





Insecticidal activity of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) oils on the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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Management of *Spodoptera littoralis* infestation has been achieved by using chemical insecticides; however, the environment-friendly methods without unwanted side effects of these chemicals are becoming very important in modern pest management strategies. Natural products including plant extracts and oils are some of the alternative approaches in pest control. In the present work, insecticidal activities of oils from garlic cloves (*Allium sativum*), and ginger rhizomes (*Zingiber officinale*) were evaluated on *S. littoralis* by means of sublethal concentrations. The essential oils were extracted and their chemical composition was identified using a GC Ultra-ISQ mass spectrometer. The results showed that there were highly significant differences between all treatments and the control in some biological aspects. The larval and pupal duration was significantly prolonged, also pupal weight increased for all treatments when compared to the control. The percentage of hatchability of deposited eggs was significantly decreased, especially when using ginger both oil extracts at LC₅₀ concentration. On the other hand, the pupation percentage, rate of adult emergence, sex ratio and female fecundity were not significantly affected by all applications. The catalase enzyme (CAT) activity showed significant difference only at LC₅₀ of ginger oil.

Key words: *Spodoptera littoralis*, environment-friendly insecticides, sublethal concentrations, *Allium sativum*, *Zingiber officinale*, CAT enzyme.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the most notorious and destructive polyphagous insect pests in Egypt. The extensive application of chemical insecticides to control this pest led to the development of resistance to conventional insecticides (Ishaaya *et al.* 1995). In addition, the environmental problems caused by overuse of these pesticides have been a matter of concern for both scientists and the public in recent years. Therefore, natural insecticides are considered a new promising alternative for pest control as they reduce negative impacts on human health and the environment (Isman 2000; Rehman *et al.* 2009).

Secondary compounds from plants such as alkaloids, terpenoids, phenolics, and flavonoids may be effective on insect pests in several ways through disruption of major metabolic pathways and cause rapid death, act as attractants, deterrents, phago-stimulants, antifeedants or

modify oviposition (Smet *et al.* 1986; Houghton 1996). Crude extracts of plants and their isolated components have shown ovicidal, repellent, antifeedant, sterilisation and toxic effects on insects (Nawrot & Harmatha 1994; Isman 2006). The active components such as monoterpenoids, cyanohydrins and cyanates, sulphur compounds (dimethyl disulphide, diethyl trisulphide, di-n-propyl disulphide, allyl disulphide, diallyl trisulphide, and allyl thiosulphates), alkaloids (Z-asarone) and others (methyl salicylate, benzene derivatives, bornyl acetate, and terpinolene) caused insect toxicity in the vapour phase (Rajendran & Sriranjini 2008). Essential oils rich in monoterpenes can cause death of insects by inhibiting acetylcholinesterase activity on the nervous system (Houghton 1996).

Garlic (*Allium sativum*) contains various chemical compounds, including flavonoid, allicin (allyl-2-propene thiosulfinate), which is responsible for garlic's pungent smell and only exists in



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crushed garlic cloves, have been reported as a phytoalexin (Cavallito *et al.* 1945; Farnsworth & Bunyapraphatsara 1992; WHO 1999; Ross *et al.* 2000; Yukihiko *et al.* 2002). Garlic oil contains some sulphur-containing compounds (alliin, ajoene, allicin, diallyl trisulphide, dithin, sallylcysteine), enzymes, minerals (Mg, Zn, Se, Ge), vitamins (C, A, B complex), amino acids, proteins, saponins and flavonoids (Block 1992; Johnson *et al.* 2013). Garlic extracts have shown bactericidal and fungicidal properties. Therefore, garlic essential oil was reported to have insecticidal effects on pests of stored products (Ho *et al.* 1996).

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a well-known spice used either fresh or dried in the daily diet in many countries (Panji *et al.* 1991; Demin & Yingying 2010). The rhizomes have been also used for medicinal purposes in India and China (Oliver 1959; Hasan *et al.* 2012). *Zingiber officinale* showed high insecticidal efficacy on *Diphtheria* spp. and proved to be highly effective against mosquito larvae (Okonkwo & Ohaeri 2013).

Most of the identified components of ginger oil were sesquiterpene hydrocarbons such as zingiberene, β -bisabolene, α -farnesene, β -sesquiphellandrene; monoterpene hydrocarbons α -curcumene and phenolic compounds as gingerol and shogaol extracted by methanol and *n*-hexane, respectively (Hasan *et al.* 2012).

The toxicity and the activity of sublethal concentrations of some bio-insecticides like spinosad, and emamectin benzoate in addition to a new group of insecticides including the diamide group, and indoxacarb have been extensively studied on different species of lepidopteran insects (Yin *et al.* 2008; López *et al.* 2010; Wang *et al.* 2011; Guo *et al.* 2013; El-Sheikh 2015; Moustafa *et al.* 2016). On the other hand, there is a lack of information about the effect of the plant oils extracts on pests and the effect of their sublethal concentrations on the oxidative stress enzymes in insects. Generally, cellular metabolism in living organisms is a continuous source of reactive oxygen species (ROS), which destroy the cell components at high concentration (Ilhan *et al.* 2005). ROS could be removed by antioxidant defence enzymes such as catalase (CAT), which has the ability to consume hydrogen peroxide (H₂O₂) at high concentration and quickly converts it to water and oxygen (Kono & Fridovich 1982; Felton 1995).

