

REPELLENT AND INSECTICIDAL ACTIVITY OF DERIVED PLANT OILS AGAINST SOME STORED GRAIN INSECTS

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ABSTRACT

Chemical composition of botanical oil garlic (*Allium sativum*) and chamomile (*Matricaria camomela*), its toxic and repellent activity were investigated against three stored product insects, *Callosobruchus maculatus* (Fab.), *Trogoderma granarium* (Khapra beetle), and *Tribolium castaneum* (Herbst). Malathion dust was used as a standard chemical insecticides against stored product insects while neem oil was implicated in this study as a known repellent agent. Based on LC₅₀ values of adults, it is quite clear that garlic, in general had a high toxic effect against adult stage of *C. maculatus*. Complete mortality was achieved at a concentration of 50 and 100 ppm of garlic against *C. maculatus* and *T. granarium* after 2 weeks post treatment. The repellent action of the tested essential oils (garlic, chamomile and neem) was increased with the increasing of concentration with the tested insect species. There was highly significant differences between the repellent effect of neem oil with the three tested insects compared with that of chamomile and garlic oils. Malathion dust had the highest action against the tested insects compared with the two oils used in this study.

INTRODUCTION

Stored product insects are a perennial problem in retail stores, where they damage and contaminate susceptible merchandise such as food products and animal feed. In stored grain, insect damage may account for 10-40% of loss worldwide (Matthews, 1993). *C. maculatus*, *T. granarium* and *T. castaneum* are of the most common species attacking stored grain and other products. Insect control in stored product relies heavily on the use of gaseous fumigants and residual contact insecticides, both of which can pose serious hazards to warm-blooded animals and environment (Shiaaya *et al.*, 1991; White, 1995). Natural products are well known to have a range of useful biological properties against insect pests (Arthur, 1996). In this regard, many plants products have been evaluated for their insecticidal properties against different stored grain pests (Mondal and Khaleqzaman, 2010). Bhuwan and Tripathi (2011) observed highest repellent activity for *Schzygium aromaticum* essential oil against (90%) and *Sitophilus oryzae* (90%).

Udo (2011) tested the biological activity of *Zanthoxylum zanthoxyloids* against *Sitophilus zeamais* and *C. maculatus*. He found that the extracts also evaluated moderate repellent effect against the two insect pests.

Essential oils, obtained by the distillation of plant foliage and even the foliage itself of certain aromatic plants have traditionally been utilized to protect stored grain and legumes (Isman, 2000 and 2006). In recent years, essential oils have received a great deal of attention as pest control agents. They are volatile and can function as fumigants, and may also be applicable

to the protection of stored products. Essential oils from plants are valuable secondary metabolites which have already been used as raw materials in many fields, including perfumes, cosmetics, phytotherapy and nutrition. These oils also offer potential as sources of insecticides with environmental compatibility (Katz *et al.*, 2008). Recently, many studies have focused on the possibility of using plant essential oils for application to stored grain to control insect pests (Collins, 2006; De Carvalho and Da Fonseca, 2006). Neem oil, a known insect repellent against *T.castaneum* and *T.granarium* they found that 10%oil treatment reduced the insect penetration more than those of 5% (Anwar *et al.*, 2005). Garlic, *Allium sativum* L. (Amarylidaceae), oil and its two major constituents, methyl allyl disulfide and diallyl trisulfide, have been demonstrated to be highly toxic to both *S. zeamais* (L.) (Coleoptera: Curculionidae)and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Ho *et al.*, 1996; Huang *et al.*, 2000) as well as other insect pests (Park and shin, 2005; Park *et al.*, 2006;; Kimbaris *et al.*, 2009), indicating their potential for use in grain protection. However, plant essential oils, like those from garlic, may leave a persistent odor and when applied at high dose could cause food to retain a strong smell and unpleasant taste (Liu and Ho, 1999; Sékou *et al.*, 2000; Benkeblia, 2004).

This study was initiated to evaluate the toxicity and repellence of garlic, chamomile and neem essential oils and malathion dust against three stored product insects *C. maculatus*, *T. granarium* (Khapra beetle) and *T. castaneum*.

