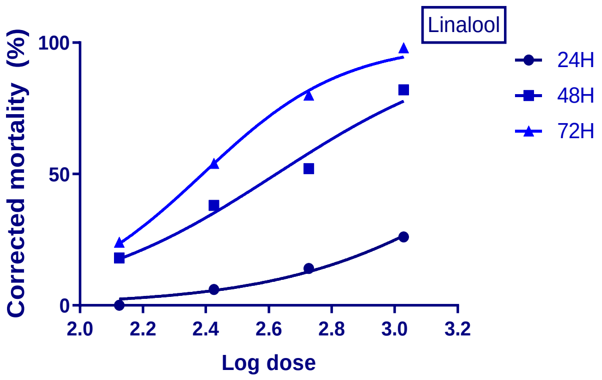


Figure 12. Effect of carvone, limonene and linalool applied through fumigation on *T. granarium* larvae at different periods: Dose-response curve expressing the percentage of corrected mortality as a function of the logarithm of doses.

Table 5. Efficacy of Carvone (A) Limonene (B) Linalool (C) applied by fumigation on *T. granarium* larvae: determination of lethal concentrations and their confidence intervals (95%).

A

Space	time	R2	Hill slope	LC 25 CI	LC 50 CI
T.granarium	**24 H**	0.91	1.45	91.09 0.25±162.10	194.10 119.00±161473
	48 H	0.99	2.60	70.04 52.90±84.29	106.80 93.55±122.50
	72 H	0.97	2.57	51.30 20.69±70.86	78.60 52.14±96.70

B

Space	time	R2	Hill slope	LC 25 CI	LC 50 CI
T.granarium	**24 H**	0.91	1.45	892.20 756.70±1383	1272 1030±5815
	48 H	0.99	2.60	632.90 344.50±797.00	803.40 658.10±1051
	72 H	0.97	2.57	517.00 204.90±684.70	651.10 446.20±793.20

C

Space	time	R2	Hill slope	LC 25 CI	LC 50 CI
T.granarium	**24 H**	0.91	1.45	1005 778.90±169.90	2350 1488±8084
	48 H	0.99	2.60	185.40 58.59±332.00	420.5 262.20±691.90
	72 H	0.97	2.57	139.10 99.23±178.90	245.4 202.80±293.90

3.1.2 Ingestion toxicity:

After a screening test, different concentrations of carvone, limonene and linalool were applied against *T. granarium* larvae through ingestion. Parallel control groups were also set up. No mortality was observed in the control series.

The corrected mortalities recorded during the contact toxicity tested by carvone ranged from 6.66 % at 12 h to 38.33 % at 48 h for the lowest dose (8 µl/ml), and from 68.33% at 12 h to 100% at 48 h for the highest dose (64 µl/ml). For limonene, the corrected mortalities ranged from 12 % at 12 h to 36 % at 48 h for the lowest dose (120 µl/ml), and from 94% at 12 h to 100 % at 48 h for the highest dose (150µl/ml). Finally, for linalool corrected mortalities ranged from 7.5 % at 12 h to 37.5 % at 48h for the lowest dose (30 µl/ml), and from 45% at 12 h to 100 % at 48 h for the highest dose (60 µl/ml).

Mortalities increase significantly according to the applied doses of and the time after treatment in *T.granarium* larvae treated by contact. For carvone, at 12 h ($F_{3,20}$= 202.3; p<0.0001), 24 h ($F_{3,20}$=181.7; p<0.0001), and 48 h ($F_{3,20}$=252.3; p<0.0001). For limonene, at 12 h ($F_{3,16}$= 242.9; p<0.0001), 24 h ($F_{3,16}$=179.6; p<0.0001), and 48 h ($F_{3,16}$=169.2; p<0.0001). Finally, for linalool, at 12 h ($F_{3,16}$= 36.86; p<0.0001), 24 h ($F_{3,16}$=48.31 ; p<0.0001), and 48 h ($F_{3,16}$=81.00; p<0.0001).

The results indicate that the three bioactive components applied through fumigation exhibit insecticidal activity with a dose-response relationship towards *T. granarium*. The ranking of doses using the Tukey's HSD test reveals the existence of 4 for carvone and limonene with the three periods. For linalool, we have 3 groups in 12, 24h and 4 groups in 48h after treatment.

The dose-response curve expressing the percentage of mortality as a function of the logarithm of the applied doses (Fig. 13) allowed for the estimation of lethal concentrations (LC) along with their confidence intervals and HillSlope (Table 7).

Table 6. Toxicity of carvone (A) limonene (B) linalool (C) applied through contact (µl/ml of air) on *T. granarium* larvae at different periods: Corrected mortality (%) (mean ± SEM, n = 4 repetitions of 10 individuals each): Tukey's HSD test.

A

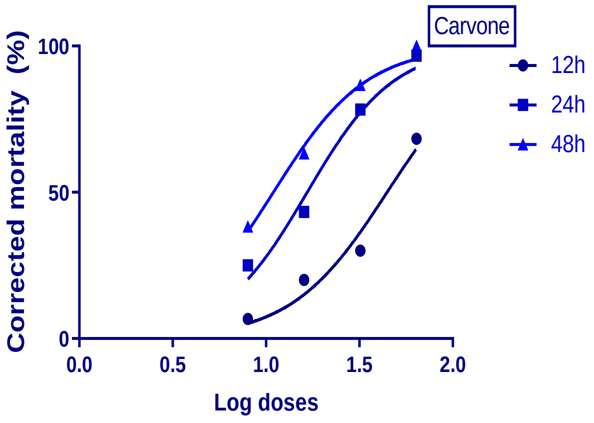
dose time	8	16	32	64
12 h	6.66±4.44 a	20.00±0.00 b	30.00±3.33 c	68.33±2.77 d
24 h	25.00±5.00 a	43.33±6.66 b	78.33±2.77 c	96.66±4.44 d
48 h	38.33±2.77 a	63.33±4.44 b	86.66±4.44 c	100.00±0.00 d

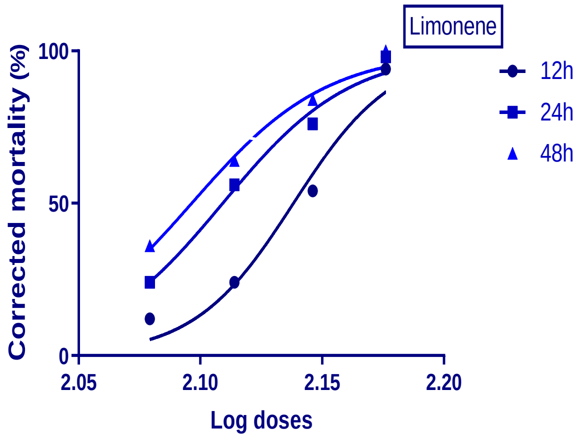
B

dose time	120	130	140	150
12 h	12.00±3.20 a	24.00±4.80 b	54.00±4.80 c	94.00±4.80 d
24 h	24.00±4.80 a	56.00±4.80 b	76.00±4.80 c	98.00±3.20 d
48 h	36.00±4.80 a	64.00±4.80 b	84.00±4.80 c	100.00±0.00 d

