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INSECTICIDAL ACTIVTY OF THREE ESSENTIAL OILS COMBINED TO MINERAL SUBSTANCES AGAINST *TRIBOLIUM CONFUSUM* DUVAL 1868 (COLEOPTERA, TENEBRIONIDAE)

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Abstract

Description of the subject: Biopesticides such as essential oils and inert dusts attract frequently attention of researchers as alternative safer to synthetic chemicals.

Objective: This study was conducted to evaluate the toxicity of three essential oils (Eos) from three plants: *Zingiber officinalis* (R.), *Syzygium aromaticum* (L.), and *Origanum floribundum* (M.) combined with two mineral substances; white clay and diatomaceous earth (DE) against a secondary pest of stored commodities: *Tribolium confusum* (Duv).

Methods: The selected plants were subjected to steam distillation using a Clevenger apparatus. The EOs compositions were identified using GC-MS analysis. The bioassays were conducted under laboratory conditions.

Results: The results revealed that the oregano EO had the highest toxicity in both contact (at 0.27µl/cm^2) and inhaled tests (at 0.23µl/cm^3) with 100% of mortality after 48h of treatment. Whereas, EOs + diatomaceous earth after 48h, exhibited an increase in mortality compared to those of EOs or DE used separately.

Conclusion: The obtained findings exerted a biocidal action of the essential oils against *T. confusum*, while the mineral substances can perform the function of carrier agent in the formulation of biopesticides. .

Keywords: Essential Oils; *Tribolium confusum*; mineral substance; combined treatment; insecticidal activity.

ACTIVITÉ INSECTICIDE DE TROIS HUILES ESSENTIELLES COMBINÉES À DES SUBSTANCES MINÉRALES CONTRE *TRIBOLIUM CONFUSUM* DUVAL 1868 (COLEOPTERA, TENEBRIONIDAE)

Résumé

Description du sujet : Les biopesticides tels que les huiles essentielles et les poudres inertes attirent fréquemment l'attention des chercheurs en tant qu'alternative aux produits chimiques synthétiques.

Objectifs : Cette étude a pour but d'évaluer la toxicité de 3 HEs de *Zingiber officinalis* (R.), *Syzygium aromaticum* (L.) et *Origanum floribundum* (M.) combinées à deux substances minérales l'argile blanche et la terre de diatomée (TD) contre un rageur secondaire des denrées stockées : *Tribolium confusum* (Duv).

Méthodes : Les plantes choisies ont été soumises à une hydro-distillation à l'aide d'un appareil de Clevenger. Les HEs ont été identifiées par GC/MS. Les tests biologiques ont été menés dans des conditions du laboratoire.

Résultats : Les résultats ont révélé que l'HE d'origan a la toxicité plus élevée par contact et inhalation avec 100% de mortalité après 48 h d'exposition respectivement à des doses de 0,27 µl / cm^2 et 0,23 µl / cm^3. Alors que l'association des HEs avec de la terre de diatomées a montré une augmentation des mortalités par rapport à celles de l'HE ou de la terre de diatomées utilisées séparément après 48 h d'exposition.

Conclusion : Les résultats obtenus ont montré une action biocide des HEs vis à vis *T.confusum*, alors que les substances minérales peuvent jouer le rôle d'un synergisant dans la formulation de ces biopesticides

Mots clés : Huiles essentielles ; *Tribolium confusum* ; substance inerte ; traitement combiné ; Activité insecticide

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INTRODUCTION

In Algeria, the cereals occupy a strategic place in both the food system and the national economy. This characteristic is visible throughout all phases of the industry [1]. In relations with the world market, Grain products represent over 40% of imports value of food products. They rank first (39.22%), consumption of these products at a level of about 205 kg/capita/year [2]. Nowadays, Algeria obtains 75% of its food requirements through imports making it the first African food importer; it is still far from satisfying its needs [3].To compensate for low returns and post-harvest losses, farmers are increasingly using pesticides. These agricultural inputs have a negative impact on the environment. The storage pests can damage much of the stored product. Overall losses to grain post-harvest insect attacks and other biological agents estimate of 10 to 40% [4]. The use of chemical insecticides is currently the most widely adopted method [5]. However, the use of these substances has led to the development of resistance in many species, poisoning, environmental pollution and ecological disturbances given the reluctance of consumers to consume products treated with pesticides [6, 7]. In order to reduce post-harvest losses while preserving the environment, more work is now geared towards the development of insecticide based on local herbs [8, 9]. This work contributes to researching Algerian plants and minerals as natural insecticides. For this, we propose to test the biocide activity of three essential oils from three plants; oregano (*Origanum floribundum* (M.), cloves (*Syzygium aromaticum* (L.), and ginger (*Zingiber officinalis* (R.) against a pest of stored product; *Tribolium confusum* (D.) and assess the insecticidal activity of two minerals; white clay and diatomaceous earth against the same pest.

