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Effect of Essential Oils of Clove and Dill Applied as an Insecticidal Contact and Fumigant to Control some Stored Product Insects

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Contact and fumigation toxicity of Clove and Dill essential oils were investigated in the laboratory against the adults of *Sitophilusoryzae* (L.), *Rhyzoperthadominica* (F.) and *Triboliumcastaneum* (Herbst.). The results showed that insect mortality was increased by increasing plant oils concentration and the period of exposure. The adults of *S. oryzae* were the most susceptible insect species under study followed by *R. dominica* then *T. castaneum* which was the least sensitive to the two plant oils. The toxicity of Clove and Dill oils against the tested insects was much higher in the fumigant bioassay tests than in the contact method. Clove oil was more effective than Dill oil against the three insect species. The results indicated also that these plant oils could be used as grain protectants or fumigants to control stored products insects

INTRODUCTION

Fumigants such as methyl bromide and phosphine are still the most effective for the protection against insect infestation of stored food, feedstuffs, and other agricultural commodities [1]. has proposed elimination of the production of methyl bromide because of its ozone depletion potential. Additionally, some stored products insects are found to have resistance to methyl bromide and phosphine [2]. These problems have high-lighted the need for the development of new types of selective insect-control alternatives with fumigant action. Natural compounds of plant origin are biodegradable, often of low mammalian toxicity, and pose low danger to the environment if used in small amounts. Plants may provide potential alternatives to currently used insect control agents, because they constitute a rich source of bioactive chemicals [3]. Recent research has focused on natural product alternatives for pest control in developing countries to develop new classes of safer insect-control agents. Recently, there has been a growing interest in research concerning the possible use of plant extracts as alternatives to synthetic insecticides. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored products insects. Some essential oils were found to have potential for the control of stored products insect pests [4-10] . Essential oils exhibit various and variable antimicrobial activities, including antifungal, antiviral, antibacterial, insecticidal, and antioxidant properties [11] . In order to keep these stored grain products free from pest attack, various synthetic chemicals have been used. Synthetic pesticides are currently the appropriate choice to protect stored grains from insect damage. However, continuous or heavy use of synthetic pesticides has created serious problems arising from factors such as direct toxicity to parasites, predators, pollinators, fish and man. It also develops pesticides resistance [12, 13] susceptibility of crop plant to insect pests [14] and increased environmental and social cost [15]. Therefore, other alternatives rather then chemical pesticides are needed to protect the environment.

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One alternative to synthetic insecticides is the botanical pesticides i.e. insecticidal plants or plant compound and the use of natural compounds, such as essential oils that result from secondary metabolism in plants. Essential oils and their constituents have been shown to be a potent source of botanical pesticide. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of bruchid pests [16-18] **.** Plant essential oils and their constituents, in relation to contact and fumigant insecticidal actions, have been well demonstrated against stored products pests. Their main compounds (monoterpenoids) offer promising alternatives to classical fumigants [19] and also have some effects on biological parameters such as growth rate, life span and reproduction [20]**.** This study presents the contact and fumigant activities of Clove and Dill oils against three of the stored products insects, namely, the rice weevil; the lesser grain borer, and the red flour beetle.

MATERIAL AND METHODS

Insect species used

Three species of stored products insects namely, the rice weevil, *Sitophilusoryzae* (L.) (Curculionidae, Coleoptera); the lesser grain borer, *Rhyzoperthadominica* (F.) (Bostrochidae, Coleoptera) and the red flour beetle, *Triboliumcastaneum* (Herbst.) (Tenebrionidae, Coleoptera) were used in this study. Tests were performed in the stored product pests Laboratory at the Plant Protection Department, Faculty of Agriculture, Moshtohor, Benha University. The insects were reared in glass jars (approx. 500 ml) containing about 200 g of sterilized and conditioned wheat kernels in case of *S. oryzae* and *R. dominica* or crushed wheat grains in case of the red flour beetle. The glass jars were covered with muslin. Insect cultures were kept under controlled conditions of 28±1° C and 65±5% R.H. at the rearing room of the laboratory. Wheat grains were treated by freezing at -18° C for 2 weeks before application to eliminate any possible infestation by any insect species. The moisture content of the food was around 14%. Mass cultures of around 1000 adults of each insect species (1-2 weeks old) were introduced into the jars for laying eggs and then kept at 28±1° C and 65±5% R.H. Three days later, all insects were separated from the food, and the jars were kept again at the controlled conditions in the rearing room. This procedure was repeated several times in order to obtain a large number of the adults needed to carry out the experiments during this study and to determine the durations of the various developmental stages under laboratory conditions. The foods in the jars were renewed when it was necessary.