MATERIALS AND METHODS

Insects:

C. maculatus, *T. granarium* and *T. castaneum* cultures were reared under laboratory conditions (27°C and 70±5 R.H.). Adult insects 1-3 weeks old were collected and used for the bioassay tests.

Botanical oils:

Three commercially available essential oils (garlic, chamomile and neem) were tested in this study. The first two oils were obtained from CAB Farm Chemical Col., Egypt. Neem oil (10% azadirachtin) was obtained. Malathion dust (1%) was purchased from local market.

Contact toxicity:

Four dilutions of each oil (12.5, 25.0, 50.0 and 100 ppm) were prepared in acetone. Aliquots of 1 ml of each dilution were sprayed on twenty grams of wheat by using Potter Precision Laboratory Spray Tower to achieve homogenous distribution of oil. Wheat moisture content was 12.5%. Treated wheat was placed in 250 cc flasks. After acetone evaporation for an hour, ten unsexed adults of *C. maculatus*, *T. granarium* or *T. castaneum* separately were introduced to each flask. Flasks were covered with a piece of muslin by the aid of rubber band. Four concentrations of malathion dust (0.08, 0.1, 0.5 and 1 w/w) were admixed with wheat grain and cowpea, ten unsexed adults of *T. castaneum*, *T. granarium* and *C. maculatus* were introduced in 250 cc flasks contained wheat and cowpea treated with malathion dust. The control

and treatments were replicated four times. Flasks were kept under laboratory conditions for two weeks. Insect mortalities were determined and calculated after 3, 7 and 14 days from exposure, according to the formula of Abott (1925).

Repellency:

Previous concentrations of plant essential oils were also assayed for their repellency to *C. maculatus* or *T. granarium* or *T. castaneum*. Whatman No. 1 filter paper was cut into two equal halves, one half was treated with essential oil solution as uniform as possible by using micro pipette. The other half of the filter paper was treated with acetone only. The essential oil treated and acetone treated half-dish were then attached length wise, edge to edge with adhesive tape and placed at the bottom in glass Petri dish (9 cm). Ten adults of insects were released at the center of Petri dish and then Petri dish as covered and kept in dark. Four replicates were set for each concentration of essential oils. Number of the insects on both the treated and untreated halves was recorded after four hours in mild light. The repellency percentage (RP) was calculated using the method of Jilani *et al.* (1988). The parent repellency of the essential oil was calculated using the formula:

$$PR(\%) = [(N_c - N_t) / (N_c + N_t)] \times 100$$

Where N_c was the number of insects on the control half and N_t was the number of insects on the treated half according to Koko and Chandrapatya (2009).

All repellency assays were conducted in the laboratory. Insects that died during experimental period were replaced by the same aged adults from the same treatment.

GC-MS conditions:

GC-MS analysis was performed with an Agilent 6890 gas chromatography equipped with a mass spectrometric detector (MSD) mode Agilent 5973 with a DB-5 column with the same characteristics as the one used in GC. The transfer line temperature was 2601. The ionization energy was 70 ev with a scan time of 1S and mass range of 40-300 amu. These method according to Negahban *et al.* (2006).

Statistical analysis:

The least significant difference (LSD) at 0.05% level was used to compare treatment means (Waller and Duncan, 1969). Computations were done using SAS (1996).

RESULTS AND DISCUSSION

Chemical constituents of gralic and chamomile:

The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Coats *et al.*, 1991; Regnault-Roger and Hamraoui, (1995) and Ahn *et al.*, 1998). these results revealed that major components of the oil from garlic were N-octadecane (11.02%), N-nonadecane (8.62), butyl hexadecyl ester (4.04%), nonadecane (12.55%), Eicosanen (12.21%), Tetratetracontane (4.15%) and Octadecann (7.70%). Also, the chamomile oil was contained seven compounds such as Tran-beta-

Fornsene (18.69%) and Bisabolol oxid (27.91%) (Table 1). The mode of action of bioactive natural monoterpenoids (hydrocarbons, alcohols and ketons) from spearmint oils may be due to inhibition of acetylcholinesterase (Lee *et al.*, 2000). The compounds may be prove toxic when penetrating the insect body via the respiratory system (Park *et al.*, 2003).

