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Original Research

Effect of Plant Oils against the Main Insect Pest of Stored Products, *Sitophilus Oryzae*, in Wheat Grains

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Abstract

Laboratory experiments were carried out to evaluate the three tested plant oils, neem (*Azadirachta indica*), moringa (*Moringa oleifera*), and clove (*Syzygium aromaticum*) against Sitophilus oryzae adults, to prevent or minimize the infestation of wheat grains by this insect. To achieve this aim, the current study examined the contact and fumigant toxicity of the oils, their effects on emerged adults, the percentage reduction, and their impact on weight loss. Further studies were conducted to investigate the side effects of these oils on the quality parameters of wheat grains by determining the percentages of carbohydrates, protein, fat, fiber, and ash. The results showed that the tested oils had marked effects on the parameters of toxicity, progeny, and weight loss. Additionally, the increase in concentration and exposure period led to an increase in the effects of these plant oils. Furthermore, the responses of insect adults to the tested oils were varied. The side effects of the tested oils on some quality parameters of wheat grains showed slight differences relative to those of the control. The current study suggests using the tested oils in an integrated pest management program for stored-product insects, especially *S. oryzae* adults.

Keywords: environment, fumigation, grain weight loss, moringa oil, neem oil

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Introduction

Wheat is one of the most important crops worldwide; therefore, many studies have been conducted to maximize the yield of wheat and some economic crops under normal conditions [1-3] or stressful conditions, such as drought [4-6], salinity [7-9], pathogens [10-13], and insects [14]. Insect infestation is a serious threat to many economically important crops [15, 16], especially stored grains. Grains and legumes are strategic crops on which the majority of the world's population depends, particularly in Egypt. The stored grains and seeds are subjected to insect attacks that cause multiple damages to the quality and weight of the grains, as well as causing great harm to seed germination. *Sitophilus oryzae* (L.) is one of the most widespread and destructive insect pests of stored cereals [17]. A previous study reported that approximately 10-40% of stored products were damaged by insect beetles [18]. The most common management tactics applied to control pests in stored grain are the frequent use of synthetic chemical insecticides and fumigants. These chemical materials lead to many problems, such as pesticide residues in grains, the occurrence of resistant insects, and other negative effects on human health and the environment [19]. Therefore, there is an urgent need for a safe alternative to chemical materials. Essential oils have excellent distribution and rapid biodegradation in ecosystems [20]. They are volatile substances categorized as plant secondary metabolites [21], such as neem *Azadirachta indica* (Sapindales: Meliaceae) [22], *Moringa oleifera* (Brassicales: Moringaceae), and clove oil *Syzygium aromaticum* (Myrtales: Myrtaceae) [23]. Additionally, essential oils may be used to control pests because of their insecticidal properties [24-26]. They are considered alternatives to conventional stored grain pesticides because of their low toxicity to warm-blooded animals and suitability for use by small-scale farmers to protect crops from a wide range of insect pests [27]. Therefore, the current study aimed to evaluate the efficiency of three plant oils, neem, moringa, and clove oils, against *S. oryzae* by studying the toxicity and fumigation activities of plant oils on the progeny of *S. oryzae* and % weight loss of wheat grains, as well as the quality parameters of the feeding medium (wheat grain). This study seeks to support the development of integrated pest management strategies that reduce reliance on synthetic chemicals and promote sustainable storage practices.

Materials and Methods

Tested Insects

Adults of *S. oryzae* were reared on the wheat grain *Triticum aestivum* (Sakha 95) in jars (500 mL). Approximately 200 adult *S. oryzae* were added to 300 g of wheat and covered with muslin. Jars were maintained under suitable conditions of 28±2°C and 70% ± 5 RH. After two weeks, the parents were removed, and newly emerged adults were used for the next laboratory experimental work [28].

Oils Tested

The three plant oils used in this study were neem oil (*Azadirachta indica*), moringa oil (*Moringa oleifera*), and clove oil (*Syzygium aromaticum*). Plant oils were purchased from a local market.

