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Insecticidal, Antifeedant and repellent efficacy of certain essential oils against adult rust-red flour beetle, Tribolium castaneum (Coleoptera: Tenebrionidae)

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Abstract

Stored grains represent main constituent of food in Egypt. Tribolium castaneum comes on the top of stored grain pests causing up to 40% weight reduction of stored grains. Therefore, its control is urgent. This study aimed to evaluate the insecticidal activity of four essential oils (Eos) extracted from Allium sativum (garlic), Cinnamomum camphora (camphor), Syzygium aromaticum (clove) and Brassica junicea (mustarda) against adults T. castaneum. Also, the effects of these oils on biology and physiology of T. castaneum were tested. In addition, the effect of the tested oils on wheat seeds germination was determined. Gas chromatograph - Mass spectrophoto- metry was used to analyze the major constituent of the tested essential oils. Results indicated that the four tested essential oils have insecticidal, anti-feedant, and repellent activities against adult T. castaneum. Clove has the highest insecticidal activity with LC50 (1.44 mg/kg) followed by garlic (2.09 mg/kg), Camphor (2.75 mg/kg), and Mustard (3.67 mg/kg). the results also showed that clove oil had the highest residual effect, its insecticidal effect extended to the seventh week from the treatment. Results also showed that the clove and camphor have adverse effect on T. castaneum adult physiology. Also, the tested Eos to some extent affect seed germination, meanwhile the garlic and camphor oils showed the lowest effect. Mass spectra results revealed different bioactive components, as Fatty acids, decanes, cosane, and sulphide groups. Finally, results demonstrated that clove and garlic oils showed low cytotoxic effect. In conclusion. The tested essential oils could be considered as promising effective and safe alternatives for synthetic insecticides to control stored grain pests.

Keywords: Essential oils; Tribolium castaneum; insecticidal activity; Biochemical responses, cytotoxicity

1. Introduction

Stored grains, cereals and their products are important sources of worldwide food; therefore, effective conservation of this key resource is important for the human survival [**1**]. Globally, pests of stored grains cause the high quality and quantity losses for stored goods [2]. FAO estimated that 10 to 25% of the harvested food worldwide is destroyed annually by insects [3]. Insect pests cause damage to stored grains that would reverse inversely on their nutritional value [1]. In addition, changes caused by insects in storage environment may cause hot spots that provide suitable conditions for microorganisms. This makes the stored grains not good for human requirements [4]. In Egypt, the rust-red flour beetle, *Tribolium castaneum* (Herbst) comes on the top of stored grain pests [5, 6, and 7]. It caused up to 40% weight reduction of stored grains [8, 9, and 10]. Combating of stored grain pests relies on chemical grain protectants. Meanwhile, these chemicals can cause harmful effects after ingestion, inhalation or skin contact. The excessive use of chemical pesticides induce insecticide resistance development [11], destroys natural enemies, harms non-target species and contaminates food which consequently leads to human and animal diseases. Therefore, there is an urgent need to find effective natural other choices to control stored grain pests without risks to non-targets and losses in grain quality [2, 12, and 13]. Botanical extracts are promising candidates which have been found to act concertedly on both behavioral activities and physiological processes of the target insect pests [14] with relatively low toxic effects on the non-target organisms [15]. Plant extracts including essential oils were reported to

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have toxic, repellent, antifeedant, ovicidal effect on insect pests [16, 17, 18,19, 20, and 21]. Earlier studies had assessed the insecticidal activity of plant extracts worldwide [19, 22, 23,24, and 25], However, few studies focused on the effect of plant extracts and essential oils on the insect biology, physiology, and their effects on seed germination. So, the present study aimed to evaluate the insecticidal, repellent, and antifeedant activities of four essential oils extracted from *Allium sativum* (garlic), *Cinnamomum camphora* (camphor), *Syzygium aromaticum* (clove) and *Brassica junicea* (mustarda) against adults *T. castaneum.* Also, the effects of these essential oils on insect biology, physiology and wheat seeds germination were determined. Gas-chromatography Mass spectrophotometry (GC-MS) analysis for the tested essential oils was conducted.

2. Materials and Methods

2.1. Experimental insects and mass rearing

In this study, *T. castaneum* was gotten from the department of stored product pests, Plant Protection Research institute, Sakha Agriculture Research Station, Egypt, where it was continuously maintained for several generations. It was mass reared according to the modified method of Jagadeesan et al. **[26]** in 500 ml glass jars covered by a muslin cloth and rubber band. The glass jar contained a mixture of broken wheat grains and wheat flour. Culture rearing and experiments were under laboratory conditions of 26 ± 2 °C and 12D:12L photoperiod.