Table (1): Chemical constituents of garlic and chamomile oils using GC-MS (MSD).

Plant oil	Chemical name	Retention index	% Composition
Garlic	N-octadecane	25.46	11.02
	N-nonadecane	25.26	8.62
	Butyl hexadecyl ester	26.94	4.04
	Nonadecane	27.23	12.55
	Eicosane	29.21	12.21
	Tetratetracontane	30.53	4.15
	Octadecan	31.07	7.70
Chamomile	Trans-beta-Fornsene	16.13	18.69
	Bisabolol oxid II	20.99	4.85
	Bisabolone oxid	21.42	3.597
	Bisabolol oxid A	23.14	27.91
	Palmitic acid	27.52	14.51
	Oleic acid	30.84	9.69
	Stearic acid	31.23	4.87

Efficacy of garlic and chamomile oils against *C. maculatus*, *T. granarium* and *T. castaneum*:

The susceptibility of *C. maculatus*, *T. granarium* and *T. castaneum* to garlic and chamomile oils were evaluated and the data are shown in Tables (2-4). It is quite clear that percent mortality of both tested oils are dependant on dosage and period of exposure time dependent. Based on LC₅₀ values data obtained cleared that chamomile oil had the highest effect on *T. castaneum* adults with LC₅₀ values of 103, 41.37 and 21.76 while the LC₅₀ values of garlic oil were 100, 91.48 and 31.35 after 3 days, one week and two weeks, respectively (Table 2). Also, the percent reduction of F₁ progeny and loss percentage of wheat grain were dependent on dosage. In general, the two tested oils had deteriorated effects on the two insect species studied, where the number of F₁-progeny and the weight loss of wheat significantly decreased compared to control. Data summarized in Table (3) revealed that garlic oil had the most effectiveness on the three parameters tested of *T. granarium*, percent mortality, reduction percentage of F₁ progeny and percent inhibition in weight loss of wheat grain compared to chamomile oil which had the least effect where the LC₅₀ values of garlic oil were 33.29, 18.39 and 14.06 after 3 days, one week and two weeks of exposure to treated wheat grain. Reduction percentage of progeny ranged from 46.9 to 79.7 with garlic oil while with chamomile oil reduction of F₁ progeny ranged from 29.7 to 66.4% at the all tested levels of tested oils. Data obtained in Table (3) exhibited that garlic or chamomile oil significantly reduced the percent loss of wheat grain where ranged from 0.5 to 3.5 and 1.3 to 5.1 with the two tested oils mentioned above, respectively compared to control which had (28%) loss

of wheat grain. Results recorded in Table (4) comprised the effect on %mortality, % reduction of F₁ progeny of *C. maculatus* and % loss of cowpea seeds. The results had the same trend with *T. castaneum* and *T. granarium* where the mortality percentage increased with the increasing of concentration and time of exposure either with garlic or chamomile oil. According to data presented in Table (4) garlic oil was generally the best where it achieved the highest mortality at the all periods of exposure and increased reduction of progeny and decreased the weight loss of cowpea seeds from 25% in control to (0.5 to 5.4%) at the all tested concentrations. The previous data in Tables (2-4) greatly show that the two tested oils are likely to be stored product protectants and may be exploited in integrated pest management programs.

Table (2): Mortality percentage of *T. castaneum* as affected by the interaction between plant oils, concentration and time of exposure

Botanical oils	Conc. (ppm)	Mortality % after			Mean	% Reduction	% Loss
		3 days	One week	Two weeks			
Garlic	12.5	13	20	60	24.4	54.3	4.5 e
	25	17.5	25	65	35.8	63.7	2.5 d
	50	25	35	80	46.6	82.4	0.3 b
	100	35	55	90	60.0	88.2	0.2 a
LC ₅₀		100	91.48	31.35			
Chamomile	12.5	17.5	40.0	65.0	35.5	18.0	5.5 f
	25	28.0	42.5	72.5	48.5	32.6	3.0 b
	50	35.0	45.0	87.5	55.0	62.0	2.3 d
	100	45.0	65.0	95.0	68.3	68.2	1.8 c
LC ₅₀		103	41.37	21.76			
Control						0.0	28