MATERIALS AND METHODS

1. Entomological material

T. confusum was reared on untreated commercial flour for 10 years in the plant protection laboratory of agricultural and forest zoology department (National High School of Agronomy, El harrach, Algeria) in an obscure set oven (temperature 30 ± 1 C and a relative humidity 70 ±5%).

2. Plant / mineral material

Three plants were used to obtain essential oils; *Origanum floribundum* (M) from El kaderia (Bouira,Algeria), while *Zingiber officinalis* (R) and *Syzygium aromaticum* (L) from India. The two minerals: white clay and diatomaceous earth used in tests were obtained from the departments of Soil Science and Food Technology (ENSA, Algiers). Diatomaceous earth underwent baking at 100 °C for 20 minutes to remove moisture.

3. Dose selection

Four doses with a geometric progression ratio of (1.2) were selected from the initial dose of 12 µl/ml (equivalent to $0.19/cm^2$) of EOs. In minerals test: selected doses were ($0.017\ g/cm^2$, $0.036\ g/cm^2$) for clay and ($0.004\ g/cm^2$, $0.008\ g/cm^2$) for diatomaceous earth. In combination test, the selected doses were a mixture of (LD_{30} of EO + preselected mineral dose).

4. Extraction method

Essential oils were obtained by steam distillation in the food technology laboratory (ENSA, Algiers), extraction of the dry matter of oregano leaves, cloves and ginger tubers was carried out using a Clevenger-type apparatus in accordance with the pharmacopeia method (European Pharmacopeia 2008). Operating conditions are illustrated in Table 1. The yields (R) in percentage were calculated from the oil weight (w_1) obtained and that of the dry matter (DM) prior to extraction according to the formula: Yield (%) = $W_1 \times 100 / W_2$. **W_1:** net weight of oils (grams). **W_2**: total weight of fresh leaves (grams)

Table 1: Operating Conditions of steam distillation.

Plant material	Ginger	Clove nail	Oregano
Quantity of plant material (g)	220	130	100
Quantity of water (l)	2	2	2
Temperature (C °)	100	100	100
Steam distillation time (h)	3	4	3
Yield (%)	1.8	10.53	0.4

5. GC and GC/MS analysis

GC/MS analyses were performed using Agilent 6890 series apparatus GC systems Agilent Technologies, Santa Clara, CA) coupled to a quadruple mass spectrometer (model HP 5973) equipped with a HP5MS capillary column (30m × 0.25mm i.d., film thickness 0.25 µm). For GC/MS detection electron ionization with ionization energy of 70 eV was used over a scan range of 30–550 atomic mass units. Helium was the carrier gas, at a flow rate of 0.5 ml/min. Injector and transfer line temperatures were set at 250 and 280°C, respectively; the temperature of the ion source was 175 °C. The oven temperature program is described above for GC analysis. The samples (0.2 µl) were injected with 1:25 split ratio. The oil components were identified by matching their recorded mass spectra with the data bank mass spectra (Wiley 7N and NIST 2005 libraries) and authentic mass spectra [10] and compared to their calculated retention indices, with literature values [10, 11] and with those of available authentic standards.

6. Operating method

-*Toxicity of essential oils by contact:*Four solutions at different concentrations; 12, 14.4, 17.3, and 20.7µl EO/mL acetone were prepared. Each solution was to be evenly spayed on a filter paper disc of 9 cm in diameter using a precision micropipette (0–50 µl) [12]. After the complete evaporation of the dilution solvent, both the treated and the control, discs (solvent alone) were carefully placed in a Petri dish of the same dimensions. Four repetitions were performed for each essential oil. A batch of 20 adults insects from a homogeneous population was introduced into each box. The number of dead insects was recorded every 24 hours for six days of treatment.