2.2. Essential oils (Eos)

Common, scientific and family names of essential oils used in this study are listed in table 1. They were acquired from the Egyptian Company of Natural Oils, Cairo.

Table1: Common, scientific and family names of the tested essential oils

2.3. Insecticidal activity

This experiment was performed according to **Jairoce et al. [**27]. Briefly, six concentrations (0.2, 0.4, 0.8, 1, 2 and 3 mg/kg) of each essential oil were prepared. Then the treatment was conducted by mixing 1kg of broken wheat grains with the desirable volume of each EO. Twenty grams were then withdrawn and introduced to ten pairs of 5±2 days-old *T. castaneum* adults in a glass jar (5 cm diameter ×7.9 cm height). A Parallel control experiment with untreated grains was conducted. Triplicates were done per each concentration and essential oil. Weevils mortality was assessed 1, 3, 5, and 7 days post treatment. LC_{50} and LC_{90}, their confidence limits and slope values were calculated using Probit analysis [18].

2.4. Residual effects

The pesticide residues effect of the tested essential oils over time was determined. Briefly, one and half kilogram of wheat grains was mixed with each of the tested essential oils at the level of LC_{50} in a glass jar (25 cm diameter ×20 cm height), then kept for 7 days at 26±2 °C and 65±5% RH. Weekly up to 10 weeks, 20 g of the treated grains were taken, transferred to a new glass jar (5 cm diameter ×7.9 cm height) and ten pairs of *T. castaneum* adults from the stock culture were added to the treated grains. A parallel control treatment with untreated grains was practiced. Adult's mortality was scored after 72h post exposure. The experiment was done in triplicate.

2.5. Anti-feedant activity of essential oils

This experiment was performed according to Aslam et al. [28] and El-Desouky et al. [7], where, 10 g of broken wheat grains treated with each essential oil at LC_{50} level were placed in a Petri dish (15 cm diameter × 3cm height). Then, ten pairs of 24 h-starved adults *T. castaneum* were introduced to the treated wheat grains. Three replicates were done and parallel control with untreated grains were performed. Seventy-two hrs post exposure, wheat grains were weighed after insect removal. The percentage of food consumed from each treatment was determined. Antifeediant index (AFI) and Feeding inhibition rate (FI) were calculated using the formula of

$$\text{AFI} = \frac{\text{weight of treated food consumed}}{\text{weight of treated food consumed+weight of control food consumed}} x1$$

$$\text{FI}\ (\%) = (50 - \text{AFI})x2$$

2.6. Repellence effect of the tested essential oils

The repellency effect of the tested essential oils against adult *T.* castaneum was evaluated using the modified method of Isman [**29**]**.** For this purpose, a

small petri dish cover (1 cm hight and 6 cm diameter) containing 10 pairs of adult *T. castanum* and 10 g of treated wheat grains (treated with the tested essential oil at LC_{50}) was putted into a large petri dish (5cm hight and 15 cm diameter) then the petri dish was covered. A parallel control experiment with untreated grains was done and three replicates were practiced. Number of insects left the petri dish cover and fall in the large petri dish was counted 24 h post-treatment and percentage of insect repellency was calculated according to the formula

$$\%\ \boldsymbol{repellency} = \frac{\boldsymbol{No\ of\ adults\ outside\ petri\ dish}}{\boldsymbol{total\ No\ of\ adults}} \times \mathbf{100}$$

2.7. Physiological effects of the tested essential oils

This assay was conducted to test the effect of the most toxic Eos (clove and garlic) on total protein, lipids, carbohydrates, and acetylcholine esterase titer of adults *T. castanum*.

2.8. Preliminary preparations

T. castanum adults were taken after 48h of exposure to the tested Eo, weighed and homogenized with 10 times volumes (w/v) of phosphate buffer (PH 7.2). The homogenate was centrifuged at 4000 rpm for 10 mins at 4ºC. The supernatant was used for the following bioassays.

2.9. Total protein:

The total protein content of adults was determined following the method of Lowry et al. [31]. The density of the developed colored product was measured spectrophotometrically at 750 nm against a blank. Bovine serum albumin was used as a standard**.**

2.10. Total carbohydrate

The whole-body total carbohydrate content was measured according to Kemp and Van Heijningen [32]**.** The total carbohydrates were determined spectrophotometrically at 546 nm against blank. The concentration of carbohydrates was expressed as mg/ml tissue compared with the standard curve of glucose.