Methods of Application

Toxicity Experiments

Contact Toxicity

Mixing with the feeding medium

At concentrations (2.5, 5, 7.5, and 10 mg/mL), one mL of each of the tested oils was mixed separately and shaken manually with wheat grains to determine their effects on the tested insect. Samples of 20 g of treated grains were infested with 20 unsexed new adults of *S. oryzae* (age 1-2 weeks) in jars (250 mL). Three replicates were made for each concentration, along with an untreated control group, and were then stored under laboratory conditions of 28±2°C and 70% ± 5 RH. The mortality percentages were recorded after 1, 2, 3, and 4 days of treatment.

Thin Film Residue

At concentrations (2.5, 5, 7.5, and 10 mg/mL), one mL of each concentration of the tested oils was used as a thin film in the jars, left to dry after 60 s, and infested with 20 unsexed new adults of *S. oryzae* (age 1-2 weeks) in the jars. Three replicates were made for each concentration and an untreated group as the control, and were stored under laboratory conditions at 28±2°C and 70% ± 5 RH. Mortality percentages were recorded after 1, 2, and 3 days of treatment.

Fumigant Toxicity

In this experiment, the tested oils were applied to filter paper at concentrations of 2.5, 5, 7.5, and 10 mg/L air and exposed to *S. oryzae* adults (without grains). The filter paper was saturated with 0.5 mL of the tested oils and transferred to the bottom of sealed glass jars as fumigation chambers. Ten *S. oryzae* adults were separately transferred into jars. Mortality rates were recorded at (12-48 h) post-exposure. Three replicates were performed for each plant oil concentration and the control.

Effect on the Progeny

After treating the grains with the tested oils, all insects, both dead and live, were removed at the end of the experiment. The jars containing the tested medium were maintained at 28±2°C and 70% ± 5 RH until adult emergence. The number of emerged adults was recorded, and the reduction in adult numbers for *S. oryzae* was calculated according to the following equation [29].

% Reduction = (mean No. of emerged adults in the control group − mean No. of emerged adults in the treatment group) / mean No. of emerged adults in the control × 100

Effect on % Weight Loss

In this experiment, the residue of the wheat grain was sieved and cleaned after three months of treatment. Dry grains and seeds were weighed separately for the treatments and the control. The % loss was estimated according to the following equation [30]. % loss = Initial dry weight of seeds − seed dry weight after 3 months / Initial dry weight of seeds × 100

Effect on Grain Quality

The effect of the tested plant oils at the lethal concentration (LC_{50}) values on the fourth day after treatment was evaluated after three months of storage, and the total ash content, crude fat, fiber, and crude protein were determined [31]. The crude protein content of each sample was calculated by multiplying the total nitrogen content by a factor of 5.75. The total carbohydrate content was estimated [32].

Statistical Analysis

Data were analyzed by one-way ANOVA using Duncan's test [33, 34]. Statistical significance was set at $P \leq 0.05$. The lethal concentration for 50% mortality (LC_{50}) was determined using log-probit analysis [34]. Data analysis was performed using SPSS software (version 24.0; IBM Corp., Armonk, NY, USA).

Results and Discussion

Contact Toxicity

Mixing with the Feeding Medium

Data obtained in Table 1 summarizes the results of a laboratory experiment conducted to evaluate the toxicity of neem, moringa, and clove oils against *S. oryzae* adults. Based on these results, neem oil achieved the highest toxicity against *S. oryzae* after one day of exposure with an LC_{50} value of 20.86 mg/mL, followed by moringa and clove oils with LC_{50} values of 26.95 and 224.15 mg/mL, respectively. After two days, the results showed the same trend as after one day, where neem oil exhibited the highest effect, followed by moringa and clove oils against *S. oryzae*. The results clearly showed that *S. oryzae* adults were more susceptible to neem and moringa oil, whereas the same insect (*S. oryzae*) was tolerant to clove oil.

The data obtained after 3 and 4 days showed that clove oil had the least effect against *S. oryzae*. In general, the toxicity results revealed that neem oil showed the highest toxicity against *S. oryzae*, while clove oil had the least toxicity with the same insect throughout the experiment.