Essential oils used

Clove and Dill essential oils were bought from Al-gomhuria Company of drugs, chemicals and medical supplies in Egypt. The contact and fumigant toxicity of this oils were tested to the adults of various insect species under study.

Contact toxicity

Ten grams of each pure oil was diluted with 50 ml. acetone to obtain 20% (w/v) stock concentration which was diluted to obtain 10, 5, 2, 5 and 1.25% (w/v) concentrations. From each concentration, one ml. was taken and added to 10 gm wheat grains to obtain 2, 1, 0.5, 0.25 and 0.125% (w/w) concentrations. In case of *S. oryzae* and *R. dominica* ten grams of wheat grains were taken while *T. castaneum* were put in crushed grains. Thirty adult insects were added to each treatment and incubated at 28±1 °C and 65±5% R.H. Three replicates were used for each treatment. As for control, only acetone was used for food treatment. Insects mortality was calculated after 1, 2, 3, 5, 7, 10 and 14 days from initial treatment.

Fumigant toxicity

In this experiment, 200 ml glass jars with tightened covers were used as fumigation chambers for the plant oil. The tested dosages of each oil inside the jars were 62.5, 125, 250, 500, and 1000 mg/l. air. Six jars were taken in each treatment. Inside every jar one filter paper was inserted at the bottom. Then one ml from each oil concentration of the different prepared concentrations (20; 10; 5; 2.5 and 1.25 % w/v) was taken and added to every glass jar on a filter paper for achieving the mentioned oil dosages inside the well closed jars. Twenty adults were put inside each jar in cotton bags (2×1 cm) with a few amount of wheat kernels in case of *S. oryzae* and *R. dominica* and crushed wheat for *T. castaneum.* The jars were well closed and incubated at 28±1 °C and 65±5% R.H. The same steps were followed in the control treatment using only acetone without oil. Mortality rates were calculated after 1, 2, 3, 5 and 7 days post treatment.

Statistical analysis

The dosage mortality response was determined by probit analysis [21] using a computer program of a pervious study [22].

Results

Contact toxicity of Clove oil against some stored product insects at 28±1° C and 65±5% R.H

The lethal concentrations (LC) values were determined for both *S. oryzae*, *R. dominica* and *T. castaneum*. The LC of Clove essential oil to the adults of *S. oryzae*, *R. dominica* and *T. castaneum* are shown in **Table (1).** The results show that the LC are exposure period dependent. The higher the exposure period was the lower the LC values were. At 7 days post treatment, the LC_{50} values were 0.13 and 0.45% (w/w), the corresponding values at 14 days were significantly lower and amounted 0.11 and 0.10 % (w/w). for *S. oryzae* and *R. dominica*, respectively. The LC_{90} values were 2.23 and 18.37% (w/w) at 7 days and declined to 0.46 and 1.37 %(w/w) at 14 days post treatment for *S. oryzae* and *R. dominica*, respectively. The LC_{95} values were 4.97 and 52.44% (w/w) at 7 days and reduced to 0.69 and 2.83 %(w/w) at 14 days from treatment for *S. oryzae* and *R. dominica*, respectively. At 10 days post treatment, the LC_{50} value was 1.02 (w/w), the corresponding value at 14 days was significantly lower and amounted 0.42 (w/w), for *T. castaneum*. The LC_{90} value was 40.73 % (w/w) at 10 days and declined to 5.62 %(w/w) at 14 days post treatment for *T. castaneum*, the LC_{95} value was 115.76 % (w/w) at 10 days and reduced to 11.69 %(w/w) at 14 days from treatment for *T. castaneum*. The results show also that *S. oryzae* was the most sensitive insect species to Clove essential oil followed by *R. dominica*and *T. castaneum* which was the least sensitive to Clove oil, when the oil was applied in the contact method.

