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Research Paper

Laboratory evaluation of garlic oil against the saw toothed beetle Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae), and its effect on serotonin and acetylcholinesterase levels

Science

KEYWORDS : Garlic oil extract, Oryzaephilus surinamensis, serotonin, acetyl cholineesterase.

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ABSTRACT

A laboratory experiment was carried out to evaluate the efficacy of three concentrations of garlic oil (Allium sativum) as a control measure against the saw toothed grain beetle Oryzaephilus surinamensis infesting milled wheat grain. Mortality increased with an increase in both garlic oil concentration and exposure period. Offering beetles milled wheat grains treated with 1 % or 0.5% garlic oil caused 100% mortality to beetles after 8 and 10 days, respectively. The lowest tested concentration of 0.25% of garlic oil led to 71.5% mortality at the termination of the investigation (i.e. 10 days). Administration of garlic oil at 1 and 0.5% concentration lead to significant decrease in serotonin and acetyl-cholinesterase levels as compared to untreated beetles. This effect was less apparent when 0.25% concentration was applied, that reflect the activity of garlic oil extract against stored product insect Oryzaephilus surinamensis which lead to insect dead. Results of the present work indicate that garlic oil could be used as a control measure against Oryzaephilus surinamensis beetles.

Introduction

The saw toothed grain beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) is a serious worldwide insect pest infesting stored products, e.g. cereal grains, flour, nuts and dried fruit. Adults and larvae of *O. surinamensis* are secondary pests unable to damage whole grains or fruits (**Hussain, 2007**). The saw toothed grain beetle is a very small insect and has the ability to hide in many places in storage facilities making it difficult to be controlled by insecticides and this insect was able to build up resistance to several insecticides **(Mowery *et al*., 2002).**The intensive use of insecticides for the control of stored product pests have resulted in serious problems including insecticide resistance, environment contamination, mammalian toxicity as well as unacceptable pesticide residues in food **(White and Leesch, 1995; Wallbank, 1996; Jovanović *et al*., 2007).**

Many natural products are known to have a range of useful biological properties against insect pests **(Isman, 2006).** Over the past two decades, botanical insecticides threw attention as an approach for the control of insect pests. The effectiveness of many botanical oils against stored grain insect pests have been well demonstrated (**Shaaya *et al*., 1991; Kim *et al*., 2003; Lee *et al*., 2003; Aslan *et al*., 2005).** Oils are safe to mammals, easily obtained, can be integrated with other pest management measures and eliminate the risk associated with application of insecticides. Thus, oils can play an important role in the protection strategy of stored products.

Garlic, *Allium sativum,* exhibits a natural defensive mechanism that deterred birds and insects from attacking the plant. **Grainge *et al.* (1985)** demonstrated that garlic showed insect controlling properties; with repellant, antifeedant, bactericidal, nematicidal and/or fumigant mode of action**. Kain (1999)** reported the ability of garlic to protect crops against a variety of insects such as aphids, mites and thrips. Garlic oil contains two major constituent, allyl methyl disulfide and diallyl trisulfide and when crushed, garlic yields allicin, a powerful anti-fungal and anti-biotic compound **(Block, 2010).**

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan, serotonin is primarily found in the gastrointestinal tract (GI tract), platelets, and the central nervous system (CNS) of animals. It is popularly thought to be a contributor to feelings of well-being **(Young, 2007).** Acetylcholinesterase, known as AChE or acetylhydrolase, is a hydrolase that hydrolyzes the neurotransmitter acetylcholine. AChE is found at mainly neuromuscular junctions and cholinergic brain synapses, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. It is the primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides **(Katzung , 2001).**

The present laboratory study was undertaken to evaluate the efficacy of garlic oil as a control measure against *O. surinamensis* infesting stored products. Furthermore, acetylcholine esterase and serotonin in *O. surinamensis* adults exposed to milled wheat grains treated with different concentrations of garlic oil was estimated.

2. Materials and Methods

2.1. Insect culture:

The saw toothed grain beetle, *Oryzaephilus surinamensis* was obtained from a infested wheat grains stock culture maintained at the Laboratory of Entomology, Department of biology, Faculty of Science, Taif University, Taif K.S.A. The insect cultures were reared in glass jars (capacity of 2L). The jars were covered with muslin cloth to prevent escaping of insects and to ensure ventilation. The jar was maintained under laboratory conditions of 25±1°C and 70±5% relative humidity. To obtain beetles of similar ages, on a daily bases the insects rearing jars were investigated and any emerged adult were collected and released in other jars to maintain a continuous insect culture of a known age. Adults of 5-7 days old age were used for the experiments.