-*Toxicity of essential oils by inhalation:*A prefixed EO concentration of 10µl was sufficient to saturate an atmospheric volume of 44 cm^3, i.e. a concentration of 0.23 µl/ cm^3. Half-cut cotton swabs (serves as diffuser) were soaked in EOs using a precision micropipette (0–50 µl). The swabs were fixed in a screw cap jar containing 20 insects of the same age. Four repetitions were performed for each test including the witness test, the mortality was determined each exposure time for 6 days.

-*Toxicity of mineral:*A prefixed mineral dose of clay or diatomaceous earth was spread uniformly in Petri dish containing 20g of crushed and untreated soft wheat grains. In each box, a batch of 20 adults of a homogeneous population was deposited. The number of dead insects was recorded every 24 hours for 6 days of treatment.

-*Toxicity of combination test:*A concentration which refers to the LD_{30} of each EO (oregano=0.13µl/cm², cloves=0.10µl/cm², and Ginger=0.07 µl/cm²) was selected in this test. For minerals, prefixed doses were 1g of white clay and 0.25 g of diatomaceous earth. The mixture (EO+mineral) was spread uniformly in Petri dishes containing 20g of untreated and crushed wheat grains. Four replicates were performed for each test including witness test. A batch of 20 adults from a homogeneous population was deposited in each box.

7. Expression of results

The mortality rate was calculated and corrected by Abbott's formula : $Mc (\%)=100(M-Mt)/(100-Mt)$. The efficacy of toxicity was measured by both LD_{50} and LT_{30}, which represents the amount of toxic substance causing death in 50% of individuals from the same batch. The corrected mortality were converted into probit as described in Finney [13].

8. Statistical analysis

To estimate the insecticidal effect of essential oils, the variance analysis (ANOVA) was carried out the number of dead insects using EXCEL STAT software v.16 (Addinsoft©) two classification criteria (concentration and time). Multiple comparisons were also performed using the Tukey–Kramer (HSD) post hoc test.

RESULTS

1. Phytochemical composition analysis

The composition of oils in *O. floribundum* (OF) and *S. aromaticum* (SA) showed complex mixtures of 34 and 15 constituents respectively. *O. floribundum* oil was characterized by dominated amount of monoterpene hydrocarbons and oxygenated monoterpenes. The main identified compounds were thymol (31.7%), γ-terpinene (21.2%) and p-cymene (16%). The *S. aromatium* EO contained 15 compounds with Eugenol (65%), Eugenyl acetate (26.9%) and sesquiterpen of β-Caryophyllene (5.8%) constituting the major constituents (Table 2).

Thirty-one (31) % of compounds in dry ginger oil have been identified. The main compound was zingiberene (40.7%), followed by geranial (8.9%), elemol (5.9%), neral (4.5%) and camphor (4.3%). The oxygenated compounds and the hydrocarbons were also recorded in a large amount. Besides, the monoterpene hydrocarbons were present; limonene (3.7%), α-phellandrene (1.2%) and sabinene (1%). Moreover, the oxygenated compounds; geranial (8.9%), followed by neral (4.5%), α-terpineol (3%), in addition to monoterpene alcohols α-terpineol (3%) and other compounds were furthermore distinguished.

For the sesquiterpene compounds zingiberene and other sesquiterpene hydrocarbons as α-curcumene (0.1%) were also detected (Table 3).

Table 2: Chemical composition (%) of O. *floribundum* and *S. aromaticum* essential oils.