Table (3): Mortality percentage of *T. granarium* as affected by the interaction between plant oils, concentration and time of exposure

Botanical oils	Conc. (ppm)	Mortality % after			Mean	% Reduction	% Loss
		3 days	One week	Two weeks			
Garlic	12.5	25.0	60.0	80.0	55.0	46.9	3.5 d
	25	38.0	65.0	87.5	63.5	53.9	2.5 c
	50	65.0	92.5	97.5	85.0	62.5	1.3 b
	100	78.0	95.0	100.0	91.0	79.7	0.5 a
LC ₅₀		33.29	18.39	14.06			
Chamomile	12.5	16.0	55.0	70.0	47.0	29.7	5.1 f
	25	52.5	60.0	80.0	64.0	42.9	4.5 e
	50	60.0	68.0	85.0	71.0	52.3	3.4 d
	100	85.0	87.5	95.0	89.0	66.4	1.3 b
LC ₅₀		31.65	24.24	18.27			
Control						0.0	28

The effectiveness of many plant extracts and essential oils as repellents, antifeedants and insecticides against *T. castaneum* and *O.*

surinamensis have been studied. Those beetles have shown susceptibility to plant-derived chemicals (Jilani *et al.*, 1988; Tripathi *et al.*, 2000; Kim *et al.*, 2003). Owsu (2001) on the other hand, reported that, extracts of *Ocimum viride* leaves at 0.1 mg/ml proved to be the most effective in the control of *T. castaneum* and *S. oryzae* after ten days of treatments.

Table (4): Mortality percentage of *C. maculatus* as affected by the interaction between plant oils, concentration and time of exposure

Botanical oils	Conc. (ppm)	Mortality % after			Mean	% Reduction	% Loss
		3 days	One week	Two weeks			
Garlic	12.5	35.0	62.5	85.0	60.8	48.7	5.4 f
	25	42.5	67.5	90.0	66.8	61.5	3.5 e
	50	70.0	92.5	100.0	87.5	65.7	1.5 b
	100	80.0	97.5	100.0	92.5	75.9	0.5 a
LC ₅₀		25.84	17.36	12.5			
Chamomile	12.5	25.0	60.0	75.0	53.3	22.6	7.8 g
	25	60.0	65.0	82.5	69.2	26.0	5.5 f
	50	62.5	70.0	90.0	74.2	52.8	3.0 d
	100	87.5	90.0	95.0	90.8	64.9	2.5 c
LC ₅₀		21.91	27.56	19.54			
Control						0	25h

Reduction %:

The percent of reduction ranged from 56-86.6% with the all tested concentrations of malathion while garlic oil gave reduction percentage between 46-88.16 and chamomile oil induced from 17.95-68.16% inhibition in progeny number at the all tested rates with the all studied insects (Tables 2-7)

Weight loss %

The percent of weight loss ranged from 0.3-10% with malathion while the same parameter ranged from 0.2-5.4% with garlic oil and from 1.32-7.8% with chamomile oil (Tables 2-7). These results show that the two essential oils (garlic and chamomile) were better than malathion where the later have hazard effect on environment compared to the tested oils.

Table (5) : Effect of Malathion dust admixed with cowpea seeds as protectants against *C.maculatus*

Insecticide	Con% (w/w)	%mortality after 3 days	After one week	After two weeks	%Reduction after one month	%Loss of cowpea
malathion 1%	0.08	60	77.5	92.5	74.9	5d
	0.1	65	82.5	95	77.6	3.5c
	0.5	72.5	90	100	80.0	2.2b
	1	82.5	92.5	100	86.6	0.3a
control		0	0	0	0	24.5e

Table (6) : Effect of Malathion dust admixed with wheat grains as protectants against *T.castaneum*

Insecticide	Con% (w/w)	%mortality after 3 days	After one week	After two weeks	%Reduction after one month	%Loss of cowpea
	0.08	35	56.9	80.3	56	10d
malathion 1%	0.1	45	68.0	84.5	70.8	9c
	0.5	55	80	88	92	7.5b
	1	75	92	96	80	5a
control		0	0	0	0	32e