Thin Film Residue

A laboratory experiment using thin film residue was conducted to investigate the oils used against *S. oryzae* adults. The results in Table 2 show the toxic effects of the oils. Based on LC_{50} values after one day post-treatment, neem oil was the most toxic agent with (2.35 mg/mL), followed by moringa oil (3.58 mg/mL) and clove oil (13.73 mg/mL). After two days, data demonstrated the same trend as after one day, while after three days, data slightly differed from one and two days, where moringa oil (0.167 mg/mL) was the most effective, followed by neem (0.208 mg/mL) and clove oil (0.667 mg/mL). The

Table 1. Toxicity of the plant oils against *S. oryzae* adults after the indicated periods post-treatment using the mixing with the medium technique.

Oil	Exposure period (days)	LC_{50} mg/mL	Confidence limits		Slop value (s.v)
			lower	upper	
Neem	1	20.86	13.99	54.52	1.67
	2	8.96	7.54	11.71	1.91
	3	5.13	3.39	6.47	1.29
	4	3.39	2.16	4.33	1.30
Moringa	1	26.95	15.61	32.34	1.32
	2	13.88	10.18	27.63	1.45
	3	8.26	6.35	13.86	1.12
	4	2.17	1.81	3.23	1.03
Clove	1	224.15	186.79	268.98	0.99
	2	43.57	20.76	70.26	1.39
	3	19.72	16.43	23.66	0.81
	4	9.76	7.19	11.71	1.04

Table 2. Toxicity of the plant oils against *Sitophilus oryzae* adults after the indicated periods post-treatment using the thin film residue method.

Oil	Exposure period (days)	LC_{50} mg/mL	Confidence limits lower	Confidence limits upper	Slop value (s.v)
Neem	1	2.35	1.88	3.19	1.37
	2	0.926	0.675	1.17	1.26
	3	0.208	0.076	0.341	1.37
Moringa	1	3.58	2.61	6.19	1.14
	2	1.69	1.32	2.27	1.16
	3	0.167	0.019	0.356	0.82
Clove	1	13.73	7.28	59.45	1.27
	2	3.77	2.63	7.49	1.01
	3	0.667	0.227	1.05	0.68

results revealed that toxicity increased with an increase in exposure period. For example, the LC_{50} values of oils dropped from 2.35, 3.58, and 13.73 mg/mL after one day against *S. oryzae* to 0.208, 0.167, and 0.667 mg/mL after three days post-treatment for neem, moringa, and clove oil, respectively. Clove oil was used as a wacking agent during the experimental period (3 days). The oils tested in this study have been extensively used to treat various ailments. The biological activity of *M. oleifera* was attributed to the presence of chavicine and piperine. The pungent odor of oil has been reported to deter weevils from feeding on grains [35]. *Schisandra chinensis* demonstrated significant contact toxicity against *S. oryzae* and *T. castaneum* adults, with LC_{50} values of 24.37 ppm and 68.47 ppm, respectively, recorded three days after treatment [36]. Wheat grains treated with clove oil had a toxic effect on *S. oryzae*, with LC_{50} values of 0.31 and 0.13% at 10 and 14 days, respectively [37]. Furthermore, clove and cinnamon essential oils exhibited insecticidal activity against *Callosobruchus maculatus*, and were similar to the synthetic pyrethroid insecticide deltamethrin [38]. Additionally, cinnamon and clove essential oils have lethal effects on *T. molitor* [39]. The results showed that cinnamon and clove essential oils were toxic to *T. molitor*, and clove oil can be used as a safe alternative to pesticides in integrated stored grain pest management programs.

Essential oils are a mixture of secondary metabolites that contain several volatiles responsible for many biological responses [35]. In this study, the marked mortality of *S. oryzae* indicated that the plant oils used in the experiment had remarkable effects on the mortality of the rice weevil. The oils in the current study showed a gradual increase in mortality with exposure time from 1-4 days (mixing with medium) to 1-3 days (thin film residue). This suggests that plant oils are slow-acting insecticides and led to an increase in the mortality of *S. oryzae*, which increased with increasing exposure [39]. The oils may be absorbed by the grains, thereby reducing their availability for pick-up by the beetles. This could also be due to a slow breakdown of the oil; therefore, additional treatments would be required if a longer storage time was needed [40].

Fumigant Toxicity

The fumigant technique is one of the most important bioassays, especially for materials with volatile

Table 3. Fumigant activity of the plant oils against *Sitophilus oryzae* adults after the indicated intervals post-treatment.