N°	Compounds	RI	% OF	% SA	Identification
1	α-Thujene	930	2.8	-	RI, MS
2	α-Pinene	937	1.9	T	RI, MS, Std
3	Camphene	951	0.1	-	RI, MS, Std
4	Sabinene	974	0.2	T	RI, MS, Std
5	β-Pinene	978	0.4	T	RI, MS, Std
6	1-Octen-3-ol	980	0.9	-	RI, MS
7	β-Myrcene	990	3.6	-	RI, MS, Std
8	α-Phellandrene	1004	0.4	-	RI, MS, Std
9	δ -3-Carene	1011	0.1	-	RI, MS, Std
10	α-Terpinene	1017	4.9	-	RI, MS, Std
11	p-Cymene	1023	16	-	RI, MS, Std
12	β-Phellandrene	1031	0.6	-	RI, MS
13	1,8-Cineole	1032	0.2	-	RI, MS, Std
14	cis-β-Ocimene	1038	0.2	-	RI, MS
15	trans-β-Ocimene	1048	0.1	-	RI, MS
16	γ-Terpinene	1060	21.2	-	RI, MS, Std
17	cis-Sabinene hydrate	1067	0.6	-	RI, MS
18	Terpinolene	1087	0.1	T	RI, MS
19	trans-Sabinene hydrate	1098	0.1	-	RI, MS
20	Linalool	1099	1.5	-	RI, MS, Std
21	Borneol	1166	0.1	-	RI, MS, Std
22	Terpinen-4-ol	1177	0.2	-	RI, MS, Std
23	Methyl salcylate	1193	-	0.1	RI, MS
24	Thymol methylether	1234	0.1	-	RI, MS, Std
25	Carvacrol methylether	1243	0.2	-	RI, MS, Std
26	Chavicol	1254	-	0.2	RI, MS
27	Thymol	1290	31.7	-	RI, MS, Std
28	Carvacrol	1300	8.6	-	RI, MS, Std
29	Eugenol	1357	-	65.0	RI, MS, Std
30	α-Copaene	1376	-	0.2	RI, MS
31	α-Gurjunene	1409	0.1	-	RI, MS
32	β-Caryophyllene	1421	0.8	5.8	RI, MS, Std
33	α-Humulene	1453	T	0.7	RI, MS
34	Germacrene D	1481	0.1	0.1	RI, MS
35	Z, E- α-Farnesene	1490	-	0.1	RI, MS
36	Bicyclogermacrene	1494	0.1	-	RI, MS
37	δ-Cadinene	1523	T	0.1	RI, MS
38	Eugenyl acetate	1520	-	26.9	RI, MS
39	Spathulenol	1575	0.1	-	RI, MS, Std
40	Caryophyllene oxide	1580	0.3	0.3	RI, MS

Components quantified on the HP 5MS capillary column; t= trace (<0.05%); identification: RI, comparison of retention indices relative to C7-C18 n-alkanes on HP5 column with those of bibliography, Std, comparison with authentic compounds; MS, comparison of mass spectra with MS libraries and literature.

Table 3: Chemical composition of *Zingiber officinalis* essential oil.

N°	Component	RI	%	Identification
1	α-pinene	933	T	RI, MS, Std
2	sabinene	973	1.0	RI, MS, Std
3	B-pinene	978	0.3	RI, MS, Std
4	myrcene	992	T	RI, MS, Std
5	α-phellandrene	1005	1.2	RI, MS, Std
6	p-cymene	1024	1.	RI, MS, Std
7	limonene	1028	3.7	RI, MS, Std
8	camphor	1045	4.3	RI, MS
9	(E)-β-ocimene	1050	1.3	RI, MS
10	linalool	1097	T	RI, MS, Std
11	terpinen-4-ol	1175	T	RI, MS
12	α-terpineol	1189	3.	RI, MS, Std
13	citronellol	1227	T	RI, MS
14	neral	1246	4.5	RI, MS
15	geranial	1277	8.9	RI, MS
16	eugenol	1370	2.4	RI, MS, Std
17	α-copaene	1378	1.2	RI, MS
18	geranyl acetate	1383	2.2	RI, MS
19	β-cubebene	1392	0.4	RI, MS
20	ar-curcumene	1485	0.1	RI, MS
21	α-zingiberene	1496	40.7	RI, MS
22	(E, E)-α-farnesene	1509	3.6	RI, MS
23	eugenyl acetate	1526	2.2	RI, MS
24	α-cadinene	1542	0.5	RI, MS
25	Elemol	1552	5.9	RI, MS
26	germacrene-D-4-ol	1578	2.7	RI, MS
27	Spathulenol	1582	2.1	RI, MS
28	α-muurolol	1651	0.5	RI, MS
29	α-cadinol	1658	2.1	RI, MS
30	(2E, 6E)-α-farnesol 1742	1748	2.0	RI, MS
31	Phytol	1949	0.6	RI, MS

Components quantified on the HP 5MS capillary column; t= trace (<0.05%); RI: identification: comparison of retention indices relative to C7-C18 n-alkanes on HP5column with those of bibliography, Std: comparison with authentic compounds; MS, comparison of mass spectra with MS libraries and literature.