The aim of this study was to evaluate the effect of

garlic and ginger essential oils on the life history parameters and on the activity of CAT enzyme in the cotton leafworm *S. littoralis*.

MATERIAL AND METHODS

Plant samples

Samples of garlic cloves and ginger rhizomes were bought from a herbarium store in Alexandria city, Egypt.

Oil extraction

Garlic cloves and ginger rhizomes were dried for two days at 45 °C in an oven, then ground to powder using a small laboratory mill. Samples of 200 g powder of dry garlic and ginger were soaked in 200 ml *n*-hexane for two weeks. The macerated samples were filtered using filter paper No. 1 and the oils were obtained after the solvent was evaporated under reduced pressure using a rotary evaporator. The extracted oils were stored at 4 °C for further use (Salem *et al.* 2013).

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of the oil extracts were identified using a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, U.S.A.); carried out at the Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 μ m film thickness). Helium was used as the carrier gas (flow rate of 1 ml/min), and the oven temperature programme was: 45 to 165 °C (4 °C/min) and 165 to 280 °C (15 °C/min) with post run (off) at 280 °C. Samples (1 μ l) were injected at 250 °C, with split/splitless injector (50:1 split ratio) in the splitless mode flow with 10 ml/min. The solvent delay was 2 min and diluted samples of 1 μ l were injected automatically using Auto-sampler AS3000 coupled with GC in the split mode. Electron ionisation (EI) mass spectra were collected at 70 eV ionisation voltages over the range of *m/z* 40 to 550 in full scan mode. The ion source and transfer line temperatures were set at 200 and 250 °C, respectively. The components were identified by comparison of their retention times and mass spectra with those of Wiley 09, mainlib, replib and NIST 11 mass spectral database (Adams 2001).

Insect culture

The laboratory strain of *S. littoralis* used in this study has been reared in the laboratory and had not previously been exposed to any insecticides as described by El-Defrawi *et al.* (1964). Larvae were reared on fresh castor bean leaves (*Ricinus communis*) at 25 ± 1 °C and 75 ± 5 % RH. The pupae and adults were transferred to suitable cages for mating and egg deposition. Emerged moths were fed on a 10 % sugar solution.

Bioassay and determination of lethal and sublethal concentrations

Insecticidal activities of the garlic and ginger oils were tested on neonate larvae of *S. littoralis*. Five different concentrations, *i.e.* 500, 1000, 2000, 4000, and 6000 mg/l (ppm) of both compounds were used in the experiment. Castor bean leaves were dipped in each concentration for 20 s after which leaves were left to air-dry. The experiment had four replicates. Each replicate consisted of 25 larvae. The larvae were kept in 1-l glass jars and were allowed to feed on a pair of treated leaves for 48 h, after which they were placed onto untreated leaves until death or pupation. Larvae in the control treatment were fed on untreated leaves. Mortality was recorded daily for seven days post-treatment to calculate the lethal and sublethal concentrations of both oils on *S. littoralis* larvae. The bioassay was repeated twice.

Effect of garlic and ginger oils on development of S. littoralis

Sublethal concentrations (LC₁₅ and LC₅₀) of both garlic, *A. sativum*, and ginger, *Z. officinale* were used in subsequent experiments to determine their effects on development time of larval and pupal stages, accumulative mortalities and adult emergence. Larval duration and mortality were recorded daily until the last instar. Non-feeding last instar larvae were transferred individually to a clean cup containing sawdust for pupation and covered. Each pupa was gently removed from the sawdust after three days, sexed and weighed, and kept separately in the same cup with moist cotton wool to record the duration of the pupal development period and percentage moth emergence.

Studies on fecundity and fertility

After emergence, groups of five females and seven males were transferred to 1-l glass jars lined

internally with white paper, covered on top with fine mesh, and fed as described above. Three replicates, each consisting of five females and seven males were used at each of the two sublethal concentrations. Mating of females was confirmed visually. Deposited eggs were counted from day 2 to day 6 of mating. The eggs were transferred to a clean Petri dish with a piece of wet cotton wool and kept for three to five days to record hatchability percentage.

Effect of garlic and ginger oils on the catalyses enzyme (CAT)

Seven days post-treatment of neonate larvae with two sublethal concentrations (LC₁₅ and LC₅₀) of both oils, all live treated and untreated larvae were transferred to clean jars and left for 24 h before the biochemical assays. CAT activity was measured using Biodiagnostic Kit No. CA 2517, which is based on the spectrophotometric method described by Aebi (1984). The activity of CAT enzyme was determined by measuring the rate of H₂O₂ consumption *via* absorbance at 240 nm.