C

dose time	30	40	50	60
12 h	7.50±3.75 a	15.00±5.00 a	27.50±3.75 b	45.00±5.00 c
24 h	17.50±3.75 a	27.50±7.50 a	50.00±5.00 b	75.00±5.00 c
48 h	37.50±7.50 a	55.50±5.50 b	85.00±5.00 c	100.00±0.00 d





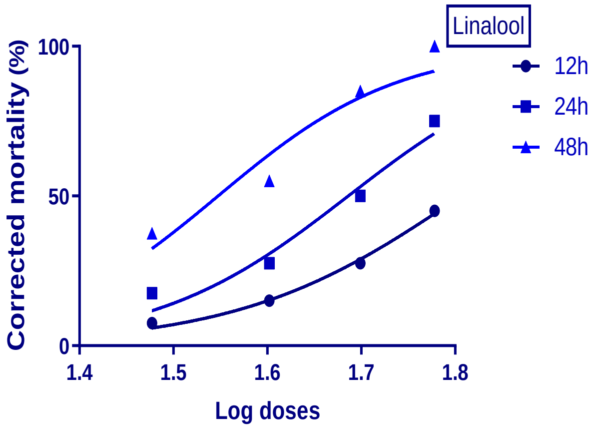


Figure 13. Effect of carvone, limonene and linalool applied through contact on *T. granarium* larvae at different periods: Dose-response curve expressing the percentage of corrected mortality as a function of the logarithm of doses.

Table 7. Efficacy of Carvone (A) Limonene (B) Linalool (C) applied by contact on *T. granarium* larvae: determination of lethal concentrations and their confidence intervals (95%).

A

Space	time	R2	Hill slope	LC 25 CI	C 50 CI
T.granarium	**12 H**	0.96	1.69	23.25 8.94±41.87	44.49 28.37±110.20
	24 H	0.97	1.86	9.22 3.74±15.19	16.62 10.72±24.00
	48 H	0.98	1.73	5.82 2.74±8.62	10.95 7.52±14.34

B

Space	time	R2	Hill slope	LC 25 CI	LC 50 CI
T.granarium	**12 H**	0.96	21.28	130.60 115.30± ??	137.50 129.60±145.00
	24 H	0.98	16.59	120.30 110.50±126.30	128.60 123.20±133.30
	48 H	0.98	15.75	226.60 ??± ??	124.90 119.40±129.00

C

Space	time	R2	Hill slope	LC 25 CI	LC 50 CI
T.granarium	**12 H**	0.99	3.65	47.39 44.17±50.34	64.00 59.73±71.84
	24 H	0.96	4.20	37.38 23.82±46.17	48.55 41.15±60.03
	48	0.92	4.53	27.69 1.70±38.81	35.28 10.23±44.77

3.2 **Repellent effect:**

The results of the repellent potential of carvone, limonene and linalool toward *T. granarium* larvae are presented in Table 8. The percentage of repellence shows an increase based on the applied concentrations and decrease with exposure time with the two bioactive molecules linalool and carvone, but for limonene it shows an increase also with exposure time.

A high rates of repellence (100%), (95%) and (80%) were observed at 1h, 24h and 6h with the highest concentration (24 µl/ml) of linalool, limonene and carvone, respectively.

Furthermore, both limonene and linalool are classified in category 5 (Highly repellent). For carvone, it is classified in category 4 (Repellent).

Table 8. Percentage (PR) and class (CR) of repellence of carvone, limonene and linalool on *T. granarium* larvae.

		Linalool		**Limonene**		**Carvone**	
dose	**Period**	**RP**	**CLASS**	**RP**	**CLASS**	**RP**	**CLASS**
6 ul/ml	30 min	20	I	15	I	20	I
	1 h	30	II	20	I	25	I
	3 h	25	II	25	II	30	II
	6h	20	II	40	II	35	II
	12 h	15	I	45	III	20	I
	24 h	10	I	50	III	10	I
12ul/ml	30 min	65	IV	40	II	30	II
	1h	75	IV	45	III	35	II
	3h	70	IV	50	III	40	II
	6h	65	IV	55	III	45	III
	12h	50	III	60	III	35	II

	24h	45	III	55	III	25	II
24ul/ml	30 min	95	V	60	III	55	III
	1h	100	V	65	IV	65	IV
	3h	95	V	75	IV	75	IV
	6 h	90	V	80	IV	80	IV
	12 h	85	V	85	V	65	IV
	24 h	75	IV	95	V	55	III

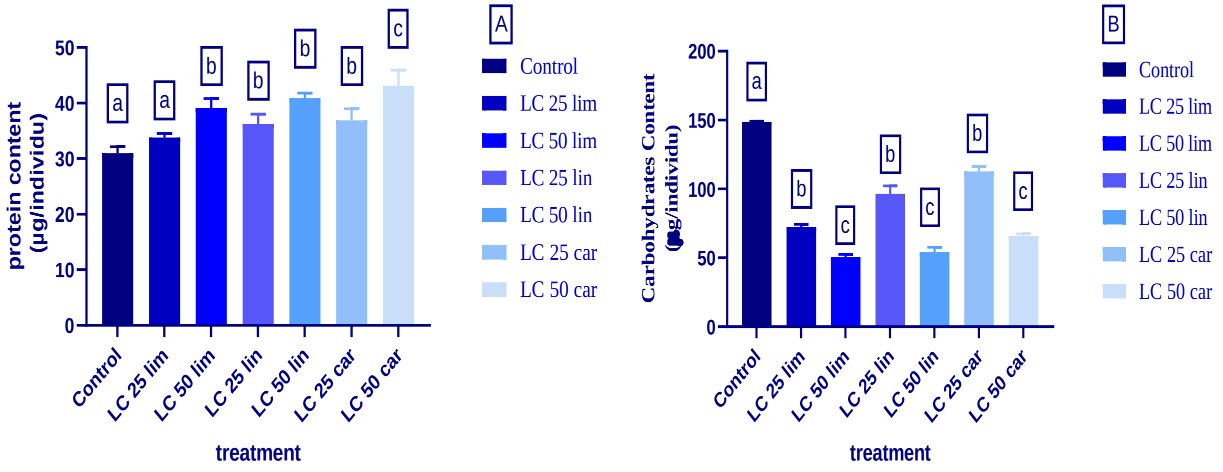
3.3 Effect of the Three Bioactive Molecules on Biochemical Composition:

Carvone, limonene and linalool were administered by fumigation to *T. granarium* larvae with two lethal concentrations (CL25 and CL50). Their effects were evaluated on the biochemical composition (energy reserves and proteins) of this species at 72h after treatment.

According to the results presented in Figure 14 (A), a significant increase in total protein content was observed after treatment with both applied concentrations ($F_{6,14}$=16.99; p<0.0001). A dose effect was observed (CL25 vs CL50: p=0.0279) in limonene and also for carvone (CL25 vs CL50: p=0.0091). Tukey's HSD test highlights 3 groups of groups.

The results of carbohydrate analysis reveal a significant decrease in the treated groups at CL25 and CL50 ($F_{6,14}$= 382.30: p<0.0001) compared to the controls, with a dose effect in the three molecules (CL25 vs CL50: p<0.0001). Tukey's HSD test highlights 3 groups (Fig. 14 B).

The results mentioned in Figure 14 C show a significant decrease in lipid content in the treated groups at CL25 and CL50 ($F_{6,14}$= 552.70: p<0.0001) with a dose effect in the three molecules (CL25 vs CL50: p<0.0001). The ranking of means by Tukey's HSD test highlights 3 groups.