Contact toxicity of Dill essential oil against some stored product insects at 28± 1°C and 65±5% R.H

The LC values were determined for both *S. oryzae*, *R. dominica* and *T. castaneum* . The LC of Dill essential oil to the adults of *S. oryzae*, *R. dominica* and *T. castaneum* are shown in **Table (2).** The results show that the L Care exposure period dependent. The higher the exposure period was the lower the LC values were.

Table (1): Lethal concentrations of Clove essential oil in the contact bioassay against some stored products insects at various exposure periods

Exposure period (days)	Lethal concentrations (w/w%) and their 95% confidence limits			Slope ± SD	R
	LC_{50}	LC_{90}	LC_{95}		
Sitophilusoryzae					
7 days	0.13 (0.06-0.26)	2.23 (0.90-5.49)	4.97 (1.43-17.33)	1.04 ± 0.03	0.981
10 days	0.11 (0.06-0.20)	1.04 (0.59-1.85)	1.94 (0.88-2.29)	1.34±0.12	0.964
14 days	0.11 (0.07-0.17)	0.46 (0.34-0.63)	0.69 (0.46-1.03)	2.12±0.55	0.938
Rhyzoperthadominica					
7 days	0.45 (0.26-0.77)	18.37 (2.36-142.81)	52.44 (3.86-711.95)	0.79±0.41	0.979
10 days	0.77 (0.08-0.31)	3.35 (1.14-9.81)	7.84 (1.83-33.42)	0.98±0.03	0.979
14 days	0.10 (0.05-0.21)	1.37 (0.68-2.79)	2.83 (1.05-7.63)	1.16±0.07	0.970
Triboliumcastaneum					
10 days	1.02 (0.53-1.94)	40.73 (3.55-467.11)	115.76 (5.76-2326.46)	0.80±0.008	0.992
14 days	0.42 (0.29-0.62)	5.62 (1.98-15.92)	11.69 (3.13-43.58)	1.14±0.005	0.997

Table (2): Lethal concentrations of Dill essential oil in the contact bioassay against some stored products insects at various exposure periods

Exposure period (days)	Lethal concentrations (w/w%) and their 95% confidence limits			Slope ± SD	R
	LC_{50}	LC_{90}	LC_{95}		
		Sitophilusoryzae			
7 days	0.18 0.10-0.33	3.70 1.26-10.83	8.63 2.05-36.39	0.98±0.0 3	0.982
10 days	0.07 0.02-0.20	1.58 0.66-3.81	3.78 1.05-13.57	0.96±0.0 1	0.992
14 days	0.06 0.02-0.15	0.60 0.36-0.99	1.12 0.54-2.32	1.33±0.3 0	0.916
		Rhyzoperthadominica			
7 days	0.31 0.19-0.50	6.25 1.80-21.74	14.60 2.92-73.00	0.98±0.0 6	0.963
10 days	0.18 0.09-0.35	4.92 1.34-18.04	12.58 2.19-72.09	0.89±0.0 1	0.990
14 days	0.12 0.06-0.24	1.83 0.83-4.01	3.89 1.31-11.48	1.10±0.0 2	0.987
		Triboliumcastaneum			
10 days	0.97 0.60-1.58	16.29 3.57-74.23	36.19 5.69-229.84	1.04±0.0 4	0.977
14 days	0.49 0.30-0.78	12.85 2.50-66.08	32.45 4.08-257.47	0.90±0.0 3	0.976