Statistical analysis

Percentage mortality of the treated larvae was adjusted by the percentage mortality of the untreated larvae using Abbott's formula and then subjected to Probit analysis version 1.5 (EPA Probit Analysis Program) to estimate the LC₁₅, LC₅₀ and LC₉₀ for each oil. Data on life history parameters, and the oxidative stress enzyme were performed using one-way ANOVA (SAS 2001) followed by Duncan's multiple range test.

RESULTS

Chemical composition of garlic oil

GC-MS analysis of oil extracted from garlic cloves showed the presence of 82 compounds (Table 1). The main chemical constituents were diallyl disulphide (6.03 %), nonadecane, 9-methyl- (4.45 %), nonadecane (3.47 %), tetradecane, 2-methyl- (3.37 %), octadecane (3.33 %), 1-decanol, 2-hexyl- (3.27 %), dodecanoic acid, hex-3-enyl ester (2.75 %), 1-dodecanol, 3,7,11-trimethyl- (2.66 %), tridecane, 5-propyl- (2.56 %), eicosane, 2,4-dimethyl- (2.48 %), cyclohexanecarboxaldehyde, 3,3-dimethyl-5-oxo- (2.36 %), disulphide, methyl 1-propenyl (2.33 %), 2-hexyl-1-octanol (2.32 %), eicosane, 2,6,10,14,18-pentamethyl- (2.21 %) and heptadecane (2.00 %).

Table 1. Chemical composition of garlic, *Allium sativum*, clove oil analysed by GC-MS.

Retention time (min)	Compound	Peak area (%)	Molecular weight	Molecular formula
2.62	1-Propene, 3,3'-thiobis-	0.22	114	C ₆ H ₁₀ S
3.27	1,3-Dithiane	0.78	120	C ₄ H ₈ S ₂
3.55	Disulphide, methyl 1-propenyl	2.33	120	C ₄ H ₈ S ₂
4.07	Dimethyl trisulphide	0.45	126	C ₂ H ₆ S ₃
5.77	Diallyl disulphide	6.03	147	C ₆ H ₁₀ S ₂
7.00	Trisulphide, methyl 2-propenyl	1.77	152	C ₄ H ₈ S ₃
8.08	3-Vinyl-1,2-dithiacyclohex-4-ene	0.52	144	C ₆ H ₈ S ₂
8.58	3-Vinyl-1,2-dithiacyclohex-5-ene	0.52	144	C ₆ H ₈ S ₂
10.34	Trisulphide, di-2-propenyl	0.47	178	C ₆ H ₁₀ S ₃
12.00	Tetradecane	0.34	198	C ₁₄ H ₃₀
13.02	Tridecane, 3-ethyl-	0.68	212	C ₁₅ H ₃₂
13.10	Tetradecane, 5-methyl-	0.44	212	C ₁₅ H ₃₂
13.22	Tetradecane, 4-methyl-	0.30	212	C ₁₅ H ₃₂
13.32	Tetradecane, 2-methyl-	0.44	212	C ₁₅ H ₃₂
13.48	Tetradecane, 3-methyl-	0.53	212	C ₁₅ H ₃₂
14.70	Nonadecane	0.76	268	C ₁₉ H ₄₀
15.20	4S,6S-Dimethyl-7R-hydroxynonan-3-one	1.33	186	C ₁₇ H ₃₂ O ₂
15.13	Nonadecane, 9-methyl-	0.67	282	C ₂₀ H ₄₂
15.25	Tetradecane, 2,5-dimethyl-	0.54	226	C ₁₆ H ₃₄
15.34	Pentadecane, 2-methyl-	0.88	226	C ₁₆ H ₃₄
15.5	Pentadecane, 3-methyl-	0.92	226	C ₁₆ H ₃₄
16.06	Hexadecane	1.30	226	C ₁₆ H ₃₄
16.22	5-Octadecene, (E)-	1.65	252	C ₁₈ H ₃₆
16.36	Hexadecane,2,6,10,14-tetramethyl-	0.50	282	C ₂₀ H ₄₂
16.51	Cyclohexane, 1,4-didecyl-	0.55	364	C ₂₆ H ₅₂
16.74	9-Tricosene, (Z)-	0.41	322	C ₂₃ H ₄₆
17.07	Tetradecane, 4-ethyl-	0.69	226	C ₁₆ H ₃₄
17.19	Hexadecane, 4-methyl-	0.66	240	C ₁₇ H ₃₆
17.29	Hexadecane, 2-methyl-	1.12	240	C ₁₇ H ₃₆
17.44	Heptadecane, 3-methyl-	1.05	254	C ₁₈ H ₃₈
17.76	1,3-Dioxolane-4-methanol,2-pentadecyl-, acetate, cis-	0.74	356	C ₂₇ H ₄₀ O ₄
17.99	Heptadecane	2.00	240	C ₁₇ H ₃₆
18.13	1-Dodecanol,3,7,11-trimethyl-	2.66	228	C ₁₅ H ₃₂ O
18.27	Ditetradecyl ether	0.52	410	C ₂₈ H ₅₈ O
18.07	Pentadecane, 8-hexyl-	0.38	296	C ₂₁ H ₄₄
18.90	2-Hexyl-1-octanol	2.32	214	C ₁₄ H ₃₀ O
19.06	1-Decanol, 2-hexyl-	0.78	242	C ₁₆ H ₃₄ O
19.20	1-Tridecane, 7-cyclohexyl-	1.24	266	C ₁₉ H ₃₈
19.30	Cyclopentane, (4-octyldodecyl)-	0.87	350	C ₂₅ H ₅₀
19.48	Heptadecane, 3-methyl-	1.05	254	C ₁₈ H ₃₈
19.63	(E,E)-3,7-Cyclodecadien-1-one,3,7-dimethyl-10-(1-methylethylidene)	0.82	218	C ₁₅ H ₂₂ O
19.71	Eudesma-5,11(13)-dien-8,12-olide	0.64	232	C ₁₅ H ₂₀ O ₂
20.07	Octadecane	3.33	254	C ₁₈ H ₃₈
20.28	Tridecane, 5-propyl-	2.56	226	C ₁₆ H ₃₄
20.53	α -Santonin	0.39	246	C ₁₅ H ₁₈ O ₃
20.65	Z,Z-4,6-nonadecadien-1-ol acetate	0.70	322	C ₂₁ H ₃₈ O ₂
20.76	Cyclopentane, (4-octyldodecyl)-	0.53	350	C ₂₅ H ₅₀
20.91	Cyclohexanecarboxaldehyde,3,3-dimethyl-5-oxo-	2.36	154	C ₉ H ₁₄ O ₂
21.09	Octane, 4,5-diethyl-	0.40	170	C ₁₂ H ₂₆