2.11. Total Lipid peroxidation (LPO) assay:

The concentration malondialdehyde (MDA) as a marker of LPO was determined according to the method of **Nair and Turner** [33] based on the reaction with thiobarbituric acid (TBA). The MDA level was measured spectrophotometrically at 532 nm, and the results are expressed as nM of MDA mg-1 wet tissue

2.12. Acetylcholine esterase (AChE) titer

Following the method of Ellman et al. [34], the enzyme activity was determined in adults using acetylthiocholine iodide and butrylthiocholine iodide substrates. The optical density of the developed yellow color product was measured spectrophotometrically at 412 nm after 10 min against blank. Then, the activity was calculated as µ mole of substrate hydrolyzed per mg AChE/ min.

2.13. Effect of the tested essential oils on developmental stages reduction of Tribolium castaneum

Ten pairs of 5±2 days-old *T. castaneum* adults were added to twenty grams of broken wheat grains treated with LC_{50} of each of the tested oil in a plastic container (4cm in height and 15cm in diameter). A parallel control was practiced with untreated grains and this was replicated three times per each treatment. Numbers of hatched larvae, developed pupae, and emerged adults from each treatment were counted. The stage reduction percentage was calculated using the equation of EL-lakwah et al. [35] as follows.

$$\boldsymbol{Reduction}\ \%\ (\boldsymbol{R}\%) = \frac{Total\ no.of\ emerged\ stage\ in\ control - Total\ no.of\ emerged\ stage\ in\ treatment.}{Total\ no.\ of\ emerged\ stage\ in\ control} x\ 100$$

2.14. Cytotoxic effect of the tested essential oils (MTT assay)

This experiment was performed to evaluate the toxic effect of the most toxic Eos (clove and garlic), This colorimetric assay depends on NADPH-dependent oxidation-reduction enzymatic activity of viable cells which indicate the metabolic processes of cells and consequently cell viability. In Brief, the normal, diploid, human lung fibroblast cell line WI-38 was acquired from ATCC via VACSERA, a holding company for biological products and vaccines (Cairo, Egypt). The cells were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a 5% CO2 incubator. For the MTT assay, the cells were seeded at a density of 104 cells/well in a 96-well plate for 48 h, following which, treatments of 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL of clove, garlic oils and doxorubicin (reference compound) were administered and incubated for 24 h. Subsequently, 20 µL of a 5 mg/mL MTT (Thiazolyl Blue Tetrazolium Bromide, Sigma, St. Louis, USA) solution was added and incubated for 4 h. Dimethyl sulfoxide (100 µL, DMSO, Sigma, St. Louis, USA) for dissolving purple formazan formed by mitochondrial succinate dehydrogenase in viable cells was added to each well. Absorbance was measured at 570 nm using a plate reader (BioTek®ELx 800, USA). The cell viability was calculated as (A570 of treated samples/A570 of untreated sample) × 100% (Mosmann, 1983; Denizot and Lang, 1986). Essential oils concentration inhibiting 50% cell growth (IC50) was extrapolated by plotting the graph of concentration vs. percentage of dead cells.

2.15. Gas chromatography-mass spectrophotometry (Gc- Ms)

The percentages of different bio-active chemicals in the used Eos were figured out using Trace GC-ISQ mass spectrometer (thermo Scientific, Austin, TX USA) with a direct capillary column oven, initial temperature was at 50 °C and then increased by 5 °C /min till 300 °C. The injector and MS transfer line temperature were kept at 270, 260 °C respectively. Helium was used as a carrier gas at a constant flow rate of 1ml/min. The solvent delay was 4 min and diluted samples of 1 µ were injected automatically using autosampler AS1300 coupled with GC in the split mode. EL mass spectra were collected at 70 eV ionized voltages over the range of m/z 50-650 in full scan mode. The ion source temperature was set at 250 °C. The components were identified by comparison of their retention times and mass spectra with those of mass spectra libraries (WileyRegistry8e, replib, mainlib and Hit spectrum database).

2.16. Effect of essential oils on wheat seeds germination

Phytotoxicity test was conducted according to Qi and Burkholder [30] with slight modification. Where, wheat grains were treated with tested oil at either LC_{50} or LC_{90} levels. One month post-treatment, twenty wheat grains were transferred to cotton bed saturated with water in a sterile Petri dish (9 cm in diameter and 3 cm height). A control experiment was performed, however with untreated wheat grains. Trireplicates were performed for each treatment. Germination percentages were recorded after seven days of treatment. Total germination percentages (GP) was calculated according the following equation

$$GP = n/N \times 100$$

Where: N: Total number of seeds used for germination test. n: Total number of germinated seeds.

2.17. Statistical analysis

The obtained data were showed as Mean ± standard Deviation (M±SD). The homogeneity of variances using the Shapiro-Wilk and Bartlett's tests. The mortality, antifeedant, and replant percentages induced by the tested essential oils were analyzed using a two-way analysis of variance (ANOVA). Data on the effect of essential oils on insect biology, physiology, and seed germination were analyzed by one-way analysis of variance. Statistical differences were considered significant at the $p < 0.05$ level. All testes were analyzed using Minitab [36].