2. Effectiveness of essential oils by contact

2.1. Oregano Essential oil

The obtained results at different concentrations and in different exposure times using contact test against *T.confusum* are illustrated in Fig.1. In all the contact tests, increasing doses are associated with increasing in mortality rate. For time interval 3h to 48h, the lowest dose 0.19 µl/cm² showed a mortality ranging from 7.5 to 88.75%, at 0.23µl/cm² mortality was from 7.5 to 86.25%. While the dose of 0.27 µl/cm² recorded 3.5 to 100% mortality. The highest dose 0.33 µl/cm² recorded 7.5 to 100% mortality. In fact, the results revealed that all doses showed mortality reaching 75% after 24h of exposure time.

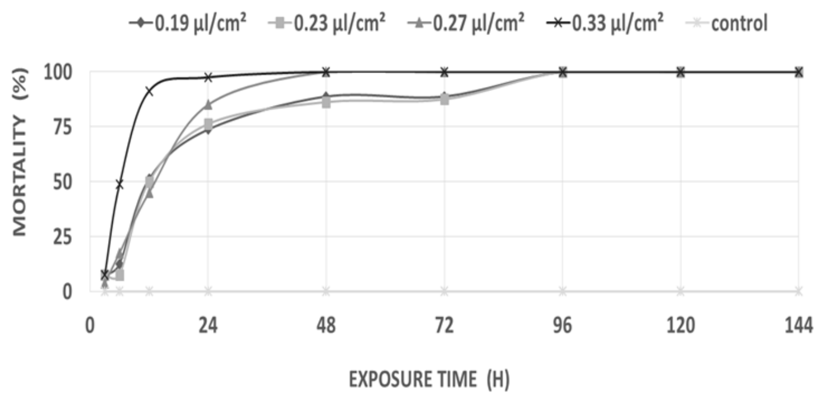

Figure 1: Contact toxicity test of oregano EO against *T. confusum.*

2.2. Ginger essential oil

After 48h of treatment, the dose of 0.19. µl/cm² revealed a mortality ranging from 2.5 to 96.25%, while at a dose of 0.23 µl/cm², the mortality ranges from 1.25 to 100%. The dose of 0.27 µl/cm² caused 0 to 100% mortality and the highest dose of 0.33 µl/cm² showed 2.5 to 100% mortality. Indeed, in all the tested doses, the best mortalities obtained after 48h and 72h of treatment. Whilst, after 24h of treatment, all mortalities rates exceed 50% (Fig. 2).

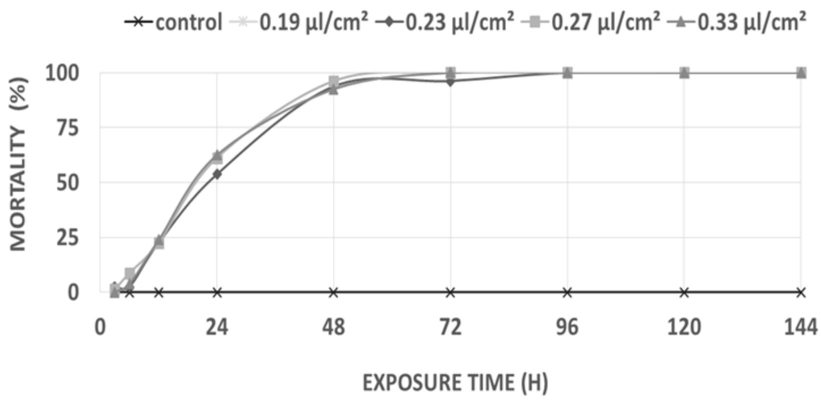

Figure 2: Contact toxicity test of ginger EO against *T. confusum.*

2.3. Cloves essential Oil

During 3h to 72h of treatment, the dose of 0.19 µl/cm² caused mortality ranging from zero to 95%. At 0.23 µl/cm², mortality was from zero to 97.5%. However, 0.27 µl/cm² dose caused 0 to 96.25%. The highest dose of 0.33 µl/cm² caused 1.25 to 97.5%. All doses exceed 50% of mortality after 24h of exposure (Fig 3).

Results of LD_{50} values from probit analysis revealed a similar toxicity of the three essential oils (Table 4).