2.2. Preparation of garlic oil extract.

Garlic *Allium sativum* oil was obtained from EL-Captain company (CAPPHARM), Alobour city (Cairo), Egypt. Three descending concentrations from garlic oil were diluted with petroleum ether (1, 0.5 and 0.25 %). **(Sharaby *et al*. 2012)**

2.3. Bioassay.

Milled wheat grains of 30 gm were sprayed with one of the three prepared garlic oil concentrations. The treated milled wheat grains were left to dry under laboratory conditions. Subsequently, the treated milled grains were placed in clean plastic gars into which 5 to 7 days old *O. surinamensis* beetles were introduced, 20 beetles per jar for each concentration. Control contained

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grains treated with the solvent only. Treatments were replicated five times. The insects were allowed to feed on treated and untreated milled wheat. The number of dead adults was recorded every 48 hours to calculate the mortality percentage.

2.4. Biochemical analysis.

2.4. 1. Preparation of insect tissue homogenates:

O. surinamensis beetles fed on wheat grains treated with garlic oil for 10 days were collected, and their whole body homogenized by a Heidolph Silent Crusher M at 4 °C for 10 seconds in homogenization buffer 0.1M sodium phosphate. Subsequently the homogenate was centrifugation by a Minispin Plus Eppendorf at 10,000 g for 15 min at 4 °C. The obtained extract was collected for biochemical analysis of serotonin and the enzyme acetyl-cholinesterase activity.

The activities were determined by measuring the absorbance of the samples in a dual beam spectrophotometer (Shimadzu-1700, UV/vis, Kyoto, Japan). All chemicals used were analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.4.2..Estimation of serotonin content:-

Estimation of serotonin was carried out according to the method described by **(Ciarlone, 1978).** This method is based on estimating serotonin derivative formed by reaction Orthophthalaldehyde

2.4.3. .Estimation of acetylcholinesterase:

Acetyl-cholinesterase was determined by biodiagnostic kit (Biodiagnositc Company, Dokki, Giza, Egypt), according to the method of **Ellman *et al.*, (1961) and Magnotti *et al.*, (1988).**

2.5. Histological study of midgut in treated *Oryzaephilus surinamensis*

Histological examination was carried out of the midgut dissected from *O. surinamensis* beetles fed for 10 days on wheat grains treated with garlic oil. Following the dissection of the beetles the midgut was removed, its tissues were gently rinsed with a physiological saline solution (0.9%NaCl) prior to their fixation in 5% formalin for 24 h. subsequently the midgut was passed through a series of ethanol alcohol then cleared in methyl benzoate and embedded in paraffin. Sections were cut by a microtome at 6µm thickness, sections were stained with hematoxylin and counter stained with eosin **(Gabe, 1968).** Sections were mounted with DPX (digital picture exchange) and observed under a microscope.

2.6.Statistical analysis.

Statistical analysis of data was based on SAS's program. The data were subject to analysis of variance (ANOVA). Means were compared by Duncan's multiple range test **(Duncan, 1955)** at $P < 0.05$. Percentages of the mortalities were corrected according to Abbott's formula **(Abbot, 1925)** as follows: % Corrected mortality = (T-C) / (100-C)

Where: T : % mortality in treatment , C: % mortality in check (control).

3- Results and discussion

3.1. Percent Mortality of *Oryzaephilus surinamensis* adult offered milled wheat treated with garlic oil extract:

As shown in Table 1 and Figure 1,The potential effect of garlic oil as a control measure against O.surinamensis beetles was dependent. treatment. Mortality of beetles was increased with the increase of garlic oil concentration as well as exposure time (Hamed et al. 2012). Statistical analysis indicated significant differences between the three considered concentrations and exposed periods. Offering beetles milled wheat grains treated with 1% garlic oil caused 100% mortality to beetles after 8 days. Similarly, 100% mortality was also recorded in beetles offered milled grains treated with a concentration of 0.5% garlic oil but which occurred at a later time of 10 days. The lowest tested concentration of 0.25 % of garlic oil led to 71.5% mortality at the termination of the investigation (i.e. 10 days). Mortality in control insects did not exceed 5% at the end of the experiment.

Table 1. Mortality of *Oryzaephilus surinamensis* beetles offered milled wheat grains treated with three concentrations of garlic oil.