R= Correlation Coefficient of regression line

SD= Standard deviation of the mortality regression line

At 7 days post treatment the LC50 values were 0.18 and 0.31% (w/w). the corresponding value at 14 days was significantly lower and amounted 0.06 and 0.12 % (w/w) for S. oryzae and R. dominica, respectively. The LC90 values were3.70 and 6.25% (w/w) at 7 days and declined to 0.60 and 1.83 %(w/w) at 14 days post treatment for S. oryzae and R. dominica, respectively. The LC95 values were8.63 and 14.60% (w/w) at 7 days and reduced to 1.12 and 3.89 %(w/w) at 14 days from treatment for S. oryzae and R. dominica, respectively. At 10 days post treatment the LC50 value was 0.97 % (w/w), the corresponding value at 14 days was significantly lower and amounted 0.49 % (w/w) for T. castaneum. The LC90 value was 16.29 % (w/w) at 10 days and declined to 12.85 %(w/w) at 14 days post treatment for T. castaneum, the LC95 value was 36.19 % (w/w) at 10 days and reduced to 32.45 %(w/w) at 14 days from treatment for T. castaneum. The results show also that S. oryzae was the most sensitive insect species to Dill essential oils followed by R. dominica and T. castaneum which was the least sensitive to Dill oil when the oil was applicated in the contact bioassay test

Fumigant toxicity of Clove essential oil against some stored product insects at 28±1° C and 65±5% R.H

The LC values were determined for both S. oryzae, R. dominica and T. castaneum. The LC of Clove oil to the adults of S. oryzae, R. dominica and T. castaneum are shown in Table (3). The results show that the LC are exposure period dependent. The higher the exposure period was the lower the LC values were. At 3 days post treatment, the LC50 value was 92.40, 121.18 and 294.61 % mg/l. air. The corresponding values at 7 days were significantly lower and amounted 23.29, 33.67 and 42.92% mg/l. air. For S. oryzae, R. dominica and T. castaneum respectively. The LC90 value was 1895.89, 2421.98 and 23905.76% mg/l. air at 3 days and declined to 222.06, 491.81 and 2124.73 % mg/l. air at 7 days post treatment for S. oryzae, R. dominica and T. castaneum respectively. The LC95 value was 4466.0, 5662.87 and 83164.9% mg/l. air at 3 days and reduced to 420.87, 1052.08 and 6424.96% mg/l. air at 7 days from treatment for S. oryzae, R. dominicaand T. castaneum respectively. The lethal time of Clove flowering buds oils against the adults of S. oryzae, R. dominica and T. castaneum is shown in Table

(4). The results reveal that the time required to obtain 50% kill (LT50) at 1000 mg/l. air concentration were1.06, 1.09 and 1.80 days for S. oryzae, R. dominica and T. castaneum, respectively. The time needed to achieve 90% mortality (LT90) was 3.25, 4.31 and 9.82 days for S. oryzae, R. dominica and T. castaneum, respectively. The time required to obtain 95% mortality (LT95) were 4.46, 6.36 and 15.88 days for the various insects, respectively. At 500 mg/l. air. the time needed to obtain 50% kill (LT50) at was 1.29, 1.36 and 2.38 days for S. oryzae, R. dominica and T. castaneum, respectively. The times needed to achieve 90% mortality (LT90) were 5.94, 9.04 and 15.98 days for S. oryzae, R. dominica and T. castaneum, respectively. The times required to obtain 95% mortality (LT95) were 9.13, 15.46 and 27.40 days for the various insects, respectively.

Fumigant toxicity of Dill essential oil against some stored product insects at 28 ±1°C and 65±5% R.H

The LC values were determined for both S. oryzae, R. dominica and T. castaneum, The LC of Dill essential oils to the adults of S. oryzae, R. dominica and T. castaneum are shown in Table (5). The results show that LC are exposure period dependent.

Table (3): Lethal concentrations of Clove essential oil in the fumigation bioassay against some stored products insects at various exposure periods