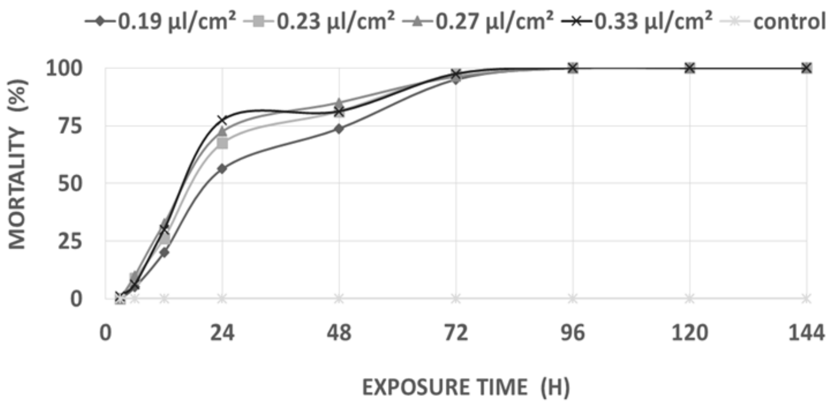

Figure 3: Contact toxicity of cloves EO against *T. confusum.*

Table 4: Probit analysis values of contact test.

Treatment	Equation	Slope ± SE	LD_{50}	95%CI	LD_{30}	95%CI	x^2
Z. officinalis	y=1.42 x+3.30	1.42± 0.14	0.16	[0.08-0.30]	0.07	[0.03-0.13]	1
S. aromaticum	Y=2.46x+2.06	2.46 ±2.06	0.16	[0.11–0.23]	0.10	[0.07–0.14]	1
O. floribundum	Y=5.48x+1.57	5.48±1.57	0.16	[0.13–0.19]	0.13	[0.10–0.15]	0.97

LD_{50}: lethal dose that kills 50% of the exposed insects, Cl: confident intervals; SE: standard error; X^2: chi-square testing goodness of the fit in µl/cm².

3. Effectiveness of essential oil by inhalation

At a prefixed dose of 0.23µL/cm³, ginger and cloves EOs showed a mortality ranging from 0 to 1.25% and from 0 to 5.75%, respectively, 6 days of treatment. However, oregano EO caused a mortality from 1.25 to 100% during 48h of treatment (Table 5).

Table 5: Inhaled toxicity of different treatments against *T. confusum.*

Treatment	Dose (µl/cm³)	Exposure time (h)						Sign[(a)]
		24	48	72	96	120	144	
Control	0.02	0±0.00[b]	0±0.00	0±0.00[c]	0±0.00[c]	0±0.00[c]	0±0.00[c]	n.d [(a)]
Z. officinalis		0±0.00[b]	0±0.00	0±0.00[c]	0±0.00[c]	0±0.00[c]	1.25±0.14c	n.s
S. aromaticum	0.23	0±0.00[b]	0±0.00	1.25±0.14[b]	1.25±0.14[b]	4.75±0.14[b]	5.75±0.14[b]	***
O. floribundum		96.25±0.14[a]	100±0	100±0[a]	100±0[a]	100±0[a]	100±0[a]	***
Sign	-	***	n.d	***	***	***	***	-

(a) Levels of significance:*p< 0.05, **p< 0.01, ***p<0.001, n.s: non-significant/ significative, n.d: not determined, Different letters indicate statistical differences within the rows of the same column.

Results of LT_{50} values from probit analysis are illustrated in Table 6, among the three EOs, oregano showed the highest toxicity in short time.

Table 6: Probit analysis of inhalation test.

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Treatment	Equation	Slope ± SE	LT_{50} (h)	95%CI	x2
Z.officinalis	-	-	-	-	-
S.aromaticum	y=3,65x-3,46	3,65 + - 0.06	207,99	(154,10-280,71)	0.65
O.floribundum	y=4,16x+0,01	4,16 + - 0.04	16.42	(12.33-21.86)	0.057

LT50: lethal time that kills 50% of the exposed insects, Cl: confident intervals; SE: standard error; X^2: chi-square testing goodness-of the fit of time mortality response.

4. Toxicity of mineral substances

The obtained results showed that white clay at 0.017 g/cm² caused a mortality ranging from 6.25 to 20% in 6 days of treatment, while DE at 0.004 g/cm² caused 75 to 100% mortality in 4 days (Table 7).

