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Biochemical effects of certain plant oils on main metabolites and several enzymes of *Sitophilus oryzae* (Coleoptera:Curculionidae)

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Abstract

The rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) is a significant stored product pest which attacks the seeds of several crops, including wheat, rice and maize. The present work was carried out to evaluate the effect of celery, camphor, and garlic oils on the main metabolites and several enzymatic activities in adults of rice weevil. Laboratory tests were conducted by treating wheat grains with plant oils. Treatment adults of rice weevil with the LC₅₀ values determined for the oils were found to be 0.8 ml/kg for celery oil, 0.85 ml/kg for camphor oil, and 1.27 ml/kg for garlic oil. All treatments resulted in a significant decrease in the contents of total proteins, lipids, and carbohydrates in the rice weevils. Additionally, varying levels of significant changes were observed in carbohydrate levels and the activities of enzymes such as protease, phosphatases, and acetylcholinesterase in the bodies of treated insects compared to the control group. These findings highlight the potential of using plant-derived oils as effective biopesticides against rice weevil.

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Keywords: Rice weevil, celery, camphor, garlic, wheat grains

INTRODUCTION:

One of the major problems with agriculture nowadays is to produce more and more in order to provide food for the population, which number is in permanent augmentation. According to the Ministry of Agriculture and Land Reclamation report MALR (2007), in Egypt, wheat is the most important cereal crop because it's the essential ingredient for a loaf of bread, which is supported by the government with billions of pounds in order to provide food for Egyptian citizens, especially low-income people. There is a significant loss in wheat which destroyed by stored insects and other pests which make us think seriously about the reduction of wheat losses.

In Egypt, the annual loss in wheat 12.3 million tons per year, and the percentage of loss due to stored insects 3.6 %, equivalent to half a million tons per year. The rice weevil alone causes 12% reduction in the wheat, occupies the second place in terms of percentage distribution of types of insects causing a reduction in wheat (MALR 2007). Rice weevil *Sitophilus oryzae*, has been reported as one of the severe pests of cereal grains and their products (Baloch 1992). It is one of the most important widespread primary pests of cereals after harvest causing a loss in weight and leading to quality deterioration and fungal growth (Park *et al.* 2003).

The control of this insect by chemical insecticides has serious drawbacks, such as, the pollution of the environment, insect's resistance, high mammalian toxicity and increasing cost of application. This had led to a search for more safe and less expensive alternative insecticides from various sources. Unlike insecticides, which mostly kill the insect, plant ingredients are known to

suppress the feeding and breeding of insects in various ways in addition to causing direct mortality (Singh *et al.* 2012). Many essential oils could be an alternative source of insect control agents (Jemaa *et al.* 2012) because they constitute a rich source of bioactive chemicals, harmless to mammals, are easily obtained and can be integrated with other pest management procedures. This method also eliminates the need and risk associated with hand mixing of insecticide. Thus, oils can play an important role in the strategy of stored products protection.

The present study concentrated on determining the effect of the celery oil (*Apium graveolens*), camphor oil (*Cinnamomum camphora*) and garlic oils (*Allium sativum*) on some biochemical enzymes activity for controlling the *S. oryzae* adults.

MATERIALS AND METHODS:

Tested insect: A laboratory strain of the rice weevil, *Sitophilus oryzae* was obtained from well-maintained culture reared under controlled conditions of $25\pm 2^{\circ}\text{C}$ and $65\pm 5\%$ RH at the research laboratory of Stored Products Department, Plant Protection Research Institute at the Agriculture Research Center, Doki, Giza, Egypt, reared on wheat grains variety, Sakha 93.

Plant oils used: Three oils were tested in this study, Celery (*Apium graveolens*), camphor (*Cinnamomum camphora*) and garlic oils (*Allium sativum*) were available in the local market in Egypt.

Biochemical tests: One-two week old adults of *S. oryzae* were selected after fed on wheat grains treated with LC_{50} of celery, camphor and garlic oils. After 48h of feeding, the insects were removed and a weight of 0.5 g of these weevils

was homogenized represented by one gm body weight, in 1 ml distilled water by using chilled glass Teflon grinder. The homogenate was centrifuged for 10 min at 7000 rpm at zero °C; the enzyme solutions (samples) were filtered, and the supernatant was used for enzyme assay. Each was replicated 3 times. Total proteins, total carbohydrates and total lipids were determined in the prepared weevil's homogenates according to the methods of Bradford (1976), Singh and Sinha (1977) and Knight *et al.* (1972), respectively. Carbohydrates, proteases, acid and alkaline phosphatases and acetylcholine esterase activity were determined according to the method described by Ishaaya and Swiriski (1976), Ishaaya *et al.* (1971), Laufer and Schin (1971) and Simpson *et al.* (1964), respectively.