Exposure period (days)	Lethal concentrations (mg/l. air) and their 95% confidence limits			Slope ± SD	R
	LC_{50}	LC_{90}	LC_{95}		
		Sitophilusoryzae			
3 days	92.40 50.43-169.28	1895.89 614.97-5844.82	4466.00 986.5-20217.5	0.97±0.06	0.964
5 days	30.86 10.06-94.61	667.66 288.4-1545.1	1596.53 455.6-5593.9	0.96±0.05	0.970
7 days	23.29 7.81-69.41	222.06 136.50-356.03	420.90 212.93-831.98	1.30±0.09	0.970
		Rhyzoperthadominica			
3 days	121.18 71.80-204.53	2421.98 747.70-7845.31	5662.87 1207.64-26554.29	0.98±0.01	0.993
5 days	39.26 13.25-116.30	1241.21 395.21-3898.18	3305.19 630.69-17321.22	0.85±0.01	0.987
7 days	33.67 12.78-88.66	491.81 251.02-963.56	1052.08 389.10-2844.67	1.10±0.10	0.954
		Triboliumcastaneum			
3 days	294.61 157.04-552.7	23905.76 1214.6-470485.3	83164.9 1927.8-3587667	0.67±0.0008	0.998
5 days	71.19 22.54-224.81	10993.99 573.7-210660.6	45903.21 845.33-2492620	0.58±0.01	0.987
7 days	42.92 13.45-136.95	2124.73 463.24-9745.30	6424.96 734.19-56225.1	0.75±0.01	0.983

Table (4): Lethal times of Clove essential oil in the fumigation bioassay against some stored product insects at two fixed oil concentrations

Conc. (mg/l. air)	Lethal times and their 95% confidence limits (days)			Slope ± SD	R
	LT_{50}	LT_{90}	LT_{95}		
		Sitophilusoryzae			
1000	1.06 0.79-1.43	3.25 2.51-4.21	4.46 3.19-6.24	2.64±0.06	0.989
500	1.29 0.92-1.80	5.94 3.94-8.95	9.16 5.29-15.87	1.93±0.06	0.981
		Rhyzoperthadominica			
1000	1.09 0.77-1.55	4.31 3.11-5.98	6.36 4.11-9.85	2.14±0.02	0.993
500	1.36 0.91-2.03	9.04 4.97-16.44	15.46 6.91-34.58	1.56±0.00 2	0.999
		Triboliumcastaneum			
1000	1.80 1.34-2.42	9.82 5.64-17.10	15.88 7.71-32.72	1.74±0.0 1	0.993
500	2.38 1.79-3.17	15.98 7.45-34.28	27.40 10.35-72.55	1.55±0.0 1	0.992

R= Correlation Coefficient of regression line
SD= Standard deviation of the mortality regression line

Table (5): Lethal concentrations of Dill essential oil in the fumigation bioassay against some stored products insects at various exposure periods

Exposure period (days)	Lethal concentrations (mg/l. air) and their 95% confidence limits			Slope ±SD	R
	LC_{50}	LC_{90}	LC_{95}		
		Sitophilusoryzae			
3 days	97.38 52.50-180.65	2313.58 674.26-7938	5681.17 1093.01-29529.0	0.93±0.01	0.9 89
5 days	30.53 8.73-106.78	1005.06 337.05-2996.9	2707.13 532.26-13768.63	0.84±0.05	0.9 63
7 days	30.76 12.35-76.61	291.49 178.24-476.70	551.52 269.42-1128.97	1.31±0.18	0.9 44
		Rhyzoperthadominica			
3 days	117.44 59.45-232.01	5430.74 842.44-35008.6	16107.84 1374.57-188759.3	0.76±0.01	0.9 90
5 days	45.64 15.29-136.20	2058.06 483.32-8763.53	6060.78 777.10-47268.81	0.77±0.003	0.9 97
7 days	44.68 19.39-102.95	716.14 330.74-1550.61	1572.79 520.60-4751.58	1.06±0.01	0.9 90
		Triboliumcastaneum			
3 days	165.71 93.54-293.54	6919.31 1010.8-47364.9	19937.77 1654.7-240225.4	0.79±0.03	0.9 68
5 days	92.66 44.38-193.43	3677.13 727.38-18588.9	10443.75 1187.87-91821.3	0.80±0.004	0.9 96
7 days	77.06 41.78-142.12	1229.28 501.66-3012.20	2696.21 797.87-9111.18	1.06±0.01	0.9 94

Table (6): Lethal times of Dill essential oil in the fumigation bioassay against some stored products insects at two fixed oil concentrations