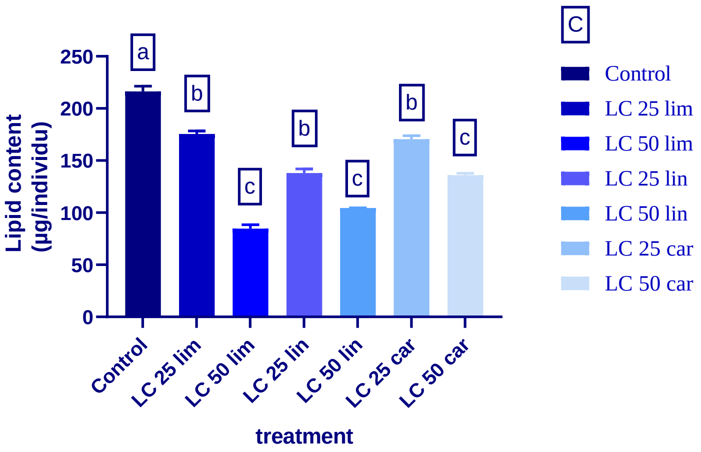


Figure 14. Effect of carvone, limonene and linalool (CL25 and CL50) applied through fumigation against *T. granarium* larvae on protein content (A), carbohydrate content (B), and lipid content (C) at 72h after treatment (mean ± SEM, n=3 repetitions, each consisting of 10 individuals).

3.4 Effect on enzymes of intermediary metabolism:

T. granarium larvae treated by fumigation with two lethal concentrations of carvone, limonene and linalool (CL25 and CL50), and their effects were evaluated on the specific activity of four enzymatic biomarkers: ALT, ASP, Esterase and ALP.

The results mentioned in Figure 15A show a significant increase in ALT activity in the treated groups ($F_{6,14}$= 45.24: p<0.0001). A dose effect was observed just with linalool (CL25 vs CL50: p<0.0001).The ranking of means by Tukey's HSD test highlights 3.

The application of the three bioactive components induced a significant increase in AST activity in the treated groups ($F_{6,14}$=12.76: p<0.0001). A dose effect was observed just with limonene (CL25 vs CL50: p<0.0001). The ranking of means by Tukey's HSD test highlights 2 groups (Fig. 15B).

An increase in esterase activity was observed ($F_{6,14}$= 3.60: p=0.0224). There is no dose effect in the three applied bioactive components. The ranking of means by Tukey's HSD test highlights 2 groups (Fig. 15C).

ALP activity shows a significant increase in the treated groups at CL25 and CL50 ($F_{6,14}$= 17.64: p<0.0001) with a dose effect in linalool (CL25 vs CL50: p=0.0001). The ranking of means by Tukey's HSD test highlights 2 groups (Fig. 15D).

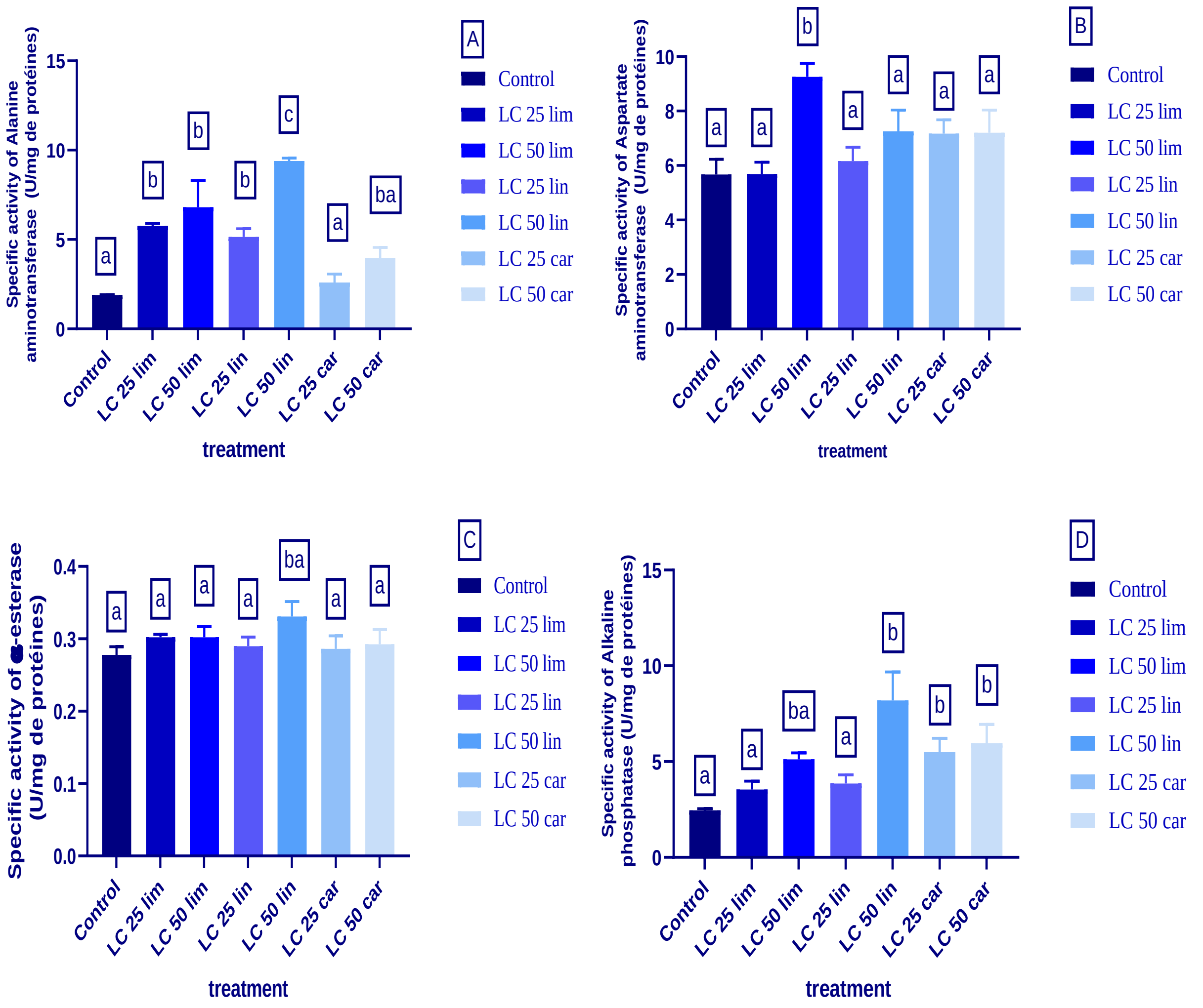


Figure 15. Effect of carvone, limonene and linalool (CL25 and CL50) applied through fumigation against T. granarium larvae on enzymatic biomarkers: ALT (A), AST (B), Esterase (C), and ALP (D) at 72h after treatment (mean ± SEM, n=3 repetitions, each consisting of 10 individuals).

The results mentioned in Figure 16 show a significant decrease in the amount of triglyceride in the treated groups ($F_{6,14}$= 44.68: p<0.0001). A dose effect was observed in linalool (CL25 vs CL50: p=0.0028) and in carvone (CL25 vs CL50: p=0.0157). The ranking of means by Tukey's HSD test highlights 3 groups.