Oil	Exposure period (hrs)	LC_{50} mg/l air	Confidence limits lower	Confidence limits upper	Slop value (s.v)
Neem	12	22.17	17.78	31.47	1.32
	24	27.18	21.01	45.85	1.23
	36	27.18	21.01	45.85	1.23
	48	14.72	11.16	18.52	1.29
Moringa	12	55.79	37.83	66.95	1.52
	24	52.94	38.73	102.98	2.09
	36	38.59	27.24	46.31	1.16
	48	14.99	12.22	17.59	1.68
Clove	12	174.89	145.75	209.87	1.07
	24	77.22	64.35	92.66	1.19
	36	31.34	24.01	37.61	1.34
	48	31.34	24.01	37.61	1.34

Table 4. Comparative competence of the three bioassay methods of toxicity after one and two days post-treatment against Sitophilus oryzae.

Oil	Time (day)	LC_{50} mg/mL	Confidence limits lower	upper	Slop value (s.v)
Mixing with the feeding medium					
Neem	1 2	20.86 8.96	13.99 7.54	54.52 11.71	1.67 1.91
Moringa	1 2	26.95 13.88	15.61 10.18	32.34 7.63	1.32 1.45
Clove	1 2	224.15 43.57	186.69 20.76	268.98 70.26	0.99 1.39
Thin film residue					
Oil	Time (day)	LC_{50} mg/mL	Confidence limits lower	upper	Slop value (s.v)
Neem	1 2	2.35 0.926	1.88 0.675	3.19 1.17	1.37 1.26
Moringa	1 2	3.58 1.69	2.61 1.32	6.19 2.27	1.14 1.16
Clove	1 2	13.73 3.77	7.28 2.63	59.45 7.49	1.26 1.01
Fumigation activity (mg/cm^3 air)					
Neem	1 2	0.027 0.014	0.021 0.011	0.045 0.018	1.32 1.23
Moringa	1 2	0.052 0.014	0.038 0.012	0.103 0.017	1.52 2.09
Clove	1 2	0.077 0.031	0.064 0.024	0.092 0.037	1.07 1.19

components such as plant volatile oils. A laboratory experiment was carried out to evaluate the fumigant action of the three tested plant oils, neem, moringa, and clove oil, against *S. oryzae* adults. Based on the results obtained from the current study through 48 h post-treatment, neem oil was effective against *S. oryzae* with LC_{50} values of 22.17 mg/L air.

In general, neem oil was the most effective against *S. oryzae*, followed by moringa and clove oil after 12 h of treatment (Table 3). Regarding the results obtained 24 h post-treatment, the rank of toxicity of the tested oils was similar to that at 12 h against the tested insect. The results showed that moringa oil and clove oil had nearly the same effect on *S. oryzae* adults, where the LC_{50} of clove oil overlapped between the confidence limits of moringa oil. Results after 36 h showed that the efficacy of the tested oils was nearly equal, with the LC_{50} values of the three oils overlapping with that of the insect. Data after 48 h showed that the response of *S. oryzae* differed in its response to exposure to the oils tested. The results also showed that the fumigant action of the tested oils increased with increasing exposure time. For example, the LC_{50} value ranged between 22.17-14.72 mg/L air for neem oil against *S. oryzae,* starting from 12 h to 48 h. These findings in the present study explain the most important role of exposure periods. Several studies have reported the toxicity and protective potential of EOs extracted from various plants against major stored-product insects [41]. The insecticidal toxicity of the oils was mainly due to the aromatic compounds. It is deduced from the present study that the effectiveness of these oils could be due to their toxicity, which blocked the insect spiracles and, as a result, hindered their movement in the treated containers. Monoterpenes are the major compounds in oils that contribute to the characteristic flavor and aroma of the plant [42, 43]. The monoterpenes and eugenol in clove oil contribute to the insecticidal and repellent activities of the oils. Furthermore, eugenol exerts a neurotoxic effect on insect pests through different mechanisms, including acetylcholinesterase inhibition and octopamine synapses [44, 45].