STATISTICAL ANALYSIS:

The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range tests ($p < 0.05$) (Duncan, 1955). All analysis was made using a software package (SPSS) computer program.

RESULTS AND DISCUSSION:

Lethal concentration LC_{50} of celery, camphor and garlic oils after three days post- treatment of *S. oryzae* adults are presented in Table 1. Based on the LC_{50} , the susceptibility of *S. oryzae* adults varied among tested compounds. Celery oil showed the highest toxicity followed by camphor which has moderate toxicity while the least effect was recorded with garlic oil with LC_{50} value of (0.8, 0.85 and 1.27 ml/kg, respectively).

Determination of the main metabolites in the whole-body homogenate of *S. oryzae* adults after treatment with LC_{50} of celery, camphor and garlic

oils are given in Table 2. Data summarized indicated that celery, camphor and garlic oils of their LC_{50} values caused a significant decrease in the total content of proteins, lipids and carbohydrates of *S. oryzae* compared with untreated weevils. However, the adults treated with celery oil recorded a remarkable decrease in the total proteins, but when treated with garlic they recorded the highest decreased in both carbohydrates and lipids compared with the untreated weevils. Total protein was decreased to 9.22, 10.99 and 12.42 mg/ml in celery, garlic and camphor oils, respectively, compared to 14.64 mg/ml for untreated weevils. Ahmad *et al.* (2000) reported that there was a direct correlation between developmental retardation and protein reduction in *S. oryzae* after treatment with *Acorus calamus* extracts. The decrease in the protein content in the treated adults might be due to inhibition of DNA and RNA synthesis as suggested by Mitlin *et al.* (1977). Or it may be due to decrease in the activity of Glutamic Pyruvic Transaminase known to be related to protein synthesis as obtained for *S. granarius* adults treated with petroleum ether extract of cinnamon (*Cinnamomum zeylanicum*) as reported by (Ahmad *et al.* 2008).

The mean values of decrease in total carbohydrates were 8.96 mg/ml for garlic oil, followed by 10.83 mg/ml for camphor oil and 11.06 mg/ml for celery oil, as compared with 12.74 mg/ml in untreated insects. Moreover, the decrease in total carbohydrates following treatment with the plant extract may be attributed to the feeding deterrence of these compounds (Salem 1994). The mean values of decrease in lipid content were 10.41, 10.69 and 12.61 mg/ml for garlic, celery and camphor oils, respectively.

Arulprakash and Veeravel (2007) reported that there was a sharp reduction in the major biochemical constituents such as protein, sugar and lipids of *S. oryzae* adults due to the treatment with extracts of *Calotropis gigantea* plant. Whole plant extract treated insects showed maximum reduction in protein, sugar and lipid contents compared to other parts of the same plant.

Determination of the carbohydrate's enzymes activity of whole-body homogenate of *S. oryzae* adults after treatment of LC₅₀ of celery, camphor and garlic oils are presented in Table 3. Our results indicated that both celery and garlic oils at LC₅₀ caused a significant increase in the activity of trehalase of *S. oryzae* adults. The mean values of this increase were 252.26 and 238.17 µg glucose/ml/min for garlic and celery respectively, compared to 197.8-µg glucose/ml/min for control. On the contrary, camphor oil caused a significant decrease in the activity of this enzyme. The mean value of this decrease was 188.87-µg glucose/ml/min. Our results confirmed with that obtained by Abou El Ghar *et al.* (1994) who found that acetone extract of *Melia azedarach* caused an increase in trehalase activity in *Agrotis ipsilon* larvae.

The present research revealed that there was a significant increase in the activity of invertase enzyme in a homogenate of the whole body of *S. oryzae* weevils when treated with the LC₅₀ of both garlic and camphor oils. The mean values of this increase were 506.2 and 267.8 µg glucose/ml/min for garlic and camphor oils, respectively, compared with 262.98 µg glucose/ml/min for untreated wheat grains. Meanwhile, a significant decrease was recorded when treated with LC₅₀ celery oil, the value of this decrease was 252.6 µg glucose/ml/min. This result agrees with the

finding of Ayyangar and Rao (1990) larvae of *Spodoptera littoralis* injected with azadirachtin oil.