Conc. (mg/l. air)	Lethal times and their 95% confidence Limits (days)			Slope ± SD	R
	LT_{50}	LT_{90}	LT_{95}		
		Sitophilusoryzae			
1000	1.15 0.87-1.53	3.61 2.77-4.71	4.99 3.53-7.04	2.59±0.11	0.9 81
500	1.36 0.95-1.95	7.41 4.52-12.15	11.98 6.18-23.22	1.74±0.02	0.9 90
		Rhyzoperthadominica			
1000	1.23 0.86-1.78	6.03 3.95-9.21	9.46 5.35-16.72	1.86±0.02	0.9 91
500	1.56 1.11-2.19	9.26 5.25-16.34	15.34 7.23-32.53	1.65±0.03	0.9 98
		Triboliumcastaneum			
1000	1.06 0.61-1.86	9.72 4.64-20.38	18.20 6.51-50.92	1.33±0.04	0.9 72
500	1.62 1.08-2.42	14.16 6.17-32.51	26.19 8.69-78.88	1.36±0.02	0.9 83

R= Correlation Coefficient of regression line
SD= Standard deviation of the mortality regression line

The higher the exposure period was the lower the LC values were. At 3 days post treatment the LC_{50} values were 97.38, 117.44 and 165.71 mg/l. air. the corresponding values at 7 days were significantly lower and amounted 30.76, 44.68 and 77.06 mg/l. air. For *S. oryzae*, *R. dominica* and *T. castaneum* respectively. The LC_{90} values were q2313.58, 5430.74 and 6919.31 mg/l. air. At3 days and declined to 291.49, 716.14 and 1229.28 mg/l. air. at 7 days post treatment for *S. oryzae*, *R. dominica* and *T. Castaneum* respectively. the LC_{95} values were 5681.17, 16107.84 and 19937.7mg/l.air.at3 days and reduced to 551.52, 1572.79 and 2696.21 mg/l. air. at7 days from treatment for *S. oryzae*, *R. dominica* and *T. castaneum* respectively. The lethal times (LT) of Dill oil against the adults of *S. oryzae*, *R. dominica* and *T. castaneum* are shown in **Table (6).** The results reveal that the time required to obtain 50% mortality (LT_{50}) at 1000 mg/l. air. was1.15, 1.23 and 1.06 days for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. The times needed to achieve 90% mortality LT_{90} were 3.61, 6.03 and 9.72 days for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. The time required to obtain 95% kill LT_{95} were 4.99, 9.46 and 18.20 days for the various insects, respectively. At 500 mg/l. air. the time needed to obtain 50% kill LT_{50} were 1.36, 1.56 and 1.62 days for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. The times needed to achieve 90% mortality LT_{90} were 7.41, 9.26 and 14.16 days for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. The time required to obtain 95% kill LT_{95} were 11.98, 15.34 and 26.19 days for the various insects, respectively.

Discussion

Several oils were tested against some stored product insects attacking grain. There were differences in oil efficacy at the doses tested under different experimental conditions, as noted by Pierrard [23]. The present results corroborate the findings of pervious studies [24] and [25**]** which reported the toxic effect of neem oil, coconut oil, rapeseed oil, mustard oil, sesame oil, dalda and palm oil on *C. chinensis*. More current research illustrated that essential oils and their constituents may have potential as alternative compounds to currently used fumigants [26, 27] and [28,29]. Cinnamaldehyde, the main constituent of cinnamon oil, exerted equal contact toxicity to both *T. castaneum* and *S.* [30]. Oil of clove was toxic to *S. oryzae* and *Rhyzopertha dominica* [31]. Non-polar extracts of the flower buds of clove, *Syzygiumaromaticum* and star anise (*Illiciumuvrum* Hook f.) are insecticidal to *T. castaneum* and *S. zeamais* Motsch., and suppress progeny production [32]. It was found that against C. *maculatus*, *C. chinensis* and *C. analis* attack in *V. radiata*, neem oil *(Azadirachtaindica* A. Juss) allowed no adult emergence, reduced oviposition, and prevented insect development [33]. In the present study the fumigation bioassay showed also that *S. oryzae* was the most sensitive insect species to Dill essential oils followed by *R. dominica* and *T. castaneum* which was the least sensitive Dill oil when the oil was applicated as fumigation. In addition, the results of the toxicity of Clove and Dill oils against the tested insects indicated clearly that effectiveness of the two oils in the fumigation bioassay tests was obviously higher than in the contact method.

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