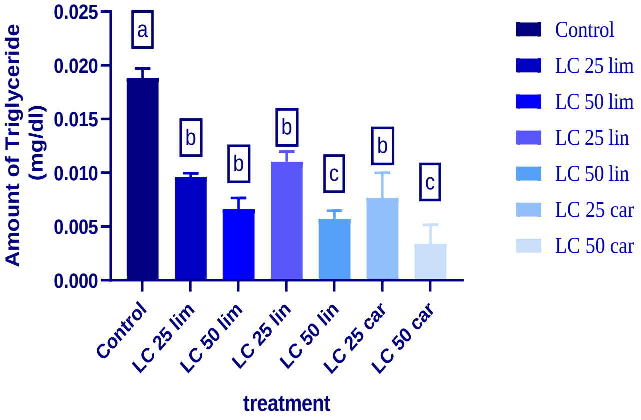


Figure 16. Effect of carvone, limonene and linalool (CL25 and CL50) applied through fumigation against *T. granarium* larvae on the amount of triglyceride, at 72h after treatment (mean ± SEM, n=3 repetitions, each consisting of 10 individuals).

3.5 Effect on digestive enzymes:

T. granarium larvae treated by fumigation with two lethal concentrations of carvone, limonene and linalool (CL25 and CL50),). Their effects were evaluated on the specific activity of four digestive enzymes: α-amylase, protease, chitinase and lipase.

Regarding the α-amylase activity, the application of the three bioactive molecules induced a significant decrease in the treated group ($F_{6,14}$= 82.97: p<0.0001) compared to the controls. A dose effect was observed just in carvone (CL25 vs CL50: p= 0.0086). Tukey's HSD test revealed 3 groups (Fig. 17A).

Furthermore, the results of the protease assay revealed a significant decrease in the treated groups at CL25 and CL50 ($F_{4,16}$= 54.67: p<0.0001) compared to the controls. A dose effect was observed in limonene (CL25 vs CL50: p=0.0019), in linalool (CL25 vs CL50: p=0.0025) and also in carvone (CL25 vs CL50: p=0.0011). Tukey's HSD test classified the means into 3 groups (Fig. 17B).

The results of the chitinase assay show a significant decrease in chitinase activity in the treated groups at CL25 and CL50 ($F_{6,14}$= 44.54: p<0.0001). A dose effect (CL25 vs CL50: p<0.0001) (CL25 vs CL50: p<0.0001) was observed in both limonene and carvone, respectively. However, no dose effect was reported in linalool (CL25 vs CL50: p>0.05) (Fig. 17C). Tukey's HSD test classified the means into three groups.

Finally, the results shown in Figure 17D demonstrate a significant decrease in lipase activity in the treated groups at CL25 and CL50 ($F_{6,14}$= 28.71: p<0.0001). A dose effect was observed in limonene (CL25 vs CL50: p=0.0011) and in linalool (CL25 vs CL50: p=0.0017). Tukey's HSD test classified the means into three groups.

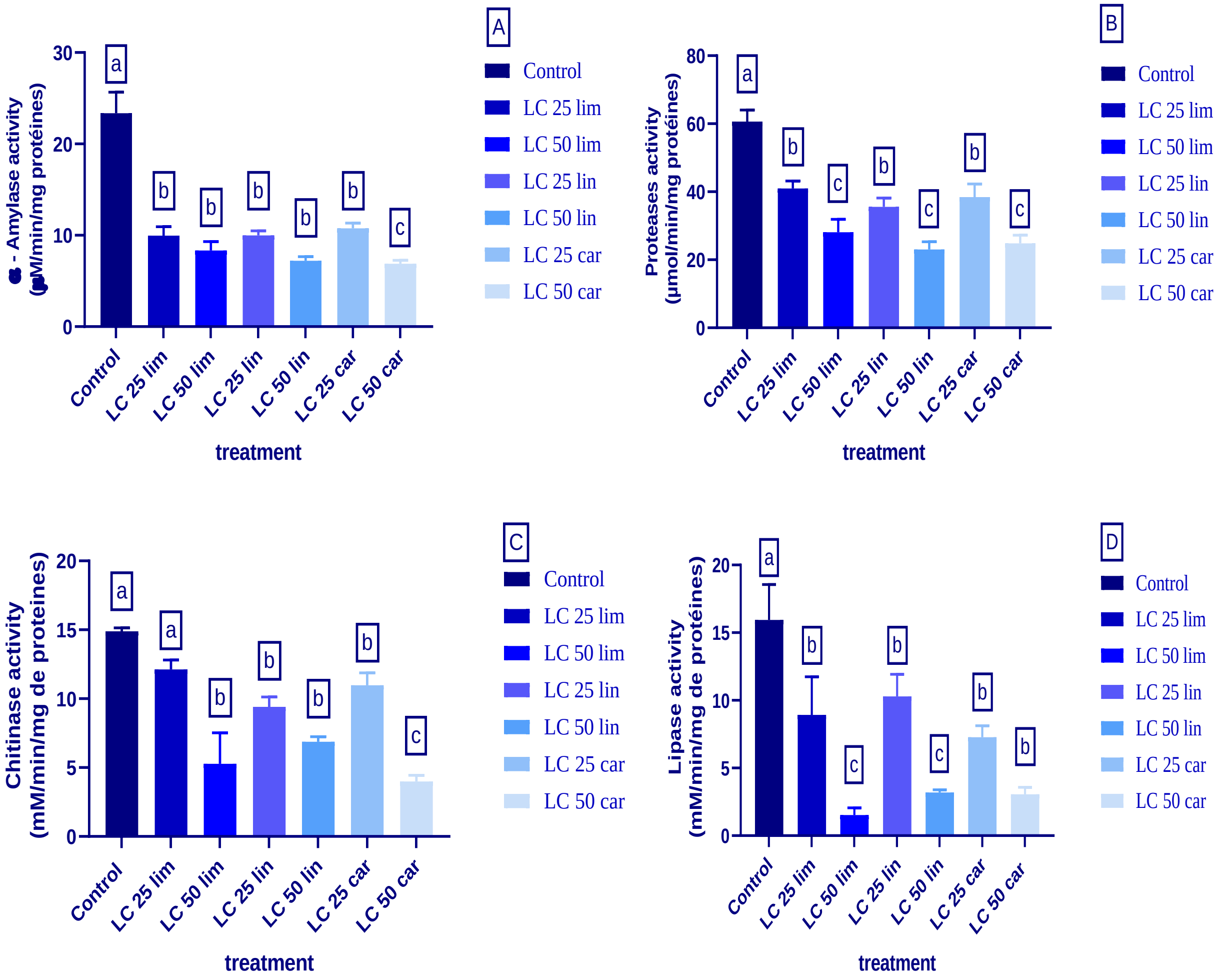


Figure 17. Effect of carvone, limonene and linalool (CL25 and CL50) applied through fumigation against *T. granarium* larvae on digestive enzymes activity: α-amylase (A) Protease (B) Chitinase (C) and lipase (D), at 72h after treatment (mean ± SEM, n=3 repetitions, each consisting of 10 individuals).

Discussion

IV DISCUSSION.