3. Results and Discussion

3.1. Insecticidal activity

Results showed that the four essential oils had a highly significant effect ($P<0.001$) on the mortality of adult *T. castaneum* (Table 2). Clove oil was the most effective, it induced 96.67±2.89% mortality at 3 mg/kg and 7 days post exposure. It was followed by garlic oil with 86.67±10.41 % mortality at 3 mg/kg and 7 days

post exposure, camphor caused 75.00±8.66 mortality at 3 mg/kg and 7 days post exposure, and mustard caused 70.00±10 mortality at 3 mg/kg and 7 days post exposure. The data also indicated that insecticidal effect significantly increased in time and concentration dependent manner (supplementary tables for more details on statistical analysis are available). The LC_{50} and LC_{90} were estimated at confidence interval 95% after 7 days post exposure (Table 3). Clove followed by garlic had the lowest LC_{50} and LC_{90} values (1.44 and 4.36 mg/kg) and (2.09 and 4.88 mg/kg), respectively.

Table2: Mortality rates of *Tribolium castanium* adults exposed to different concentrations of the tested essential oils at different time intervals.

Essential oils	Exposure time (days)	Mortality % ± S.D. Essential oils concentrations (ml/kg)					
		0.2	**0.4**	**0.8**	**1**	**2**	**3**
Clove	1	0	0	0	3.33±2.89	6.67±2.89	15.00±2.00
	3	0	8.33±2.89	11.67±2.89	21.66±2.89	28.33±2.89	38.33±2.89
	5	5±0.02	28.33±2.89	33.33±2.89	40.00±5	55.00±5	70.00±5
	7	16.67±2.89	45±3.00	60.00±5	78.33±2.89	88.33±2.89	96.67±2.89
Garlic	1	0	0	1.67±0.5	8.33±5.77	13.33±5.77	18.33±5.77
	3	0	0	6.67±2.89	20.00±5	33.33±5.77	43.33±5.77
	5	3.33±2.89	6.67±2.89	25.00±5	35.00±8.66	46.67±7.36	58.33±10.41
	7	20.00±5	33.33±2.89	45.00±5.00	56.67±7.64	70±10	86.67±10.41
Camphor	1	0	0	0	3.37±2.89	8.33±5.77	15.00±5
	3	0	0	0	5.00±0.02	10.00±2.88	36.67±5.77
	5	1.67±0.57	5±0.02	16.67±7.64	28.33±5.77	35.00±5	53.00±7.64
	7	10.00±2.5	20±5	33.33±2.89	46.67±2.89	58.33±2.89	75.00±8.66
Mustard	1	0	0	0	3.33±2.8	5.00±0.02	18.33±2.89
	3	0	0	0	5.00±0.02	16.67±2.89	36.67±2.89
	5	0	3.33±2.89	6.67±2.89	10.00±3.00	28.33±2.89	43.33±5.77
	7	8.33±2.89	18.33±2.89	31.67±2.89	43.33±2.89	56.67±2.89	70.00±10

Table3: Toxicity of the tested essential oils against adults *Tribolium castaneum* 7-days post exposure.

Eos	LC_{50} and LC_{90} values (ml/kg grains)		X^2	Slope
Clove	**LC_{50}**	**1.44** (1.22- 1.77)	7.73	1.64
	LC_{90}	**4.36** (1.38-7.94)		
Garlic	**LC_{50}**	**2.09** (1.28-3.42)	4.17	1.82
	LC_{90}	**4.88** (1.14-23.25)		
Camphor	**LC_{50}**	**2.75** (1.82-5.68)	4.39	1.87
	LC_{90}	**5.43** (1.51- 55.08)		
Mustard	**LC_{50}**	**3.67** (2.82- 7.82)	1.87	1.97
	LC_{90}	**6.35** (2.43- 37.14)		

3.2. Residual effect of the tested essential oils

The obtained data in table 4 showed that clove oil had the highest residual effect, its insecticidal effect extended for the seventh week of treatment. It followed by garlic oil and there was no significance between camphor and mustard. Also, it was shown that the residual effect decreased by time.

3.3. Antifeedant and repellant activity

The results showed that the four tested essential oils at LC_{50} level significantly ($P<0.001$) repel and deter the feeding in adults of *T. castaneum* (Fig.1 A and B), respectively. Clove followed by garlic essential oils had the highest antifeedant and repellent activity. Both essential oils significantly *($p<0.001$)* repel the adults of *T. castaneum* far from treated grains and reduced the percentage of food consumed.