Table 7: Toxicity of mineral substances against *T.confusum.*

Treatment	Dose[b]	Exposure Time (h)						Sign[a]
		24	48	72	96	120	144	
Control	0	0	0	0	0	0	0	-
clay	0.017	6.25±0.14	10.00±0.23	16.25±0.9	22.50±0.59	20.00±0.61	20±0.61	n.s
	0.036	7.5±0.36	13.75±0.48	17.50±0.36	21.25±0.48	21.25±0.48	23.75±0.63	n.s
Sign		n.s	n.s	n.s	n.s	n.s	n.s	-
DE	0.004	75.25±0.23[a]	86.25±0.14[b]	96.5±0.16 [b]	100±0	100±0	100±0	***
	0.008	38,75 ±1,21[b]	97,50 ±0,16[a]	100 ±0 [a]	100 ±0	100 ±0	100 ±0	***
Sign	-	*	***	**	n.d	-	-	-

(a) Levels of significance:*: p< 0.05, **: p< 0.01, ***: p< 0.001, n.s: non-significant, n.d: not determined, Different letters indicate statistical differences within the rows of the same column. (b) Unit: mg/cm²

5. Toxicity of mineral substances combined with essential oils

The mixture of EO + clay revealed unsatisfactory results whatever the applied EO: mortalities remained below than those reported from EOs treatment except oregano, which marked an increase in mortality rate (+2.50%) (Fig. 4). However, mortalities recorded during 48 to 72h of treatment by EO + DE mixture were superior to those with EOs or diatomaceous earth that were applied separately (Fig. 5).

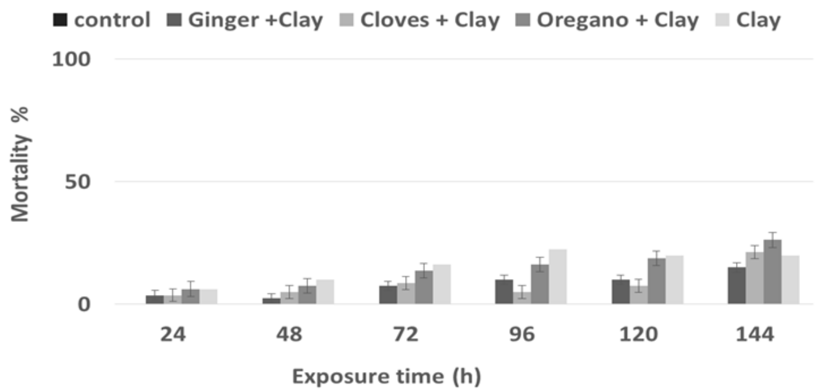

Figure 4: Toxicity of essential oil combined with clay.

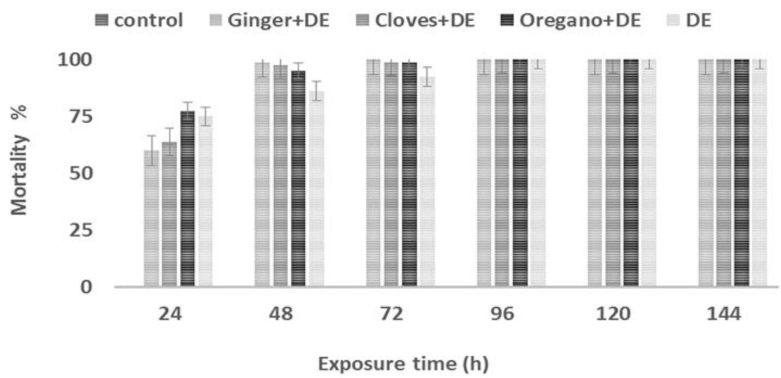

Figure 5: Toxicity of essential oil combined with diatomaceous earth.