4.1.Insecticidal activity :

Aromatic plants possess and release fragrant volatile substances in the form of essential oil, gum exudate, oleoresin, and balsam in different parts; thus, the chemical composition of these compounds includes plant-specific properties.

Essential oils are complex substances derived from plants which are mainly composed of aromatic (alcohols, aldehydes, phenols, methoxy compounds, and methylene dioxy derivatives) and aliphatic compounds (lactones, alcohols, acids, acyclic esters or aldehydes, coumarins, and compounds bearing nitrogen and sulfur), hydrocarbon terpenes (isoprenes, monoterpenes, sesquiterpenes, hemiterpenes, diterpenes, triterpenes, and tetraterpenes), and terpenoids (isoprenoids, phenols, alcohols, ketones, aldehydes, acids, esters, and ethers) (Eslahi et al. 2017).

So far, on commercial scale, essential oils have been obtained from 400 plant species belonging to 67 families, and in this respect, Lamiaceae, Asteraceae, and Apiaceae families are at the forefront. Fabaceae, Lauraceae, Rutaceae, Myrtaceae, Cupressaceae, Pinaceae, Zingiberaceae, Burseraceae, and Rubiaceae are also known as important essential oil reserves (Duke et al. 2002; Kalia 2005). Because essential oils and their fractions have various biological properties, they exhibit significant insecticidal activity as well as bactericidal, virucidal, and fungicidal use in the management of various microorganisms (Bakkali et al. 2008). Essential oils contain several volatile compounds that affect the biology and behavior of insects; thus, their insecticidal, repulsive, and deterrent effects can also significantly reduce the reproductive potential of insects (Regnault-Roger 1997; Isman 2000; Karabörklü et al. 2019).

Many essential oil components were tested on the storage pests for various purposes; they exhibit pronounced effectiveness and cause significant insecticidal effects. In our study, carvone, limonene, linalool applied on *T. granarium* were evaluated. Corrected mortality of *T. granarium* treated by fumigation and contact increases according to the applied doses and the time after treatment. Carvone was the most effective among the three applied bioactive molecules. Also, it is noted that ingestion is the most effective method of application against this specie compared to fumigation, but fumigation is still the most practical mode.

Many studies have reported the insecticidal activities of the above components against stored grain pests: Carvone obtained from *Anethum graveolens*, *Carum carvi*, *Coriander sativum* and *Ocimum basilicum* essential oils exhibited excellent potency as a fumigant toxin on *Blattella germanica*, *Callosobruchus maculatus*, *Cryptolestes pusillus*, *Rhyzopertha dominica*, *Sitophilus granaries*, *Sitophilus oryzae* and *Sitophilus zeamais* (Lopez et al. 2008; Yeom et al. 2012; Kim et al. 2013; Mbata and Payton 2013; Yildirim et al. 2013; Herrera et al. 2017; Kordali et al. 2017).

Likewise, essential oil limonene reported in *A. dubia*, *Amomum villosum*, *C. sinensis*, *C. sativum*, *Eucalyptus sp.* and *Z. armatum* proved to be an efficient fumigant toxin on *C. maculatus*, *L. serricorne*, *L. bostrychophila*, *P. interpunctella*, *R. dominica*, *S. oryzae*, *S. cerealella*, *T. castaneum*, *T. confusum*, and *T. putrescentiae* (Prates et al. 1998; Lee et al. 2001, 2018; Tripathi et al. 2003; Stamopoulos et al. 2007; Kim and Lee 2014; Wang et al. 2015a; Chen et al. 2018; Liang et al. 2018; Oyedeji et al. 2020). Similarly, the limonene component found in *C. sinensis* and *C. sativum* plants showed effective contact toxicity on *C. maculatus*, *P. interpunctella*, *R. dominica*, *S. oryzae*, *S. zeamais*, *S. cerealella*, *T. castaneum*, and *T. putrescentiae* (Prates et al. 1998; Tripathi et al. 2003; Kim and Lee 2014; Lee et al. 2018a; Oyedeji et al. 2020).

Moreover, linalool obtained from *Artemisia rupestris*, *Carum carvi*, *Citrus sinensis*, *Coriander sativum*, *Evodia lenticellata*, *L. nobilis*, *L. angustifolia*, *L. spica*, *Litsea cubeba*, *Ocimum basilicum*, *Origanum majorana*, *R. ofcinalis*, *S. lavandulifolia*, *T. quinquecostatus*, *T. vulgaris*, *T. zygis*, and *Z. bungeanum* essential oil displayed effective fumigant toxicity on *Callosobruchus maculatus*, *C. pusillus*, *L. serricorne*, *L. bostrychophila*, *Plodia interpunctella*, *R. dominica*, *S. granarius*, *S. oryzae*, *S. zeamais*, *Sitotroga cerealella*, *T. castaneum*, *T. confusum*, and *T. putrescentiae* (Lopez et al. 2008; Yang et al. 2014; Rozman et al. 2007; Stamopoulos et al. 2007; Mbata and Payton 2013; Kim and Lee 2014; Kordali et al. 2017; Cao et al. 2018; Lee et al. 2018a, b; Kheloul et al. 2020; Lu et al. 2021; Liang et al. 2022). Besides, the linalool addressing *C. sinensis*, C. *sativum*, L. *cubeba*, *O. basilicum*, and *S. lavandulifolia* also demonstrated important contact toxicity on *C. maculatus*, *L. serricorne*, *L. bostrychophila*, *P. interpunctella*, *S. zeamais*, *S. cerealella*, *T. castaneum*, and *T. putrescentiae* (Kim and Lee 2014; Yang et al. 2014; Lee et al. 2018a, b and Oyedeji et al. 2020).

4.2 Repellent activity:

In recent decade, there has been great interest in research on behavioral manipulation of insects as an alternative to broad-spectrum insecticide application for crop protection (da Camara et al. 2015). Essential oil components have direct toxic effect such as contact, ingestion, and fumigation, as well as repellent and deterrent effect on insects (Akhtar et al. 2010). Repulsion is a defense mechanism exerted by plants against insects (Jayakumar et al., 2017). This physiological phenomenon can be used to drive away these pests from the treated materials. Repellent substances act locally or at a distance, preventing an insect from flying, landing, or biting an animal or human (Blackwell et al., 2003; Nerio et al., 2009; Ebadollahi et al., 2013). This activity is linked to the active ingredients and the chemical constituents of oils (Damalas & Eleftherohorinos, 2011).

Our findings demonstrate that linalool, limonene and carvone exhibit a high rates of repellence (100%), (95%) and (80%) at 1h, 24h and 6h respectively, with the highest concentration (24 µl/ml).

Previous results have revealed the repellent power of a wide range of essential oils and their constituents. Linalool found in *Aframomum melegueta*, *E. lenticellata*, and *L. spica* has significant repellent effect on *L. serricorne*, *L. bostrychophila*, *R. dominica*, *T. castaneum*, and *T. confusum* (Ukeh and Umoetok 2011; Cao et al. 2018; Kheloul et al. 2019). Cao et al. (2018) reported that linalool showed 76 and 84% repellency (Class IV) on *L. serricorne* at high concentrations (78.63 and 15.83 nL cm−2) and at 2 h. Some other studies reported that limonene found in *Amomum villosum*, *Artemisia dubia*, *Atalantia guillauminii*, *Baccharis reticularia* and *Litsea cubeba* essential oil showed important repellent activity on *Lasioderma serricorne*, *Liposcelis bostrychophila* and *Tribolium castaneum* (Yang et al. 2014 ; Liang et al. 2018 ; Chen et al. 2018 ; Lima et al. 2021 ; Pang et al. 2021). Furthermore, the studies conducted by Ojimelukwe & Adler (1999) demonstrated the repellent effect of several constituents, with Zimtaldéhyde (98.53%) being the most effective compared to Eugenol (80%), Thymol (70.39%), Hydroxy-anisol (50.37%), Terpinéol (42.97%), and Menthol (40.74%) against *T. confusum*. Hence, The repellent potential of phytochemical compounds against pests depends on several factors, such as the oils' chemical composition and the insect's sensitivity (Casida & Quistad, 1995).