3.4. Effect of clove and garlic essential oils on the physiological parameters of adults of Tribolium castaneum

The obtained data in table 5 explained that both garlic and clove essential oils significantly ($P<0.01$) decreased the concentrations of total lipid, protein,

carbohydrate, and activity of acetyl choline esterase enzyme compared with control. However, the effect of clove essential oil was higher than garlic essential oil.

Table4: Residual toxicity of the tested essential oils against adults of *Tribolium castaneum* up to 10 weeks post-treatment of wheat grains.

Weeks post-treatment	Mortality % (mean±SD)			
	Clove	Garlic	Camphor	Mustard
1st	61.00±5.82	43.35±2.89	21.65±2.89	15.00±3.89
2nd	46.65±2.89	35.00±5.00	8.35±2.89	5.00±0.02
3rd	35.00±10.00	23.35±2.89	1.00	0
4th	26.65±7.64	4.00±1.00	0	0
5th	11.65±2.89	0	0	0
6th	7.49±2.43	0	0	0
7th	5.00±0.02	0	0	0
8th	0	0	0	0
9th	0	0	0	0
10th	0	0	0	0

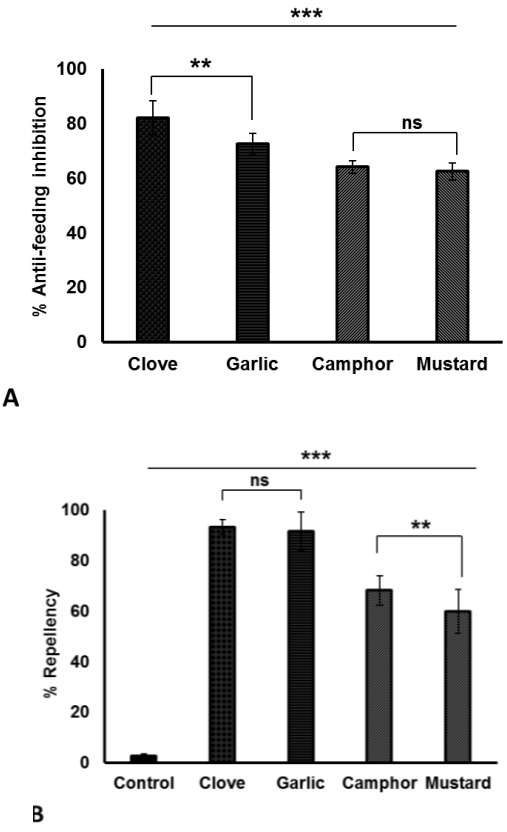

Figure 1: Anti-feeding and repellency activities of the four tested essential oils against *T. castaneum* adults

Table5: Effect of clove and garlic essential oils on the physiological parameters of adults of *Tribolium castaneum*

Essential oil	AchE	Total Lipids	Total protein	Total Carbohydrates
Clove	0.021^{b} ± 0.003	36.42^{c} ±5.84	2.41^{c} ±0.09	125.03^{a} ±20.73
Garlic	0.018^{b} ± 0.002	46.23^{b}± 4.79	4.63^{b} ±1.04	141.08^{a} ±19.83
Control	0.034^{a} ± 0.006	64.39^{a} ±10.73	8.80^{a} ±5.17	151.17^{a} ±14.85

3.5. Effect of the tested essential oils on developmental stages reduction of Tribolium castaneum

Clove and garlic essential oils significantly (*P<0.001*) decreased the percentages of hatched larvae, developed pupae, and emerged adults compared to control (table.6).

The above results indicated that the four tested essential oils had significant insecticidal, residual, antifeedant, and repellent activity against adult *T. castanum.* Meanwhile, clove and garlic oils were the best. These results were in agreement with earlier studies where tested plant oils have been proved to have insecticidal effects [37, 38,39,40,41, 42,43,44, and 45], repellant activity [46,47,48,49,and 50] and feeding inhibition activity [51,52,and 53].

The results also indicated that Clove and garlic essential oils significantly affected *T.* castanum physiology and biology. Both essential oils decreased the concentration of total lipid, protein, carbohydrate, and acetylcholine esterase titer. Also, they reduced the percentage of hatched larvae, developed pupae and emerged adults. Similar reductions in total proteins, lipids, and carbohydrates as a result of plant oils treatment were recorded in various insect [54, 55,56,and 57]. The reduction of lipid content after treatment with essential oils may be attributed to several mechanisms including increases lipase activity [58], formation of lipoproteins responsible for repairing damaged cells, direct utilization by cells for energy demands [59], or increasing the titer of hormones controlling the metabolism of lipid [60 and 61]. The reduction of total body protein in *T. castaneum* adults treated with the tested oils may be due to the low assimilation of food and low amino acid uptake required for protein synthesis [62]. Decrease of total body carbohydrate content in plant oil treated insects was also reported before. The total carbohydrates level in *Plodia interpunctella* significantly decreased following the treatment with

oils extracted from *Trigonella foenumgraceum*, *Rumex dentatus*, *Acacia nilotica*, *Piper cubebae* and *Salvia officinalis* [63].