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Table 1 (continued)

Retention time (min)	Compound	Peak area (%)	Molecular weight	Molecular formula
21.22	Octadecane, 4-methyl-	0.56	268	C ₁₉ H ₄₀
21.32	Hexadecane, 5-butyl-	0.73	282	C ₂₀ H ₄₂
21.50	1-Hexadecanol, 2-methyl-	1.68	256	C ₁₇ H ₃₆ O
21.68	Naphthalene, 1-(1-decylundecyl)decahydro-	0.79	432	C ₃₁ H ₆₀
21.90	Eicosane	0.73	282	C ₂₀ H ₄₂
22.05	Nonadecane	3.47	268	C ₁₉ H ₄₀
22.27	Phytol	1.02	296	C ₂₀ H ₄₀ O
22.32	Propanoic acid, 2,2-dimethyl-,5-(phenylthio)-3-methyl-3-Pentenyl ester	1.28	292	C ₁₇ H ₂₄ O ₂ S
22.42	E,E,Z-1,3,12-nonadecatriene-5,14-diol	0.65	294	C ₁₉ H ₃₄ O ₂
22.80	Eicosane, 2,4-dimethyl-	2.48	310	C ₂₂ H ₄₆
23.12	Heptadecane, 9-octyl-	1.08	352	C ₂₅ H ₅₂
23.21	Nonadecane, 2-methyl-	0.93	282	C ₂₀ H ₄₂
23.37	Nonadecane, 2,3-dimethyl-	1.14	296	C ₂₁ H ₄₄
23.46	Oxalic acid, cyclohexyl decyl ester	0.90	312	C ₁₈ H ₃₂ O ₄
23.61	Dodecane, 2,6,10-trimethyl-	1.26	212	C ₁₅ H ₃₂
23.92	Tetradecane, 2-methyl-	3.37	212	C ₁₅ H ₃₂
24.11	Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)-	1.22	348	C ₂₅ H ₄₈
24.23	Oxalic acid, butylcyclohexylmethyl ester	1.45	242	C ₁₃ H ₂₂ O ₄
24.57	Nonadecane, 9-methyl-	4.45	282	C ₂₀ H ₄₂
24.77	Heptadecane, 9-octyl-	0.58	352	C ₂₅ H ₅₂
24.89	Octadecane,3-ethyl-5-(2-ethylbutyl)-	0.89	366	C ₂₆ H ₅₄
24.98	Eicosane, 2-methyl- 1.48 296	1.48	296	C ₂₁ H ₄₄
25.17	1-Decanol, 2-hexyl-	3.27	242	C ₁₆ H ₃₄ O
25.29	Dodecanoic acid, hex-3-enyl ester	2.75	282	C ₁₈ H ₃₄ O ₂
25.60	Octadecane, 5,14-dibutyl-	1.78	366	C ₂₆ H ₅₄
25.67	Sulphurous acid, decyl pentyl ester	1.14	292	C ₁₅ H ₃₂ O ₃ S
25.87	1-Heneicosanol	1.10	312	C ₂₁ H ₄₄ O
25.97	Eicosane, 2-cyclohexyl-	0.95	364	C ₂₆ H ₅₂
26.17	Tetratetracontane	0.77	618	C ₄₄ H ₉₀
26.28	Eicosane,2,6,10,14,18-pentamethyl-	2.21	352	C ₂₅ H ₅₂
26.57	Eicosane, 3-methyl-	0.35	296	C ₂₁ H ₄₄
27.30	Cyclohexane,1,3,5-trimethyl-2-octadecyl-	0.40	378	C ₂₇ H ₅₄