Table (7) : Effect of Malathion dust admixed with wheat grains as protectants against *T.granarium*

Insecticide	Con% (w/w)	%mortality after 3 days	After one week	After two weeks	%Reduction after one month	%Loss of cowpea
malathion 1%	0.08	55	75	86.6	57.5	6.4d
	0.1	60	80.0	84.5	66.4	5.3c
	0.5	70	85	95	75	2.4b
	1	77.5	92	100	83.5	1.2a
control		0	0	0	0	35e

Effect of malathion dust:

Results included in Tables (5-7) showed the toxicity of malathion dust on the adults of *C. maculatus*, *T. castaneum* and *T. granarium*.

Toxicity:

Results obtained that the toxic action of malathion of the highest concentration (1%) nearly equal that of garlic and chamomile oil (100 ppm) where they achieved 100% mortality after two weeks of exposure.

Repellent action of essential oils:

Data recorded in Table (8) cleared that the repellent activity of garlic chamomile and neem oils increased with the increasing rate of concentration for the three tested insect species.

Table (8) : Repellency of essential oils against three stored product insects *C. maculatus*, *T. granarium* and *T. castaneum*, after 24hr. of exposure.

Plant oils	Insect	Repellency at concentration (%)				Mean
		12.5	25.0	50.0	100	
Garlic	<i>C. maculatus</i>	45	57.5	60.0	67.5	57.50 d
	<i>T. granarium</i>	35	50.0	55.0	60.0	50.00 e
	<i>T. castaneum</i>	25	35.0	47.5	55.0	40.63 g
Chamomile	<i>C. maculatus</i>	50	52.5	60.0	65.0	56.88 d
	<i>T. granarium</i>	25	47.5	52.5	55.0	45.00 f
	<i>T. castaneum</i>	30	50.0	55.0	62.5	49.38 e
Neem	<i>C. maculatus</i>	72.0	75.0	76.0	84.7	76.93 a
	<i>T. granarium</i>	64.0	68.0	70.0	81.0	70.75 b
	<i>T. castaneum</i>	47.7	65.5	70.0	78.3	65.37 c

For *C. maculatus* garlic and chamomile had the same effect while *T. granarium* and *T. castaneum* exhibited significant differences in the response to the two mentioned oils. Neem oil had the highest repellent effect compared to garlic and chamomile oils against the three tested insects where the % mean of neem ranged from 65.37 to 76.93% compared to that of garlic and chamomile which ranged from 40-57.5% with the three tested insect species.

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التأثير الطارد والإبهادي لبعض الزيوت النباتية ضد بعض حشرات الحبوب المخزونة

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تم إجراء تحليل كيميائي لزيوت الثوم والشيح لمعرفة المواد الفعالة فيهما والمحمّل ان تكون المسؤولة عن الفعل السام على الحشرات وقد تم اختبار تأثيرهما السام والطارّد ضد ثلاثة أنواع من حشرات الحبوب المخزونة وهي خنفساء اللوبيا وخنفساء الصعبد وخنفساء الدقيق الصدفية، ومسحوق الملاثيون كأحد المبيدات الموصى بها للمقارنة ضد هذه الحشرات بينما استخدم زيت النيم كمادة طاردة وبناءً على التركيز النصفى ٥٠% من الحشرات البالغة فانه من الواضح أن الثوم له تأثير سام عالي ضد خنفساء اللوبيا وأعطت النتائج نسبة موت ١٠٠% عند تركيزات ١٠٠، ٥٠ جزء في المليون من زيت الثوم ضد خنفساء اللوبيا وخنفساء الصعبد بعد أسبوعين من المعاملة .

وقد اتضح أن التأثير الطارد للزيوت النباتية المختبرة (الثوم، الشيح، النيم) يزيد بزيادة التركيزات للحشرات المختبرة ووجدت فروق معنوية عالية للتأثير الطارد لزيت النيم للثلاثة أنواع من الحشرات المختبرة حشرات المختبرة بالمقارنة بزيوت الشيح والثوم . وقد أوضحت النتائج أن مسحوق الملاثيون كان له تأثير عالي السمية بالمقارنة بكل من زيت الثوم والشيح المستخدمين في هذه الدراسة .

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