Comparative Competence of the Three Bioassay Methods of Toxicity

Based on the results presented in Table 4, the LC50 levels of the three methods, mixing with the feeding medium, thin film residue, and fumigation, obviously show that the fumigation method achieved the best effectiveness against the tested insect adults, *S. oryzae*, while the LC_{50} levels of these methods ranged from 0.022 to 0.174 mg/L air, followed by thin film residue with LC50 values of 0.926-13.73 mg/mL and mixing with the medium, with LC_{50} concentrations of 8.96-43.57 mg/mL against *S. oryzae* adults. The current arrangement of the three toxicity methods showed that fumigation was an effective method for controlling the adult stage of the tested insect. The fumigation method has some advantages, such as minimizing the contamination of stored products and the environment, repelling insects far away from grain storage, reducing the doses needed to achieve control, and leaving fewer residues. Meanwhile, mixing with the medium requires high doses to achieve control, which contaminates the product with its residues. In addition, its toxic effects on humans and the environment result from residues. However, thin film residues are less dangerous than mixing with the medium.

Effect of Tested Oils on Adult Progeny and % Weight Loss

The results presented in Table 5 show the effects of the oils used on *S. oryzae* as biological parameters at different concentrations. The results showed that clove oil was superior against *S. oryzae* in terms of biological parameters among the three oils used. Mean numbers of emerged adults, % reduction, and % weight loss were affected by all concentrations of clove, which differed significantly relative to the control. Neem at a concentration of 10 mg/mL and moringa at 5, 7.5, and 10 mg/mL significantly achieved a deterrent effect on the studied parameters; the remaining concentrations had no effect, similar to that of the control.

Based on the data in Table 5, clove oil had the greatest effect with 1.33-4.0 emerged adults, 93.78-97.93% progeny reduction, and 0 % weight loss followed by moringa, which produced 22.0-51.67 adults and caused 19.67-65.8% F1 reduction and reduced the % weight loss (6.4-10.3) compared to control (17.2% loss). Neem oil ranks third, with 9.84-38.86% reduction in F1 and 9.3-12.4% weight loss. The results showed that there was no significant effect of any concentration used in all treatments. This means that the lowest concentration (2.5 mg/mL) is sufficient to achieve the results of the remaining concentrations (5, 7.5, and 10 mg/mL) to minimize cost and pollution.

The mechanism of progeny inhibition by EOs is not yet clear; however, Shukla et al. [46] indicated that the reason for the early embryonic stage susceptibility is the entry of volatile constituents through the funnel present at the posterior pole of eggs, or by changes in the permeability of the chorion and/or vitelline membrane during embryogenesis, which negatively affects vital physiological and biochemical processes. Zayed and Manal [47] reported that plant oils (groundnut, parsley, sunflower, and nutmeg) were more effective against *C. maculatus* with lower egg laying, a lower number of emerged adults, and weight loss of seeds. The efficacy of the oil extended to a significant reduction in progeny production and grain damage, reinforcing its potential as a broad-spectrum botanical insecticide. Clove oil exhibits strong progeny-suppressive effects. This can be linked to its high eugenol content, a phenolic compound with well-documented neurotoxic and fumigant actions against storage insects [48, 49]. Clove oil also showed moderate effectiveness in reducing grain weight loss, likely owing to its repellent activity.

Moringa oil, although less effective than neem and clove oils as a fumigant agent, still exhibited moderate insecticidal activity. It showed a measurable effect on reducing adult survival and grain loss, but its effect on progeny suppression was relatively limited. This may be due to the lower concentrations of bioactive compounds or limited penetration through the insect cuticle. The observed reductions in grain weight loss across all treatments suggest that these oils inhibited both adult feeding and larval development inside the grain, providing dual protection. These findings align with previous studies that support the use of essential oils in pest control of stored products, particularly in developing countries where such resources are locally and economically available [50].

Comparative Efficacy of the Oils on the Different Bioassay Parameters

The results in Table 6 indicate that the tested oils had marked effects on the tested parameters. For *S. oryzae*, neem oil had the greatest effect on toxicity bioassays, followed by moringa and clove oils. However, the latent effect of the oils revealed that clove oil was distinct in reducing progeny and weight loss. Concerning the effect on progeny and weight loss of wheat grain, the results proved that moringa oil was the most effective oil compared to the other oils. The results demonstrated that the tested plant oils exerted varying degrees of toxicity and developmental inhibition against the tested insect species, *S. oryzae.* Neem oil showed the highest adult mortality rate in *S. oryzae*, which can be attributed to the presence of azadirachtin, a compound known for its antifeedant and growth-regulatory effects [51, 52].