Chemical composition of ginger oil

Data on the composition of ginger oil is provided in Table 2. Ginger oil is composed of 41 compounds and the main components were isopropyl myristate (16.41 %), tetratetracontane (13.16 %), α -zingiberene (12.26 %), 17-pentatriacontene (10.68 %), eucalyptol (4.5 %), celidoniol, deoxy- (4.47 %), octadecane, 3-ethyl-5-(2-ethylbutyl)- (4.21 %), phenol, 2-methoxy-4-(2-propenyl)- (3.25 %), cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5 α)- (3.12 %), α -phellandrene (2.66 %), (Z,Z)- α -farnesene (2.55 %), heptadecane, 9-hexyl- (2.55 %), benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- (2.5 %), and 9-octadecenoic acid (Z)-, 9-hexadecenyl ester, (Z)- (2.25 %).

Toxicity of garlic and ginger on neonate larvae of *S. littoralis*

Both of garlic and ginger exhibited low toxicity against neonate larvae of *S. littoralis* with LC₅₀ values of 3889 and 4330 μ g/ml, respectively, while LC₉₀ values were 7315 and 10369 μ g/ml, respectively (Table 3).

Sublethal effect of garlic and ginger on developmental aspects of *S. littoralis*

The results presented in Table 4 showed that both garlic and ginger significantly increased larval and pupal developmental time. Duration of larval and pupal period were significantly longer in case of ginger treatments with LC₅₀ value

Table 2. Oil composition of ginger, *Zingiber officinale*, analysed by GC-MS.

Retention time (min)	Compound	Peak area (%)	Molecular weight	Molecular formula
4.06	(Z,Z)- α -farnesene	2.55	204	C ₁₅ H ₂₄
4.98	Eucalyptol	4.5	154	C ₁₀ H ₁₈ O
5.99	α -Phellandrene	2.66	136	C ₁₀ H ₁₆
8.04	Decanal	1.56	156	C ₁₀ H ₂₀ O
8.98	Heptadecane, 9-hexyl-	2.55	324	C ₂₃ H ₄₈
11.43	Phenol,2-methoxy-4-(2-propenyl)-	3.25	164	C ₁₀ H ₁₂ O ₂
11.97	Docosane	0.55	310	C ₂₂ H ₄₆
12.17	Thiophene, 2-decyl-	0.89	224	C ₁₄ H ₂₄ S
13.71	Celidoniol, deoxy-	4.47	408	C ₂₉ H ₆₀
13.96	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	2.5	202	C ₁₅ H ₂₂
14.20	α -Zingiberene	12.26	204	C ₁₅ H ₂₄
14.82	α -Sesquiphellandrene	0.10	204	C ₁₅ H ₂₄
16.31	Phenanthrene,9-dodecyltetradecahydro-	0.13	360	C ₂₆ H ₄₈
17.30	2,2-Dimethyl-3-[3-methyl-5-(phenylthio)pent-3-enyl]oxirane	0.01	262	C ₁₆ H ₂₂ OS
18.77	Cyclohexane,1,1'-dodecylidenebis[4-methyl-	0.02	362	C ₂₆ H ₅₀
19.86	Cyclohexane,1,1'-dodecylidenebis[4-methyl-	0.40	362	C ₂₆ H ₅₀
20.64	Isopropyl myristate	16.41	270	C ₁₇ H ₃₄ O ₂
20.82	Cyclohexane, (1-hexadecylheptadecyl)-	0.32	546	C ₃₉ H ₇₈
20.88	2H-Pyran,2-(3-heptadecyloxy)tetrahydro-	0.13	336	C ₂₂ H ₄₀ O ₂
21.46	2-Nonadecanone	0.12	282	C ₁₉ H ₃₈ O
21.76	17-Pentatriacontene	0.18	490	C ₃₅ H ₇₀
22.52	1-Dotriacontanol	0.56	466	C ₃₂ H ₆₆ O
22.69	Cyclohexane,1,4-dimethyl-2-octadecyl-	0.58	364	C ₂₆ H ₅₂
23.05	Cyclohexane,1,1'-dodecylidenebis[4-methyl-	0.14	362	C ₂₆ H ₅₀
23.15	Pentalene,octahydro-1-(2-octyldecyl)-	0.17	362	C ₂₆ H ₅₀
24.09	17-Pentatriacontene	10.68	490	C ₃₅ H ₇₀
24.30	1-Pentatriacontanol	0.82	508	C ₃₅ H ₇₂ O
25.63	Cyclohexane,1,3,5-trimethyl-2-octadecyl-	0.38	378	C ₂₇ H ₅₄
26.06	14- α -H-Pregna	1.57	288	C ₂₁ H ₃₆
26.65	Eicosane, 7-hexyl-	1.52	366	C ₂₆ H ₅₄
27.21	Eicosane, 3-cyclohexyl-	0.15	364	C ₂₆ H ₅₂
29.65	17-Methyl[2.2.2](1,3,5)benzeno(3,3',3'')triphenyl methanophane	1.94	414	C ₃₂ H ₃₀
30.51	Octadecane,3-ethyl-5-(2-ethylbutyl)-	4.21	366	C ₂₆ H ₅₄
31.01	9-Octadecenoic acid (Z)-, 9-hexadecenyl ester, (Z)-	2.25	504	C ₃₄ H ₆₄ O ₂
33.00	Octadecane,9-ethyl-9-heptyl-	0.83	380	C ₂₇ H ₅₆
34.25	Octadecane,1,1'-[1,3-propanediylbis(oxy)]bis-	0.32	580	C ₃₉ H ₈₀ O ₂
34.46	Stearic acid,3-(octadecyloxy)propyl ester	0.68	594	C ₃₉ H ₇₈ O ₃
34.85	Tetratetracontane	13.16	618	C ₄₄ H ₉₀
35.33	Cholestan-3-one, cyclic1,2-ethanediyl aetal, (5 α)-	3.12	430	C ₂₉ H ₅₀ O ₂
36.80	4H-1-Benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di- α -d-Glucopyranosyl-5,7-dihydroxy-	0.76	610	C ₂₇ H ₃₀ O ₁₆