4.3 Effect on biochemical composition:

Essential oils and their constituents affect various insect functions, including metabolic, physiological, and behavioral processes (Mann and Kaufman 2012). They can cause biochemical disturbances manifested by an increase or decrease in various metabolites (proteins, carbohydrates, lipids) (Yazdani et al., 2013 ; Dris et al., 2017 ; Gnanamani & Dhanasekaran, 2017).

The results obtained from this study showed that the application of carvone, limonene and linalool by fumigation to *T. granarium* resulted in a general increase in the total proteins levels and a significant decrease in lipids and carbohydrates content.

Proteins are crucial constituents of cells and living systems since the various enzymes responsible for metabolic cascades in organisms are predominantly proteins (Preet & Sneha, 2011). They serve various functions, such as hormonal regulation and enzymatic catabolism; also incorporated into the cellular structure alongside carbohydrates and lipids (Cohen, 2010; Sugumaran, 2010).

The decrease in insect body protein levels indicates a reduction in protein synthesis, limited food assimilation, and decreased amino acid uptake for protein synthesis under insecticide stress conditions (Ribeiro et al., 2001; Vijayaraghavan et al. 2010). In the other hand, the increase in the protein content could be the result of an elevation in tissues metabolic activity to compensate the stress caused by the bioactive molecules through the synthesis of various protein-based regulators involved in regulatory and defense mechanisms in the organism, such as enzymes and hormones. This was shown in previous reports: *R. dominica* treated with *S. molle* (Tine et al. 2021a), *T. granarium* treated with *E. globulus* (Tine et al. 2021b), *R. dominica* (Tine et al. 2017), *Sitophilus granarius* treated with azadirachtin (Guettal et al. 2021a, b) and in *R. dominica* (Bouchagra & Farhi, 2022) and *T. confusum* (Lahmar & Benhadda, 2022) treated with Linalool.

Carbohydrates play a crucial metabolic role in the developmental cycle (Steele 1981) and serve as a vital source of energy. Several investigations revealed that pesticides interfere with carbohydrate metabolism in different species. Some authors have reported elevated carbohydrate contents in some insects, while others have noticed either the opposite or no change.

Similar results were reported in *S. granarius* treated with citrus oil and azadirachtin (Guettal 2021a), *T. castaneum* treated with *Agastache foeniculum* EO (Ebadollahi 2013), *S. granarius* treated with *C. limonum* EO (Guettal et al. 2020), *T. granarium* treated with *S. molle* (Tine et al. 2021b), and in *R. dominica* treated with *E. globulus* (Tine et al. 2021a). Under stressful conditions, insects may metabolize more carbohydrates to meet their energy requirements (Yazdeni et al., 2014). This could potentially explain their depletion in treated insect pests. However, the increase in the total body carbohydrates might be because they are unable to assimilate the food thereby increasing the level of carbohydrate in their tissues, or by enhancement of glycogenolysis leading to the hyperglycemia (Sharma et al.2011).

Lipids are also essential components consist of fatty acids, phospholipids, and sterols that are integral to the cell walls of insects and contribute to various other functions (Chapman, 1998). Lipid reserves appear to be the result of a balance between food intake and the energy expenditures necessary for processes such as growth (Beenakkers et al., 1981). Their reduction that induced by the three bioactive ingredients could be due to several mechanisms, including formation of lipoproteins utilized for repair of damaged cell and tissue organelles, direct utilization by cells for energy requirements, increased lipolysis and damage to cellular organization and adipokinetic hormone and other hormones controlling the metabolism of lipid (Steele,1985; Lohar & Wright, 1993). Several studies have shown fluctuation of lipid content in insects treated with different bioinsecticides: *R. dominica* adults treated with menthol (Tine et al. 2023), *S. granarius* treated with Citrus oil (Guettal et al. 2020), azadirachtin (Guettal et al. 2021b) and combination Aza-EO (Guettal et al. 2021a), in *R. dominica* treated with *E. globulus* EO (Tine et al. 2021a), and in *T. granarium* treated with *S. molle* EO (Tine et al. 2021b).

4.4 Effect on intermediary-involved enzymes:

Intermediary metabolism is a complicated phenomenon in which energy is provided for biological activities via several processes, such as detoxification of xenobiotics, etc

Our results revealed an increase in GOT, GPT, Esterase and PAL activities in *T. granarium* larvae treated with carvone, limonene and linalool.

Transamination is a crucial physiological process within insects' bodies, responsible for synthesizing proteins necessary for diverse functions (Chapman et al., 2013). Additionally, it plays a pivotal role in the energy processes of insects, including the conversion of alanine to proline (Hakkak et al., 2018; Sugeçti and Büyükgüzel 2018). ALT catalyzes the two parts of the alanine cycle in proline metabolism, while AST facilitates the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate, both of them are found in the hemolymph and fat bodies of insects (Nation, 2008). They are released in the hemolymph of insects only when the cells are damaged or destroyed (Abo El Makarem et al. 2015; Pradel and Albert 2021). Their elevated activities could suggest a higher protein content, potentially facilitating the use of amino acids for tissue development, excretion, and energy requirements. The surplus proteins need to be stored in fat bodies, and any excess amounts must be excreted due to their detrimental effects. However, Decreased alanine aminotransferase activity may be due to a lack of energy supply through proline or the need for amino acids due to tissue damage caused by the compounds used (Goharrostami et al. 2022). Our results agreed with other findings on the effect of some plant essential oils on GOT and GPT activities (Abdel-Latif and Al Moajel 2004; Arshad et al. 1999; Hassan 2002; Tabassum et al. 1994). However, decreased enzymatic activity of transaminases was reported in *Helicoverpa armigera* fed on a diet containing β-cytosterol (Mishra et al. 2020) and in in *Tribolium castaneum* treated with *Wedelia trilobata* and *Melissa ofcinalis* EOs (Khater and El-Shafey 2015).

ALP hydrolyse phosphate groups of several molecules, including nucleotides, proteins, and alkaloids in alkaline and acidic conditions through dephosphorylation. Several phenomena, such as efficiency of digestion and transportation of nutrients in the midgut as well as hemolymph, significantly affect the activity of this enzyme (Zibaee et al., 2011). The induction of this enzyme in our study agrees with the results of Mardani-Talaee et al. (2014) and disagrees with other found: *G. pyloalis* larvae treated with thyme EO, thymol, and carvacrol (Goharrostami et al. 2022), and *E. kuehniella* treated with *Teucrium polium* EO and α-pinene (Shahriari et al. 2019). In these latter, the lack of digestive function and decreased metabolism caused by a decrease in the release of phosphate groups for the production of energy can be indicated by a drop in the activity of ALP (Selin-Rani et al. 2016; Senthil-Nathan 2006).