Time following exposure (days)		Concentrations of garlic oil (%)				L.S.D
		1%	0.5 %	0.25%	Control	
2	Mean No of dead beetles ± se	7.3 ± 0.76^{a}	4.3 ± 0.57^{b}	2 ± 1^{c}	0^{d}	0.272
	% Corrected mortality	38	21	10	0	
4	Mean No of dead beetles ± se	11.3 ± 0.5^{a}	7 ± 0.28^{b}	5.03 ± 0.57^{c}	0^{d}	0.161
	% Corrected mortality	56	35	26.6	0	
6	Mean No of dead beetles ± se	16 ± 0.28^{a}	11 ± 1^{b}	7 ± 0.76^{c}	0.6 ± 0.31^{d}	0.226
	% Corrected mortality	79.3	53.5	32.7	3.3	
8	Mean No of dead beetles ± se	20 ± 0^{a}	18 ± 0.57^{b}	11 ± 0.28^{c}	0.6 ± 0.31^{d}	0.199
	% Corrected mortality	100	91.3	53.5	3.3	
10	Mean N° of dead beetles ± se	20 ± 0^{a}	20 ± 0^{a}	14.6 ± 0.56^{b}	1.6 ± 0.31^{c}	0.151
	% Corrected mortality	100	100	71.5	5	

Means within the same rows in each category refers to different letters are significant at (P ≤ 0.05) using Duncan's multiple range test **(Duncan 1955).**

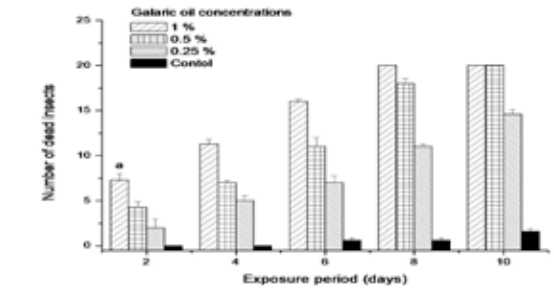

Fig 1: Mortality of *Oryzaephilus surinamensis* **beetles offered milled wheat grains treated with three concentrations of garlic oil.**

These results were agreed with **(Popoola 2013)** who showed that garlic powder caused highly mortality to different developmental stages of *Oryzaephilus surinamensis*. It is yet to be determined the effect of garlic oil on *O. surinamensis* beetles,**(Richards 1978)** reported that oils of plant origin had a highly lipophilic effect and hence have the ability to penetrate the cuticle of insects and therefore may act as contact poison**. (Hamed *et al*. 2012)** indicate that a gradual increase in the mortality rate of *Sitophilus oryzae* adults with increasing garlic oil concentrations and the exposure period. **(Adedire and Ajayi 1996)** showed that garlic oil was potent to weevil of *Sitophilus zeamais* due to its strong choky odor which might have exerted a toxic effect by disturbing respiratory activity of exposed beetles, thereby resulting in asphyxiation and subsequent death. (**KWON & SANG 2005)** studded the effect of garlic essential oils against the Japanese termite, garlic gave 100% mortality within 2 days of treatment, three major compounds from garlic oil were identified as tri and disulfide. **(George *et al*. 2010)** found that the garlic oil was highly toxic against the red poultry mite, *Dermanyssus gallinae* . **(Asawalam *et al.*, 2007)** found that *Capsicum frutescens* had high percentage mortality on *Sitophilus zeamais* and significantly reduced in adult emergence. It was demonstrated that some essential oils are able to kill the hatched larvae of *Sitophilus zeamais and Tribolium castaneum* **(Huang *et al*., 2000).** By this method the plant material apart from its odor, may have also acted as a contact poison.

Table 2: Serotonin and acetylcholinesterase levels in *Oryzaephilus surinamensis* beetles homogenate 10 days offered milled wheat grains treated with garlic oil at three concentrations.

Enzyme	Garlic oil concentrations (%) ± se				L.S.D
	Control	1%	0.5%	0.25 %	
Serotonin (µg/ ml)	0.66±0.011^{a}	0.18±0.030^{d}	0.28±0.075^{c}	0.47±0.098^{b}	0.015
Acetylcholinesterase (mmol/min/ml)	612.60±25.04^{a}	286.00±13.52^{d}	330.80±15.14^{c}	481.80±13.73^{b}	2.11

Means within the same rows in each category carrying different litters are significant at (P ≤ 0.05) using Duncan's multiple range tests. **(Duncan 1955).**