compared to garlic LC₅₀, while garlic LC₅₀ was significantly longer than garlic LC₁₅ and ginger LC₅₀ was significantly longer than garlic LC₅₀ and ginger LC₁₅. There were, however, no significant differences in percentage of adult emergence between the two oil treatments and the control. Female and male pupal weight increased signifi-

cantly after larval treatment with the LC₁₅ and LC₅₀ dosage of both oils compared to control values (0.273 mg female and 0.265 mg male).

Fecundity and fertility

Both garlic and ginger significantly decreased the percentage of hatchability at all concentrations

Table 3. Toxicity of the garlic, *Allium sativum*, and ginger, *Zingiber officinale*, oils on neonate larvae of *Spodoptera littoralis*.

Oil extract	^a LC ₁₅ (µg/ml) 95 % confidence limits	^b LC ₅₀ (µg/ml) 95 % confidence limits	^c LC ₉₀ (µg/ml) 95 % confidence limits	Slope ± S.E.
Garlic	2333.58 (1818.54–2729.83)	3889.56 (3488.27–4231.31)	7315.98 (6520.10–8709.95)	4.67 ± 0.594
Ginger	2137.36 (1561.07–2584.35)	4330.57 (3857.32–4813.18)	10369 (8508.19–14410.21)	3.37 ± 0.471

^aLC₁₅: concentration causing 15 % mortality.

^bLC₅₀: concentration causing 50 % mortality.

^cLC₉₀: concentration causing 90 % mortality.

tested compared to the control as shown in Table 5. The percentage eggs that hatched after treatment of the larvae they originated from at the LC₁₅ and LC₅₀ of garlic and ginger was 72.02, 62.96, 75.70 and 61.64 %, respectively. The value for untreated larvae was 86.78 %. Fecundity of the females was not affected by the treatments of both oils at all concentrations and there were no significant differences between the oil treatments and control (Table 5).

Catalase enzyme (CAT) activity

After seven days post-treatment of neonate larvae of *S. littoralis* with the LC₁₅ and LC₅₀ of ginger, the CAT activity was only significantly higher in larvae exposed to the LC₅₀ value (2.19 ± 0.36 IU/mg of protein) compared to the untreated

larvae (0.26 ± 0.06 IU/mg of protein). There was no significant difference between CAT activity of larvae treated with the LC₁₅ and LC₅₀ rates of garlic (0.48 ± 0.08, and 0.42 ± 0.12 IU/mg of protein, respectively) and the control (0.26 ± 0.06 IU/mg of protein) (Fig. 1).

DISCUSSION

Over the past 25 years, interest in botanical insecticides including essential oils and plant extracts increased due to environmental concerns and problems experienced with insect resistance evolution to conventional chemical insecticides. Essential oils and of plant extracts, which are used as insecticides, presently constitute 1 % of the world insecticide market (Rozman *et al.* 2007).