AChE is the enzyme catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid [64] to terminal the neural signaling in appropriate time. Results of this study indicated the decrease of AChE titer after tested oils application. This result is in agreement with previous studies which reported the adverse effect of some of essential oil components especially monoterpenoids on insect AChE (65,66, and 67). Which in turn lengthened stimulation of post synaptic membrane and consequently lack of coordination between nervous and muscular systems which leads finally to insect death [68].

The proven insecticidal activity of the tested essential oils may be attributed to the active chemical constituents of these oils. This matter leads us to analyze clove, garlic, camphor and mustard oils using gas chromatography mass spectrophotometry.

Table6: Effect of Clove and Garlic oils on the population reduction of *Tribolium castaneum*

Essential oil	Larvae (%)	Pupae (%)	Adults (%)
Clove	83.63^{b}±3.61	90.09^{a}±2.52	95.76^{a} ±2.00
Garlic	86.36^{a}±2.52	90.59^{a}±3.06	91.53^{b} ±2.52

3.6. GC_MS anyalsis of the tested essential oils

GC_MS anyalsis of clove essential oil revealed eighteen components (Table 7). The Major constituents were 1- Heptacosene (53.31%), eugenol (9.86%), Triacontane (6.20 %) , heptacosane (4.94%),2H-Pyran-3-ol,tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S-[3à,6à(R*)]]- (4.60 %), Dotriacontane (3.29%) and 1- Eicosanol (3.18%).

Also, thirty-seven components were identified from the analysis of garlic essential oil (Table 8). The Major constituents were 14-á-H-Pregna (58.86 %), 1-(4-Bromobutyl)-2-piperidinone (20.53 %), 3S,6S)-2,2,6-Trimethyl-6-((S)-4-methylcyclohex-3-en-1-yl)tetrahydro-2H-pyran-3-ol (3.63 %), 1-Heptatriacotanol (2.43 %), eugenol (1.32 %), 1-Chlorooctadecane (1.24 %), N,N-Diethanoldodecyl sulfonamide (0.6 %), Disulfide, (1E)-1-propen-1-yl 2-propen-1-yl (0.3 %), 1-Allyl-3-methyltrisulfane (0.12 %), Diallyldisulphide (0.11 %).

For camphor essential oil, forty components were identified (Table 9), which were Alcanfor (17.59 %), Methyl salicylate (14.49 %), triacontane(6.39%), pentacosane (6.14%), (3S,6S)-2,2,6-Trimethyl-6-((S)-4-methylcyclohex-3-en-1-yl)tetrahydro-2H-pyran-3-ol (5.93 %) andheneicosane (5.46 %). Meanwhile, the analysis of mustard oil revealed twelve components (Table 10), the major constituents were 4-allyl-2-methoxy-phenol (59.52 %), heptacosane (25.49 %), 4-allyl-3-methoxyphenyl acetate (6.72 %) and 3s,6s)-2,2,6-trimethyl-6-((s)-4-methylcyclohex-3-en-1-yl)tetrahydro-2h-pyran-3-ol (4.05 %).

Chemical constituents of the tested essential oils grouped as monoterpenes, sesquiterpenes, decanes and other aliphatic compounds. These compounds may be responsible for the insecticidal activity of the tested oils. [22, 69, and 70] attributed the insecticidal activity of different plant extracts and essential oils to the chemical constituents of these extracts. In addition, the chemical constituents of essential oils affect the insect's respiration rate, impair muscle activity and disrupt the nervous system and physiological processes which leads to paralysis or eventually death [71,72, and 73].

Table7: The major constituents of clove essential oil

Molecular formula	Name	Area %	RT
$C_{10}H_{12}O_2$	Eugenol	9.86	15.72
$C_{15}H_{26}O_2$	2H-Pyran-3-ol,tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S-[3à,6à(R*)]]-	4.60	27.00
$C_{18}H_{36}O_2$	Ethyl hexadecanoate	1.19	33.44
$C_{20}H_{36}O_2$	Ethyl linoleate	1.25	37.32
$C_{18}H_{31}ClO$	Linoleoyl chloride	0.82	37.45
$C_{20}H_{42}O$	1-Eicosanol	3.18	38.69
$C_{30}H_{62}$	Triacontane	6.20	38.92
$C_{41}H_{77}F_5O_2$	Octatriacontylpentafluoropropionate	2.12	39.42
$C_{27}H_{54}$	1-Heptacosene	53.31	39.97
$C_{27}H_{56}$	Heptacosane	4.94	40.09
$C_{27}H_{30}O_{16}$	6,8-DI-C-á-Glucosylluteolin	067	40.33
$C_{32}H_{66}$	Dotriacontane	3.29	40.90
$C_{39}H_{76}O_3$	Oleic acid, 3-(octadecyloxy)propyl ester	2.05	41.64
$C_{35}H_{70}$	17-Pentatriacontene	2.16	42.65