Esterases play a crucial role in the metabolism of insects. They are enzymes that catalyze the hydrolysis of ester bonds, which are found in various compounds, including insecticides, pheromones, and other endogenous substances. The primary function of esterases in insects is the detoxification and elimination of foreign compounds, including insecticides, by breaking them down into less toxic or inactive forms. This enzymatic activity is essential for the development of resistance to insecticides, as certain esterases can effectively metabolize and degrade these chemicals, rendering them ineffective. Esterases also have roles in the regulation of physiological processes, such as hormone metabolism, pheromone production, and lipid metabolism. Additionally, they are involved in the regulation of neurotransmitters and neurotransmission, making them significant players in insect physiology and behavior. However, terpenes are known to compete for the active site of the compounds to be detoxified and thus reduce the activity of these enzymes (Khosravi et al. 2013a; War et al. 2014; Tarigan et al. 2016; Oftadeh et al. 2020).

Excess calories are often stored in the form of triacylglycerol or glycogen (Cohen, 2018). TAGs are packed into lipid droplets in the cytosol. These organelles undergo active biogenesis and maturation. At the time of need, such as during stress, caloric restriction or starvation, TAGs are hydrolyzed to generate energy (Walther and Farese, 2012), which explains the reduction of the amount of triglyceride in treated *T. granarium* larvae in our study.

4.5 Effect in digestive enzymes:

Digestion is a vital process, where insects transform complex macromolecules from their diet into smaller, more manageable forms that can be absorbed by the epithelial cells of their midgut. This intricate process heavily relies on specific enzymes that are tailored to the various types of food they consume. If there is any disruption or impairment in the activity of these enzymes, insects become unable to effectively extract the essential nutrients required to meet their biological needs. Furthermore, this decrease in the ability to utilize nutrients can have consequences on energy conversion essential for biomass production and induction of enzymes activity required for detoxification purposes (Tanzubil & McCaffery, 1990; Senthil-Nathan & Kalaivani, 2005).

Proteins, carbohydrates, and lipids constitute the majority of food macromolecules, hydrolyzed by proteases, amylases, and lipases, respectively. In this study, exposure of *T. granarium* larvae to sublethal doses of carvone, limonene and linalool, reduced digestive enzyme activities, suggested the reduced phosphorous liberation for energy metabolism and decreased rate of metabolism and rate of metabolite transportation.

These results are consistent with previous studies which were demonstrated the reduction of α-amylase, proteases, lipases and chitinase activities in pests such as: *R. dominica* treated with *Lavandula angustifolia* EO (Sayada et al. 2021a), *Trogoderma granarium* treated with *Eucalyptus globulus* EO (Tine et al. 2021a), and *R. dominica* treated with *Schinus molle* EO (Tine et al. 2021b).

α-Amylases (EC 3.2.1.1) endo-digestive enzyme that catalyzes the endohydrolysis of long a-1,4-glucan chains such as starch and glycogen which are the storage carbohydrates forms (Terra and Ferriera, 2005; Shekari et al. 2008). It converts starches into maltose (disaccharide) and glycogen into glucose. Our result is consistent with previous studies which were demonstrated the reduction of α-amylase activities in pests such as *E. kuehniella* (Shahriari et al. 2017), *Ectomyelois ceratoniae* (Ramzi et al. 2014), *Glyphodes pyloalis* (Yazdani et al. 2013), *Pieris rapae* (Hasheminia et al. 2011) after treatment with botanical toxins.

Different types of proteases are necessary because the amino acid residues vary along the peptide chain (Terra & Ferriera, 2005). There are three subclasses of proteinases involved in digestion classified according to their active site group (and hence by their mechanism): serine, cysteine, and aspartic proteinases. Studies by Johnson et al. (1990), Senthil-Nathan et al. (2006) and Zibaee and Bandani (2010a) inferred that botanical insecticides may interfere with the production of certain types of proteases and disable them to digest ingested proteins. Similar results have been reported in *Periplaneta americana* (Paranagama et al. 2001) and *G. pyloalis* (Khosravi and Sendi 2013) treated with azadirachtin, in *T. granarium* larvae treated with *E. globulus* EO (Tine et al. 2021a), in *R. dominica* treated by *S. molle* EO (Tine et al. 2021b), and *Lavandula angustifolia* (Sayada et al. 2021a).

Lipases (EC 3.1.1) are enzymes that preferentially hydrolyze the outer links of fat molecules, they play a very important role in the storage and mobilization of lipids. These enzymes are also involved in several physiological processes such as reproduction, growth, and defense against pathogens (Lemaitre and Miguel-Aliaga 2013). Although, there are a few studies on insect digestive lipases but the enzyme activity significantly changes due to using botanical insecticides. Senthil Nathan et al. (2006) showed that treating *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae), the rice leaffolder, with Btk, NSKE and VNLE (azadirachtin and neem components) sharply decreased the activity level of lipase in the midgut. Zibaee et al.

(2008b) found inhibition of lipase activity in the midgut of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) when they add *A. annua* extract to enzyme samples in vitro. Zibaee & Bandani (2010a) found similar results when adults of *E. integriceps* fed on food containing *A. annua* extract. Similar observations were noted in *T. granarium* larvae treated with *E. globulus* EO (Tine et al. 2021a) and *R. dominica* exposed to *S. molle* EO (Tine et al. 2021b).

Insects produce chitin, a widespread biopolymer found in nature. It serves as a major component of the insect cuticle, forming its exoskeleton. Additionally, chitin is present in the peritrophic matrix, a layer that shields the midgut of insects from pathogens and toxins (Merzendorfer & Zimoch, 2003). Chitinases are enzymes that play a crucial role in breaking down the glycosidic bonds of chitin molecules during the growth processes of insects. Specifically, they target and hydrolyze the β-(1-4) bonds of N-acetyl-β-D-glucosamine residues (Merzendorfer & Zimoch, 2003), contributing to the formation and degradation of the protective peritrophic membrane in the insect's intestinal epithelium (Shen & Jacobs-Lorena, 1997). Inhibiting chitinases shows promise as a pathway for developing novel bioinsecticides and serves as an interesting target for alternative methods of controlling crop pests, reducing reliance on conventional pesticides (Saguez, 2007). Our findings demonstrate that the three applied bioactive molecules effectively reduces chitinase activity. Similar results have been reported in studies where larvae and adult males and females of *D. melanogaster* were treated with azadirachtin (Bezzar, 2016), larvae of *T. granarium* treated with the essential oil of *E. globulus* (Brahmi & Yousfi, 2021), *T. confusum* treated with *E. globulus* oil and Eucalyptol (Debab & Mesloub, 2022), *R. dominica* treated with *Schinus molle* (Soltani & Abes, 2022) and with menthol (Trad & Tine, 2022).

CONCLUSION
AND
PERSPECTIVES

V. CONCLUSION AND PERSPECTIVES.

The work carried out allowed us to evaluate the effect of three bioactive molecules, carvone, limonene and linalool on one of the most hundred hazardous grain pests in the world, *T. granarium* larvae. Their effects were tested on various aspects: toxicity, biochemical composition, enzymatic biomarkers, and digestive enzymes.