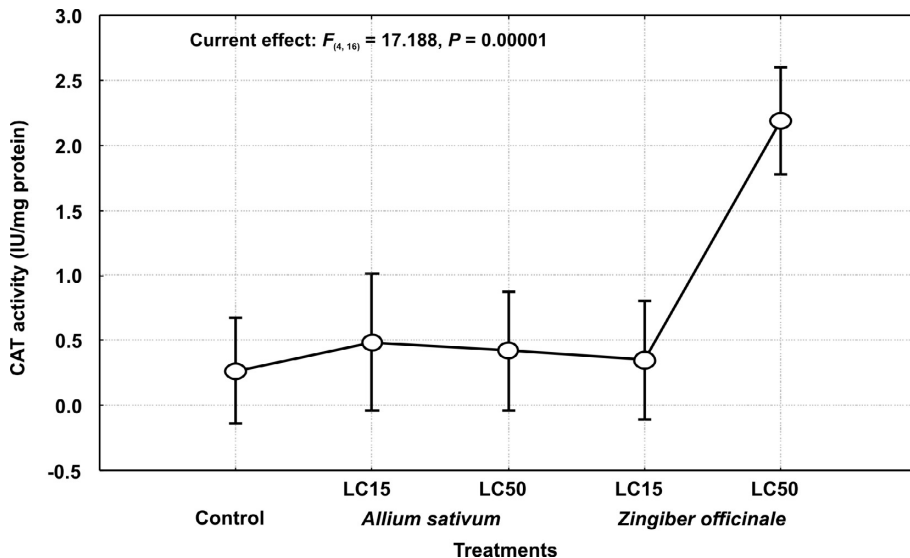


Fig. 1. Effect of sublethal concentrations (LC₁₅ and LC₅₀ values) of garlic and ginger oils on CAT activity in *Spodoptera littoralis*.

Table 4. Sublethal effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) oils on life history parameters of *Spodoptera littoralis*.

Treatment	*Larval duration	Pupation %	**Pupal duration		Pupal weight (mg)		Sex ratio		Emergence %
			Female	Male	Female	Male	Female	Male	
Control	17.58 ^c ± 0.103	99.25 ^a ± 0.750	9.34 ^d ± 0.060	0.273 ^e ± 0.005	0.265 ^e ± 0.004	52.55 ^a ± 3.34	47.44 ^a ± 3.344	98.46 ^c ± 0.885	
Garlic LC ₁₅	19.28 ^b ± 0.061	98.55 ^a ± 0.835	9.72 ^c ± 0.087	0.339 ^b ± 0.006	0.311 ^b ± 0.003	48.56 ^a ± 4.27	51.44 ^a ± 4.279	96.74 ^a ± 0.311	
Garlic LC ₅₀	19.50 ^b ± 0.088	95.77 ^a ± 3.076	10.14 ^b ± 0.107	0.349 ^b ± 0.007	0.314 ^b ± 0.005	44.93 ^a ± 6.36	55.06 ^a ± 6.360	95.41 ^a ± 2.056	
Ginger LC ₁₅	19.42 ^b ± 0.074	97.44 ^a ± 1.076	10.01 ^b ± 0.073	0.357 ^b ± 0.004	0.320 ^b ± 0.004	53.01 ^a ± 3.47	46.98 ^a ± 3.479	93.62 ^a ± 2.162	
Ginger LC ₅₀	20.28 ^a ± 0.122	93.15 ^a ± 4.135	10.47 ^a ± 0.134	0.379 ^a ± 0.009	0.346 ^a ± 0.010	58.83 ^a ± 6.28	41.16 ^a ± 6.284	91.52 ^a ± 4.198	

Values marked with the same letter within the same column are not significantly different ($P > 0.05$; Duncan's multiple range test).

*Number of days from second instar larvae till pupation.

**Number of days from the pupation till the emergence.

Table 5. Mean fecundity and egg hatch (%) (\pm S.E.) of *Spodoptera littoralis* after exposure of neonate larvae to sublethal concentrations (LC₁₅ and LC₅₀ values) of garlic and ginger oils.

Treatment		*Fecundity	**Hatchability %
Control		486.31 ^a ± 118.77	86.78 ^a ± 4.79
Garlic	LC ₁₅	561.53 ^a ± 27.13	72.02 ^{ab} ± 8.52
	LC ₅₀	499.15 ^a ± 54.11	62.96 ^b ± 3.22
Ginger	LC ₁₅	592.26 ^a ± 9.40	75.70 ^{ab} ± 6.68
	LC ₅₀	444.44 ^a ± 25.21	61.64 ^b ± 7.12

Values with the same letter within the same column are not significantly different ($P > 0.05$; Duncan's multiple range test).

*Fecundity was estimated by counting the eggs from the first day till the sixth day (total number of eggs laid by one female).

**Fertility is calculated by counting of the emerged larvae from collected eggs batches.

Using LC₁₅ and LC₅₀ concentrations of oils extracted from garlic cloves and ginger rhizomes on neonates of *S. littoralis* indicated that these essential oils have insecticidal activity and toxic effects on this species. Results of the study showed that both oils significantly prolonged the larval and pupal development periods. Application of ginger oil at the LC₅₀ rate provided improved control of larvae compared to garlic oil. These results might be attributed to the major constituents of ginger oil (monoterpenes and sesquiterpene hydrocarbons) and garlic oil (organosulphur compounds) that affected the insect neuroendocrine system and juvenile hormone causing hormonal unbalance and interrupting the ecdysis process, thus leading to the prolongation of larval and pupal developmental time. These findings are in accordance with that reported by Moawad & Ebadah (2007) for potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae), and Sharaby & El-Nojibian (2015) on the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae).