Effect on the Wheat Grain Components

After three months of treatment, there was no significant difference in carbohydrate content between the treatment and the control groups. This result indicates that plant oils, including neem, moringa, and clove, did not affect the carbohydrate content of wheat grains (Table 7). For percentage protein content, except for moringa oil, the two plant oils increased the protein content compared to the control. In addition, neem oil showed a significant increase in protein percentage compared with the control, and a higher fat percentage than the control. Meanwhile, the percentage of fiber

Table 5. Effect of plant oils on the progeny of Sitophilus oryzae and weight loss of wheat grain at the indicated concentrations.

Oil	Conc. mg/mL	Mean no. of emerged adult	% reduction F_1 progeny	% loss of wheat grain
Neem	2.5	54±3.52ab	9.84	12.4
	5	50±1.73ab	22.27	10.6
	7.5	43.67±5.92abc	32.11	9.7
	10	39.33±3.84bcd	38.86	9.3
Moringa	2.5	51.67±0.606ab	19.67	10.3
	5	35.67±2.72bcd	44.55	8.7
	7.5	27.33±4.88cd	57.51	8.1
	10	22±1.73de	65.80	6.4
Clove	2.5	4.00±1.00 e	93.78	0
	5	3.33±0.881 e	94.82	0
	7.5	1.67±3.33 e	97.40	0
	10	1.33±3.33 e	97.93	0
	control	64.33±6.06 a		17.2

Table 6. Comparative efficacy of the oils on the different bioassay parameters, toxicity, biology, and % weight loss.

Oil	Bioassay toxicity pattern				
	Mixing with medium	Thin film residue	fumigation	% progeny reduction	% weight loss
			Periods (by day)		
			One		
Neem Moringa Clove	neem	neem	neem	clove	clove
Two					
Neem Moringa Clove	neem	neem	neem	clove	clove
Three					
Neem Moringa Clove	neem	moringa	neem	clove	clove
Four					
Neem Moringa Clove	moringa	-	moringa	clove	clove

Table 7. Side effects of the tested materials on the quality parameters of wheat grain.

Toxicant	% carbohydrate	% protein	% fat	% fiber	% ash
Neem	71.23±0.51a	11.96±0.86a	2.57±0.04a	4.09±0.03a	3.25±0.02a
Moringa	70.26±0.58ab	10.90±0.86 b	2.27±0.04 b	3.69±0.03a	3.03±0.02a
Clove	70.82±0.56 b	11.26±0.80 b	2.39±0.04b	3.94±0.02a	3.16±0.02a
Control	69.70±0.51 b	10.62±0.86 c	2.22±0.40 b	3.62±0.03 a	2.95±0.02 a

increased with the treatments (oils) compared to the control. In terms of ash percentage, moringa oil did not affect this criterion relative to the control, whereas the other treatments resulted in an ash content higher than that of the control.

An increase or decrease in quality parameters indicates some reactions between these components and plant oils. Oils that did not affect the studied parameters may have degraded during storage. Also, essential oils led to changes in wheat flour physicochemical parameters but did not affect the rheological properties of the dough, which correspond to international ISO standards [53].

Conclusions

Neem, moringa, and clove oils have demonstrated strong potential as plant-based insecticides against major storage insects. Their incorporation into programs can reduce post-harvest losses, protect consumer health, and preserve grain quality. Further research is recommended to explore formulation stability, residual toxicity, and compatibility with other control methods such as hermetic storage or irradiation. The application of neem, moringa, and clove oils represents an important botanical insecticide strategy because of their effects on the toxicity and progeny of *S. oryzae*; however, these plant oils did not affect some quality parameters of wheat grains, such as carbohydrates. These results could be used in pest management programs for the control of *S. oryzae* in stored grains as an eco-friendly strategy to achieve agricultural sustainability.

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Conflict of Interest

The authors declare no conflict of interest.

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