Table8: The major constituents of Garlic essential oil

Molecular formula	name	Area %	RT
$C_{11}H_{20}O_3$	Tert-butyl 4-hydroxy-4-methyl-5-hexenoate	0.05	4.47
$C_{17}H_{32}O_2$	(8Z)-7-Methyl-8-tetradecenyl acetate	0.03	4.92
$C_{19}H_{32}O_2$	Methyl (6e,9e,12e)-6,9,12-octadecatrienoate	0.03	5.78
$C_6H_{10}S_2$	Diallyldisulphide	0.11	7.08
$C_6H_{10}S_2$	Disulfide, (1E)-1-propen-1-yl 2-propen-1-yl	0.03	7.80
$C_{16}H_{35}NO_4S$,	N,N-Diethanoldodecylsulfonamide	0.06	8.72
$C_4H_8S_3$	1-Allyl-3-methyltrisulfane	0.12	8.84
$C_6H_8S_2$	3-Vinyl-3,6-dihydro-1,2-dithiine	0.40	10.27
$C_{14}H_{29}C_l$	Tetradecane, 1-chloro-	0.02	10.77
$C_{22}H_{30}N_2O_3$	Aspidospermidin-17-ol,1-acetyl-16-methoxy-	0.02	12.42
$C_{17}H_{32}O_2$	4-Cyclopropylcarbonyloxytridecane	0.02	12.78
$C_6H_{10}S_3$	Trisulfide, di-2-propenyl	0.28	13.82
$C_2H_7NO_3S_2$	2-Aminoethanethiolsulfuric acid	0.05	13.93
$C_{18}H_{30}D_6O$	2,2,3,3,4,4 Hexadeuterooctadecanal	0.03	15.54
$C_{10}H_{12}O_2$	Eugenol	1.32	15.73
$C_{32}H_{66}$	Dotriacontane	0.04	16.15
$C_{13}H_{20}O$	4a,7,7-Trimethyl-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone	0.02	16.98
$C_{18}H_{37}C_l$	1-Chlorooctadecane	0.08	17.09
$C_{13}H_{20}O$	2,5,5,8A-Tetramethyl-3,5,8,8a-tetrahydro-2h-chromene	0.04	17.58
$C_{18}H_{34}D_2O$	2,2-Dideutero octadecanal	0.05	18.72
$C_{15}H_{24}O$	cis-Z-à-Bisabolene epoxide	0.04	18.89
$C_{17}H_{32}O$	8-Hexadecenal, 14-methyl-, (Z)-	0.05	19.04
$C_{15}H_{26}O_2$	Geranylisovalerate	0.09	19.25
$C_{17}H_{32}O_2$	(8Z)-7-Methyl-8-tetradecenyl acetate	0.02	19.54
$C_{19}H_{36}O$	2-Methyl-Z,Z-3,13-octadecadienol	0.02	19.91
$C_{16}H_{28}O_3$	(11Z)-12-(2-Oxiranyl)-11-dodecenyl acetate	0.02	20.02
$C_{16}H_{30}O_2$	1,2-15,16-Diepoxyhexadecane	0.12	20.15
$C_{10}H_{12}O_2$	4-Allyl-2-Methoxy-phenol	0.30	20.94
$C_{32}H_{66}$	Dotriacontane	0.1	22.23
$C_{15}H_{24}O_2$	Baimuxinal	0.34	24.66
$C_{15}H_{20}O_2$	Costunolide	0.68	26.59
$C_{15}H_{26}O_2$	(3S,6S)-2,2,6-Trimethyl-6-((S)-4-methylcyclohex-3-en-1-yl)tetrahydro-2H-pyran-3-ol	3.63	27.00
$C_{19}H_{36}O$	2-Methyl-E,E-3,13-octadecadien-1-ol	0.42	27.84
$C_{18}H_{37}C_l$	1-Chlorooctadecane	1.24	28.56
$C_{37}H_{76}O$	1-Heptatriacotanol	2.43	33. 15
$C_{21}H_{36}$	14-á-H-Pregna	58.86	35.35
$C_9H_{16}BrNO$	1-(4-Bromobutyl)-2-piperidinone	20.53	35.90