Toxicological assays conducted through fumigation and ingestion helped to determine the lethal concentrations (LC25 and LC50). The applied bioactive ingredients exhibited an insecticidal effect with a dose-response relationship. Moreover, ingestion was found to be the most effective mode of application compared to fumigation. The repellency test revealed their repulsive potentials towards this specie.

The study of the biochemical composition demonstrated that when we applied these components through fumigation, they induce depletion of energy reserves and a significant increase of protein content in *T. granarium*.

The applied doses of each molecule (LC25 and LC50) on *T. granarium* larvae appear to affect the enzymatic biomarkers relatively, leading to an increase in alkaline phosphatase, Glutamate Pyruvate Transaminase (GPT), and Glutamate-Oxaloacetate Transaminase (GOT). Lastly, the evaluation of an oxidative stress biomarker indicates that the three molecules cause no change in esterase activity in the treated groups compared to the control groups.

The specific activities of digestive enzymes are also disturbed under the influence of carvone, limonene and linalool showing a significant decrease in α-amylase, chitinase, lipase, and protease activity.

As a future perspective, we recommend:

- Evaluate the effect of carvone, limonene and linalool on the development and the reproductive potential of *T. granarium*.
- Targeting other pests to broaden the spectrum of action of the three applied components.
- Determine the persistence of these molecules and their combined effect.
- Finally, we suggest conducting pilot trials in storage warehouses to better assess the effectiveness of this in-situ treatment.

ABSTRACT

VI. ABSTRACT

Plant-derived insecticides such as plant essential oils and their extracts or components have been an important component that integrates into many agricultural control methods.

This present study aims to evaluate the insecticidal activity of three bioactive molecules, carvone, limonene, and linalool against a pest specie, The khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). Effects were examined on mortality, enzymes of intermediary metabolism (ALP, ALT and AST), nutritional reserves, digestive enzymes, and oxidative stress biomarker.

Toxicological tests revealed the insecticide activity of these ingredients with a dose-response relationship. Ingestion was the most effective mode of application compared to fumigation and carvone was the most effective treatment compared to other molecules. In addition, the obtained results revealed an increase in the percent repellency as a function of concentrations.

The biochemical study shows that the treatments increase the protein content and decrease the energy reserves.

Moreover, they activate enzymes of intermediary metabolism via an increase in Alanine aminotransferase (ALT), Aspartate transaminase (AST) and alkaline phosphatase (ALP). They also reduce the amount of triglyceride in treated *T. granarium* larvae.

Furthermore, the three bioactive compounds also disrupt the activity of digestive enzymes in treated larvae compared to controls. Indeed, the treatments reduce the specific activity of α-amylase, chitinase, protease, and lipase.

Finally, Future investigations are necessary for practical use of compounds as novel insecticides, because of their effect on various physiological targets on insects, which delay their resistance evolution.

Keywords: Bioactive molecules, *Trogoderma granarium*, Toxicity, Biochemical composition, physiological parameters, digestive enzymes

Resume:

Les insecticides d'origine végétale tels que les huiles essentielles des plantes, leurs extraits et leurs composants bioactives sont des éléments importants intégrés à des nombreuses méthodes de lutte agricole.

Cette étude vise à évaluer l'activité insecticide de trois molécules bioactives, carvone, limonène et linalol, contre une espèce de ravageur, le coléoptère khapra, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). Les effets ont été examinés sur la mortalité, les enzymes du métabolisme intermédiaire (ALP, ALT et AST), les réserves nutritionnelles, les enzymes digestives et sur un biomarqueur du stress oxydatif.

Les tests toxicologiques ont révélé l'activité insecticide de ces ingrédients avec une relation dose-réponse. L'ingestion était le mode d'application le plus efficace par rapport à la fumigation, et le carvone était le traitement le plus efficace par rapport aux autres molécules. De plus, les résultats obtenus ont révélé une augmentation du pourcentage de répulsion en fonction des concentrations.

L'étude biochimique montre que les traitements augmentent la teneur en protéines et diminuent les réserves d'énergie. De plus, ils activent les enzymes du métabolisme intermédiaire par une augmentation de l'alanine aminotransférase (ALT), de l'aspartate transaminase (AST) et de la phosphatase alcaline (ALP). Ils réduisent également la quantité de triglycérides dans les larves traitées de *T. granarium*.

De plus, les trois composés bioactifs perturbent également l'activité des enzymes digestives chez les larves traitées par rapport aux témoins. En effet, les traitements réduisent l'activité spécifique de l'α-amylase, de la chitinase, de la protéase et de la lipase.

Enfin, des investigations futures sont nécessaires pour l'utilisation pratique des composés en tant que nouveaux insecticides, en raison de leur effet sur diverses cibles physiologiques chez les insectes, ce qui retarde leur résistance.

Mots-clés : Molécules bioactives, *Trogoderma granarium*, Toxicité, Composition biochimique, Paramètres physiologiques, Enzymes digestives

ملخص:

مبيدات الحشرات المشتقة من النباتات مثل الزيوت الأساسية، مستخلصاتها ومكوناتها النشطة بيولوجيًا تعتبر عنصرا مهما يتكامل مع العديد من أساليب مكافحة الآفات الزراعية.

تهدف هذه الدراسة إلى تقييم النشاط المبيد للحشرات لثلاثة جزيئات حيوية هي الكارفون، الليمونين و اللينالول ضد نوع من الآفات وهو خنفساء الخابرا *Trogoderma granarium* (everts). تم دراسة تأثيرهم على الوفيات، إنزيمات عملية الايض (AST ، ALP، ALT) ، الاحتياطات الغذائية ، الإنزيمات الهاضمة ومؤشر التوتر الأكسيدي.

أظهرت اختبارات السمية النشاط المبيد لهذه المكونات مع وجود علاقة الجرعة والاستجابة. الابتلاع هو الطريقة الأكثر فعالية مقارنة بالتدخين والكارفون هو العلاج الأكثر فعالية مقارنة بالجزيئات الأخرى. بالإضافة إلى ذلك أظهرت نتائج اختبار التنافر القوة الطاردة لهذه العلاجات.

تظهر الدراسة البيوكيميائية أن تطبيق هذه الجزيئات يزيد من محتوى البروتين ويقلل من الاحتياطات الطاقية. بالإضافة إلى ذلك فإنها تنشط إنزيمات الايض الوسيطة المتمثلة في (ALT), (AST) و (ALP). كما أنها تقلل من كمية الدهون لدى يرقات *T. granarium* المعالجة .

علاوة على ذلك تعمل الجزيئات الثلاثة الحيوية المفعول على تعطيل أنشطة الإنزيمات الهضمية المتمثلة في الالفا اميلاز ,الكيتناز , البروتيازو الليباز لدى اليرقات المعالجة مقارنة بالشواهد .

و أخيرا، تعد الدراسات المستقبلية ضرورية للاستخدام العملي للمركبات النشطة بيولوجيًا كمبيدات حشرية جديدة , نظرا لتأثيرها على العديد من المؤشرات الحيوية الفيزيولوجية في الحشرات , مما يؤخر مقاومتها .

الكلمات المفتاحية: جزيئات نشطة بيولوجيًا، *Trogoderma granarium*، سمية، تركيب بيوكميائي، مؤشرات فيزيولوجية، إنزيمات هضمية.

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