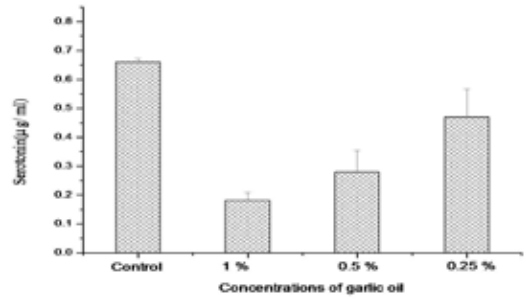

Fig 2: : Serotonin level in *Oryzaephilus surinamensis* **beetles homogenate 10 days offered milled wheat grains treated with garlic oil at three concentrations.**

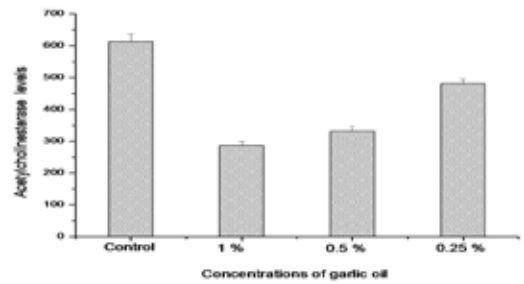

Fig 3: : Acetylcholinesterase levels in *Oryzaephilus surinamensis* **beetles homogenate 10 days offered milled wheat grains treated with garlic oil at three concentrations.**

3.2. Serotonin and Acetylcholinesterase level in *O. surinamensis* beetles homogenate, offered milled wheat grains treated with garlic oil at three concentrations:

3.2.1. Serotonin level in *O. surinamensis* beetles homogenate:
Statistical analysis showed highly significant differences between the levels of serotonin in the insect, while it decrease in *O. surinamensis* beetles offered milled wheat grains treated with 0.25, 0.5 or 1 % garlic oil being 0.47 , 0.28 and 0.18 respectively, as compare to 0.66 in untreated beetles. The "F value" recorded was instead of were 401.9. (Table 2 and Figure 2).

The biogenic amine serotonin (5-hydroxytryptamine, 5-HT) plays a key role in regulating and modulating various physiological and behavioral processes in insects. The specific functions of serotonin are mediated by its binding to and subsequent activation of membrane receptors. The vast majority of these receptors belong to the superfamily of G-protein-coupled receptors. **(Blenau and Thamm ,2011)**, and accordingly to the results are in accordance with the inhibition of serotonin toxicity level in insects was recorded in the concentration 1% group by garlic oil revealed the great toxicity induced by garlic oil on insects, they also reported that their functional studies in these two species have shown that serotonergic signaling participates in various behaviors including aggression, circadian rhythms, responses to visual stimuli, and associative learning.

3.2.2. Acetylcholinesterase level in *O. surinamensis* beetles homogenate:
As shown in Table 2 and Figure 3, Exposure of *O. surinamensis* beetles to milled wheat grains treated with garlic oil at a concentration 1 or 0.5 % decreased acetylcholinesterase level to 286 and 330.80 respectively, as compared to its level 612.60 in the control. Acetylcholinesterase level was slightly reduced to 481.80 in

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beetles offered milled wheat treated with 0.25 % garlic oil. These results supported with statistical analysis which appear highly significant differences between three concentrations, where F value= 101.6.

This oil decreased and inhibition of acetylcholinesterase level which reflect the increasing level of acetylcholine in post synapase, that causing accumulation of acetylcholine and the synaptic nerves become continuously on.

Acetylcholinesterase (AChE; EC 3.1.1.7) is a key enzyme of the cholinergic system because it regulates the level of acetylcholine and terminates nerve impulses by catalyzing the hydrolysis of acetylcholine. Its inhibition causes death and this is greatly agreed with the results as the great inhibition was reported in Concentration 1% treated group by garlic oil, so irreversible inhibitors have been developed as insecticides such as organophosphates and carbamates **(Aldridge, 1950).** The first case of AChE with a reduced sensitivity to pesticides was explained by **(Smissaert 1964).**

3.3. Histological studies:

The midgut is an important organ for insects not only because it occupies a large space in their hemocoel, or because it is a major part of the digestive system; it also plays critical roles in other physiological regulation such as metabolism, immune response, homeostasis of electrolytes, osmotic pressure, circulation and more. Therefore disturbance of these functions could provide a target and strategy for future pest management practice. The midgut is a complex organ that undergoes a gross transformation at metamorphosis to support different feeding habits at different developmental stages. It consists of epithelium and basal lamina with endodermal and mesodermal origins, respectively and the latter is innervated, i.e., an ectodermal element. **(Takeda, 2014).**