The results showed that there was an increase in amylase activities of treated adults with all tested plant oils. The mean values of these increases were 779.87, 683.8 and 683.57 µg glucose/ml/min for celery oil, camphor and garlic, respectively, compared to 666.4-µg glucose/ml/min for control. In spite of increases in the amylase activities, there are no significant differences among tested oils.

Results in Table 4 indicated the effect of LC₅₀ of celery, camphor and garlic oils on proteases, acid phosphatase, alkaline phosphatase and acetylcholine esterase activity in the whole-body homogenate of *S. oryzae* adults. The results showed that only camphor oil caused a non-significant increase in the activity of the protease enzyme (347.3 OD×1000). While celery and garlic oils caused a significant decrease in the activity this enzyme the mean values of this decrease were 232.3 and 302.3 OD×1000 for celery and garlic oils, respectively, compared with 309.67 OD×1000 for the control. Similarly, Parangama *et al.* (2001) who found that the neem oil caused a 50% reduction in proteases enzymes of the cockroaches in vivo. Bigham *et al.* (2010) found that the essential oil from *Teucrium polium* caused a reduction in proteases of *Musca domestica* larvae.

Acid and alkaline phosphatases have been shown to be associated with insect development especially in relation to nutrition and egg maturation. Acid phosphatase has received considerable attention in developmental studies because of its association with histolysis (El-Sheikh 2007). Determination of proteases, acid

phosphatase, alkaline phosphatase and acetylcholine esterase activity in whole body homogenate of *S. oryzae* adults after treatment with LC₅₀ of celery, camphor and garlic oils are shown in Table 4. Results showed that there was a highly significant increase in the activity of acid phosphatase enzyme when adults of *S. oryzae* were treated with three tested oils at their LC₅₀ value. The highest increase was recorded in the case of celery followed by garlic then camphor one. The mean values were 19.49, 12.52 and 11.5 µg phenol/ml/min for celery, garlic and camphor oils, respectively, compared with 6.01 µg phenol/ml/min for the control. For, the alkaline phosphatase activity, all tested oils caused a significant decrease in this enzyme at LC₅₀ as compared with control. Camphor oil induced the highest inhibition followed by garlic and celery oil. The mean values were 16.35, 14.4 and 13.1-µg phenol/ml/min for camphor, garlic and celery oils, respectively, compared with 18.4 µg phenol/ml/min for the control. In contrast with the results of the present work, Naqvi *et al.* (1991) reported that neutral fraction of winter neem leaves containing the tetranor-triterpenoids in nimo-cinolide and isonimocinalide (NFD) caused a reduction in acid phosphatase activity of *S. oryzae*. Also, Ahmad *et al.* (2006) reported that petroleum cinnamon extracts caused a significant decrease in the activity of acid phosphatase in *S. oryzae* adults. This difference may be attributed to different oils mode of action.

Regarding the alkaline phosphatase activity, celery, camphor and garlic oils caused a significant decrease in the activity of this enzyme. This finding agrees with the results obtained by Ayyangar and Rao (1990), who reported that injection of the azadirachtin into 6th instars larvae

of *S. littoralis* decreased the activity of alkaline phosphatase. A similar finding was reported by Imtiaz- Ahmed *et al.* (2001) who reported that there was a decrease in alkaline phosphatase activity in *S. oryzae* treated with neem leaf extract. The alteration in the activity of phosphatases caused by the effect of celery, camphor and garlic oils on *S. oryzae* could be the reason why eggs did not hatch and consequently, no progeny was produced. The results indicated that treatment of *S. oryzae* adults with LC₅₀ of the three tested plant oils induced a significant increase in acid phosphatase activity as compared with control.

Acetylcholine esterase has a vital role in the maintenance of the nerve activity by removing ACh released in the passage of an impulse in the synapses and possibly along the axon (O'Brien 1961). The levels of acetylcholine esterase, activity in the whole-body homogenate of the *S. oryzae* adults, after treatment with the LC₅₀ of the three tested plant oils are demonstrated in Table 4. The higher activity in this enzyme was induced by garlic oil followed by celery and then camphor oil that gave the lower activity of this enzyme. The mean values were 687.84, 634.04 and 518.56 µg AchBr/ml/min for garlic, celery and camphor oils, respectively, compared to the control 425.73 µg AchBr/ml/min. Ahmad and Al-Moajel (2005) reported that there was a significant increase in the activity of the cholinesterase enzyme in *S. oryzae* treated with *Sesban aegyptica* extracts at LC₅₀ level. Also, Ahmad and Shereef (2000) found that the petroleum ether extract showed a different pattern, where an activation appeared first after 12 h of exposure of *Callosobruchus maculatus* to the lupin seeds. A similar finding

was reported, after 24 h exposure of *S. oryzae* to *Ricinus communis* seed extracts (Ahmad, 2000).