DISCUSSION

GC/MS analysis findings have shown variance in plant compounds, these results are similar to those of Hazzit [14] who also found that the EO of *O. floribundum* from Lakhdaria (1170 m altitude) was characterized by a higher amount of thymol (27.9%) compared to that of the same species from Hammam Melouane, while other previous studies showed that the chemical composition of *O.floribundum* EO was rich in carvacrol (35 and 40 %) and poor in thymol (9.9 and 1.1 %) [15, 16]**.**

Compared with previous studies; Fayemiwo [17] has identified differences in *S. aromatium* essential oil composition from Nigeria (containing 28 compounds) with Eugenol (80.5% vs 65%) Eugenyl acetate (5.01% vs 26.9) and sesquiterpen of β-Caryophyllene (3.14% vs 5.8%). Furthermore, the earlier analysis of the dry ginger oil composition showed that zingiberene (28.6 vs 40.7%) was the main compound followed by geranial (8.5% vs 8.9%). However, differences were be noted in oxygenated compounds: Monoterpene, hydrocarbons, sesquiterpene and monoterpene alcohol compounds [18].

The evaluation of toxicity by the contact of EOs has indicated a biocide action against *T. confusum* at higher doses and after 48 hours of exposure time; oregano showed the highest efficiency with 100% mortality, whereas, ginger and cloves caused 81.25% and 80%, consecutively. The efficiency of cloves and ginger oils has been recorded in accordance with previous studies in Maedeh [19], Wang [20] and Jeyasankar [21]. Whereby, the Mortality was observed in witness experiments under laboratory conditions. In addition, the inhalation test at a dose of 0.23 µl/cm^3 of the oregano revealed a higher mortality rate of 100% after 48h of treatment. While, lower toxicity was showed by both the cloves and the ginger. These findings are in agreement with those obtained by Theou [22], who confirmed that the oil vapors of *Origanum vulgare* at LC_{50}= 165 µl/l air showed high fumigant toxicity at all developmental stages of *T.confusum*. The results of mineral substances exhibited a high toxicity of DE that reached 100% after 4 days of treatment. Our results can be comparable to those obtained by Baldassari [23], who examined the vulnerability of different stages of *T.castaneum* and *R.dominica* exposed to DE revealing that efficacy was only observed against *R. dominica*. Recently, Kabir [24] has also revealed that pure DE has higher effectiveness against *T. castaneum* and *R. dominica* at a dose of 1000 ppm. These results are similar to those reported by several authors; in which they concluded that DE is considered as bioinsecticide. [25, 26, 27]. On the other hand, no significant insecticidal activity in clay has been recorded in the present study; nevertheless, similar studies have shown the efficacy of clay against both *T.castaneum* (herb) and *T.confusum* and even against *C.maculatus* (coleoptera, bruchidae) chickpea pest and it seems to have the potential for use against stored products insects in integrated pest programs [28 , 29]. In combination test, the mixture EO + diatomaceous earth caused the highest toxicity reaching 100% within 2 to 4 days of treatment, depending on the EO applied. This finding can be comparable to that revealed by Paul and Korunic [30] who reported that the DE formulation (Protect-It ™) was efficient against *C. ferrugineus* at 0.15 kg in silo barley. Besides, clay with adsorbent capacity has been suggested as an essential oil carrier by some authors [31-33]**.** Furthermore, the effect of flavored clay powders with *O.basilicum*, *O.gratissimum* and *C.citratus* EOs against *T.castaneum* was tested by Camara [34], the results showed significantly reduction of species proliferation. Fouabi [35] reported that 3% of a clay powder-grain mixture caused a high mortality rate against *T.Castaneum*. Consequently, using a mineral substances flavored with essential oil can have a double effect against stored product pests.

CONCLUSION

The goal of this study is to analyze the efficacy of three essential oils from three plants: cloves, oregano, and ginger, as well as of two mineral substances: diatomaceous earth and clay, and their mixture against a stored grain pest: *T.confusum*. The phytochemical analysis of the three essential oils of oregano, cloves, ginger revealed three main constituents: thymol, eugenol and α-zingiberene, consecutively. All the three essential oils revealed a high toxicity against *T.confusum* in contact test at the upper dose, with mortality reaching 75% after 48 h of treatment. Among these experiments, oregano EO showed higher inhalation and contact test efficiency; after 48 h of exposure, mortality reached 100 %.

In addition, mineral substances showed that diatomaceous earth was more effective than clay; in which the adults were totally controlled within 48 h of treatment, whereas combined EOs with minerals can produce additive effect. Based on these findings, bioinsecticids from aromatic plants or minerals can provide an alternative way of synthetic one's against stored product insects, for this reason, other formulations need to be investigated as a part of integrated pest manager programs.

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