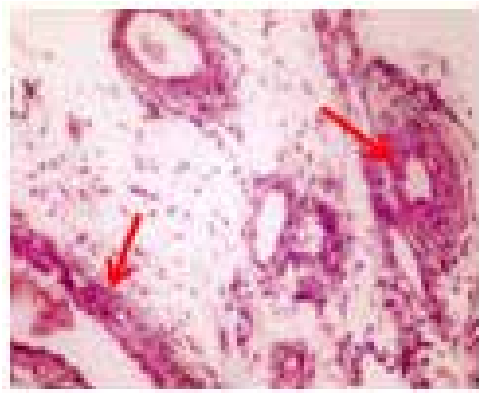
As seen in photomicrograph **(Fig.4)** in Group 1: Control group of *O. surinamensis,* photomicrograph of midgut sections of control group showing normal structure and appearance of epithelium and basal lamina cells (Red arrows). Effect of treatment with 1% of garlic oil was presented in Group (2): showing highly severe congestion in the midgut section and atrophy of midgut layers (Green arrows). Photomicrograph Group (3) showing the effect of treatment with 0.5 % of garlic on midgut sections, it was a mild congestion in the mid gut layers (Orange arrows).

The lowest congestion in the epithelium layers with nearly appeared normal structure was found

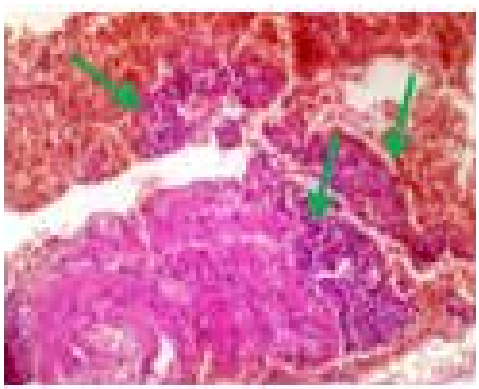
after treated with 0.25 % of garlic oil as shown in Photomicrograph Group (4) (Blue arrows). These results were agreed with **(Sharaby *et al.* 2012)** who reported that histological changes that observed in the digestive system of grasshopper *Heteracris littoralis* that treated with garlic oil showed disturbances in the foregut, midgut, and hindgut, it also caused disturbances in the metabolic process of digested food and decrease the distribution of the nutritive metabolites materials.

The atrophy and sever congestion that has been occurred in *O. surinamensis* adult midgut treated with (1 % of garlic oil) indicated that this concentration of garlic oil was the effective and best concentration in inducing toxicity in the most important organ of the insect (midgut) and thus causing severe damage to the insect followed by the concentration 0.5%, but the concentration 0.25% was the lowest in the effect on mid gut of the insect.

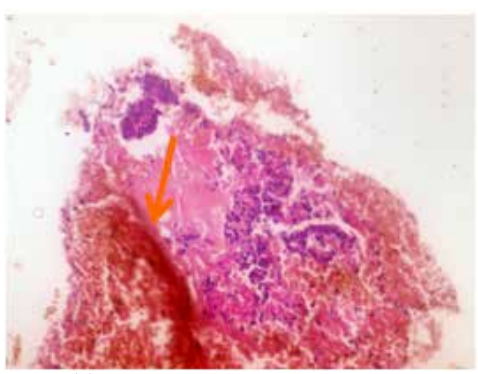
Thus, it could be concluded that essential oil of Garlic plant could be used as an effective natural products to be included in the integrated pest management program of stored insect pests.



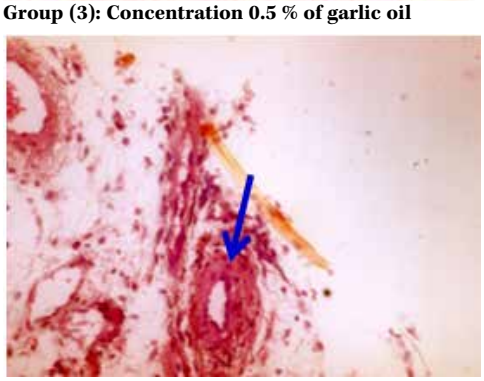
Group (1): Control group



Group (2): Concentration 1 % of garlic oil



Group (3): Concentration 0.5 % of garlic oil



Group (4): Concentration 0.25 % of garlic oil

Fig. (4): Photomicrograph sections of mid gut of O. surinamensis adult showed the effect of treatment with three concentrations of the garlic oil and untreated one.

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