Meanwhile, Tulyabaev *et al.* (1995) reported that a number of plant-derived alkaloids and their derivatives were acted as inhibitors to the activity of the cholinesterase in *S. oryzae*. Also, Lopez and Pascual-Villalobos (2010) found that some oils that contain camphor caused inhibition to the acetylcholine esterase (AChE) activity of the *S.*

oryzae. Rajendran and Sriranjini (2008) who mentioned that essential plants oils and their components (cyanohydrins, monoterpenoids, Sulphur compounds, thiocyanates and others) caused inhibition of acetylcholine esterase enzyme activity in *Tribolium castaneum*, *Rhyzopertha dominica*, *S. oryzae* and *Sitophilus zeamais*.

Table 1: Lethal concentration LC₅₀ of celery, camphor and garlic oils after three days post- treatment of *S. oryzae* adults

Plant oils	LC ₅₀ ml /kg	95% Fiducial limits of LC ₅₀	
		Lower	Upper
Celery	0.8	0.5	1.03
Camphor	0.85	0.6	1.0
Garlic	1.27	0.9	1.7

Table 2: Determination of the main metabolites in the whole -body homogenate of *S. oryzae* adults after treatment with LC₅₀ of celery, camphor and garlic oils

Plant oils	Total proteins content (mg/ml)	Total Carbohydrates content (mg/ml)	Total Lipids content (mg/ml)
	Mean ±SE		
Celery	9.22 ± 0.34 ^a	11.06 ± 0.3 ^b	10.69 ± 0.59 ^c
Camphor	12.42 ± 0.01 ^b	10.83 ± 0.34 ^b	12.61 ± 0.3 ^b
Garlic	10.99 ± 0.35 ^c	8.96 ± 0.3 ^c	10.41 ± 0.03 ^c
Control	14.64 ± 0.35 ^d	12.74 ± 0.33 ^d	15.56 ± 0.37 ^d

Means with the same letter are not significantly different (Duncan's multiple tests at p<0.05).

SE: Standard error.

Table 3: Determination of the carbohydrate 's enzymes activity of whole -body homogenate of *S. oryzae* adults after treatment of LC₅₀ of celery, camphor and garlic oils

Plant oils	Trehalase (µg glucose/ml/min.)	Invertase (µg glucose/ml/min.)	Amylase (µg glucose/ml/min.)
	Mean ± SE	Mean ± SE	Mean ± SE
Celery	238.17± 0.04 ^b	252.6±0.02 ^b	779.87±100 ^a
Camphor	188.87 ±0.1 ^c	267.8±0.01 ^c	683.8 ± 0.13 ^a
Garlic	252.26 ±0.1 ^d	506.2±0.01 ^d	683.57±0.08 ^a
Control	197.8±0.01 ^a	262.98± 0.01 ^a	666.4± 0.05 ^a

Means with the same letter are not significantly different (Duncan's multiple tests at p<0.05).

SE: Standard error.

Table 4: Determination of proteases, acid phosphatase, alkaline phosphatase and acetylcholine esterase activity in whole body homogenate of *S. oryzae* adults after treatment with LC₅₀ of celery, camphor and garlic oils

Plant oils	Proteases OD×1000	Acid phosphatase (µg phenol/ml/min)	Alkaline phosphatase (µg phenol/ml/min)	Acetylcholine esterase (µg AchBr/ml/min.)
	Mean ±SE			
Celery	232.3 ± 1.7 ^b	19.49±0.01 ^b	13.1±0.04 ^b	634.04±0.03 ^b
Camphor	347.3 ± 1.8 ^a	11.5 ± 0.01 ^c	16.35±0.01 ^c	518.56±0.03 ^c
Garlic	302.3 ± 35.2 ^a	12.52±0.01 ^d	14.4±0.008 ^d	687.84±0.03 ^d
Control	309.67±2.3 ^a	6.01±0.01 ^a	18.4±0.01 ^a	425.73±0.02 ^a

Means with the same letter are not significantly different (Duncan's multiple tests at $p < 0.05$).

SE: Standard error.

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