Table9: The major constituents of Camphor essential oil

Molecular formula	name	Area %	RT
$C_{10}H_{16}$	camphene	0.65	4.08
$C_{10}H_{18}O$	Eucalyptol	2.42	5.85
$C_{10}H_{16}O$	Alcanfor	17.59	9.00
$C_{10}H_{20}O$	Levomenthol	3.79	9.93
$C_8H_8O_3$	Methyl salicylate	14.49	10.67
$C_{10}H_{12}O_2$	4-allyl-2-methoxy-phenol	1.29	15.73
$C_{16}H_{34}$	Nonadecane	0.79	32.08
$C_{15}H_{26}O_2$	Bisabolol oxide B	0.76	24.54
$C_{19}H_{40}$	Nonadecane	2.46	25.89
$C_{15}H_{26}O_2$	(3S,6S)-2,2,6-Trimethyl-6-((S)-4-methylcyclohex-3-en-1-yl)tetrahydro-2H-pyran-3-ol	5.93	27.00
$C_{19}H_{40}$	Nonadecane	4.80	28.56
$C_{19}H_{32}$	6-phenyltridecane	2.39	29.24
$C_{25}H_{52}$	pentacosane	6.14	31.12
$C_{30}H_{62}$	triacontane	6.39	33.56
$C_{32}H_{66}$	dotriacontane	4.66	35.04
$C_{21}H_{44}$	heneicosane	5.46	35.89

Table10: The major constituents of Mustard essential oil

Molecular formula	name	Area %	RT
$C_{10}H_{12}O_2$	4-allyl-2-methoxy-phenol	59.52	15.82
$C_{12}H_{14}O_3$	4-allyl-3-methoxyphenyl acetate	6.72	20.95
$C_{15}H_{26}O_2$	3s,6s)-2,2,6-trimethyl-6-((s)-4-methylcyclohex-3-en-1-yl)tetrahydro-2h-pyran-3-ol	4.05	27.01
$C_{27}H_{56}$	heptacosane	25.49	37.95
$C_{27}H_{54}O_4Si_2$	2,3-bis[(trimethylsilyl)oxy]propyl (9z,12z)-9,12-octadecadienoate	0.37	40.94

Finding new alternatives to synthetic pesticides was a primary objective of this study, however, before recommendation these essential oils based on their insecticidal results, the cytotoxic effect of these oils was tested.

3.7. Effect of four essential oils on wheat seeds germination

As shown in table 11, both garlic and camphor oils were the least influential on the growth of the wheat grains. The percentage of seeds germination at treatment with both oils at LC_{50} value was approximately 90%. Whereas at LC_{90} value, garlic essential oil was the safest one for wheat grain growth, the percentage of germination was 83.35 ± 3.54%.

Table11: Effect of four essential oils on wheat seeds germination

Essential oils	**Percentage of seed germination ± SD at LC_{50} and LC_{90}**	
	LC_{50}	**LC_{90}**
Syzygium aromaticum	73.35^{d}± 5.77	61.65^{e}± 2.89
Allium sativum	91.65^{b} ± 3.54	83.35^{b} ± 3.54
Cinnamomum camphora	90.00^{b} ± 5.00	76.35^{c} ± 7.64
Brassica junicea	78.35^{c} ± 7.64	66.65^{d} ± 7.64
control	96.65^{a} ± 2.89	96.65^{a} ± 2.89

3.8. Cytotoxic effect of the tested essential oils

In this test, we focus only on clove and garlic essential oils due to their highest insecticidal, antifeedant and repellence effects. Results in Table 12 revealed that the IC_{50} values were 85.38 ± 4.2 and 68.45±3.8 µg/ml for clove and garlic, respectively indicating weak *in vitro* cytotoxicity of the tested essential oils on the WI-38 cells.

In conclusion, the insecticidal, antifeedant, and repellent activity of tested essential oils as well as low phytotoxic effect indicated the potential use of these oils as grains protectants against the attack of stored grain pests. we recommend these essential oils to be tested against another insect pests and under field conditions. For more safety, we recommended the washing of treated grains before grinding.

Table12: cytotoxic activity of clove and garlic oils against normal WI -38 cells.

Essential oil	IC_{50} (µg/ml)*
Clove	85.38±4.2
Garlic	68.45±3.8

*$\mathbf{IC_{50}}$: The half-maximal inhibitory concentration
**IC_{50} (µg/ml):1-10 (very strongly toxic). 11-20 (strongly toxic). 21-50 (moderately toxic). 51-100 (weakly toxic) and above 100 (non-toxic).

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