The identified components of garlic which include diallyl sulphide, diallyl di-sulphide and diallyl trisulphide have antagonistic properties against several pests including potato tuber moth, red cotton bug, red palm weevil, houseflies and mosquitoes (Amonkar & Banerji 1971). Many of the insecticidal properties of ginger are ascribed to the presence of compounds in the rhizomes known as 'oleoresins' especially those known as gingerols and shogaols (pungent component) (Sekiwa *et al.* 2000). Generally, Onyenekwe & Hashimoto (1999) reported that ginger contains approximately 1-4 % volatile oils, the aromatic

components include zingiberene and bisabolene with other components of their volatiles include zingiberene and arcurcumene, β -bisabotene arcucumene, D-camphor, and arylalkane (Chio & Laursen 2000).

The percentage of egg hatch was significantly reduced after exposure of larvae to both oils at LC₅₀ concentrations compared to the LC₁₅ and control. The effect of ginger oil application at the LC₅₀ concentration had the most pronounced effect on life history parameters of *S. littoralis* compared to other treatments. At the same time, the fecundity of the females that developed from treated larvae were not affected and there were no significant differences between the control and the treatments. The decrease in hatchability percentage might be due to some physiological changes that caused disturbance of the embryonic development. Some of the chemical components (mainly flavonoids) are thought to be responsible for the developmental defects that occurred during the embryonic development of the eggs and caused the adverse results. These results are supported by other studies which indicated that essential oils of different plant species caused a decrease in egg hatchability (Gurusubramanian & Krishna 1996; Osman 1999; Khalil & Ismail 2001). The results of the latter studies, however, do not support observations made in this study that female fecundity was not affected by the plant oil extracts.

The catalase enzyme (CAT) activity was highly significant only in the LC₅₀ treatment of ginger oil compared to other treatments and the control.

REFERENCES

- ADAMS, R.P. 2001. *Essential Oil Components by Quadrupole GC/MS*. Allured, Carol Stream, IL, U.S.A.
- AEBI, H. 1984. Catalase *in vitro*. *Methods in Enzymology* **105**: 121–126.
- AMONKAR, S.V. & BANERJI, A. 1971. Isolation and characterization of the larvicidal principle of garlic. *Science* **174**: 1343–1344.
- BLOCK, E. 1992. The organosulfur chemistry of the genus *Allium* – implications for organic sulfur chemistry. *Angewandte Chemie International Edition* **140**: 1158–1203.
- CAVALLITO, C.J. & BAILEY, J.H. 1945. Allicin: the antimicrobial principle of *Allium sativum* L. Isolation, physical properties and antibacterial action. *Journal of the American Chemistry Society* **66**: 1950–1951.
- CHIO, K.H. & LAURSEN, R.A. 2000. Amino-acid sequence and glycan structures of cysteine proteases with proline specificity from ginger rhizome *Zingiber officinale*. *European Journal of Biochemistry* **267**: 1516–1526.
- DEMİN, G. & YINGYING, Z. 2010. Comparative antibacterial activities of crude polysaccharides and flavonoids from *Zingiber officinale* and their extraction. *American Journal of Tropical Medicine* **5**: 235–238.
- EL-DEFRAWI, M.E., TAPPOZADA, A.T., SALAMA, A. & EL-KHISHEN, S.A. 1964. Toxicological studies on the Egyptian cotton leafworm *Prodenia litura* F. II. Reversions of toxaphene resistance in the Egyptian cotton leafworm. *Journal of Economic Entomology* **18**: 265–267.
- EL-SHEIKH, E.A. 2015. Comparative toxicity and sublethal effects of emamectin benzoate, lufenuron and spinosad on *Spodoptera littoralis* Bois (Lepidoptera: Noctuidae). *Crop Protection* **67**: 228–234.
- FARNSWORTH, N.R. & BUNYAPRAPHATSARA, N. 1992. *Thai Medicinal Plants*. 210–287. Prachachon, Bangkok, Thailand.
- FELTON, G.W. 1995. Oxidative stress of vertebrates and

This result might be related to increased levels of peroxides in the gut tissues of treated larvae feeding on castor bean leaves treated with ginger oil at LC₅₀ concentration. This hypothesis is supported by the fact that gut tissues are subjected to oxidative stresses when the larvae feed on a diet rich in allelochemicals. Krishnan & Kodrik (2006) found that the gut of *S. littoralis* larvae has conditions that are very favourable for oxidation and is reason why these allelochemicals exhibit oxidation conditions inside the gut and generate oxidative radicals. Polyphenols, which are abundant in ginger rhizomes, are good example of these allelochemicals. The larvae are thought to up-regulate their antioxidant levels to overcome these stresses imposed on them. The CAT activity in the treated larvae is enhanced to accommodate this up-regulation process.

CONCLUSIONS

Results showed that some of the life history parameters were significantly affected after treatment of larvae with garlic and ginger essential oil extracts. Ginger oil was more effective than garlic oil in the bioassays conducted to evaluate potential insecticidal activities on *S. littoralis*.

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- invertebrates. In: Ahmad, S. (Ed.) *Oxidative Stress and Antioxidant Defenses in Biology*. 356–424. Chapman and Hall, New York, U.S.A.
- GURUSUBRAMANIAN, G. & KRISHNA, S.S. 1996. The effects of exposing eggs of four cotton insect pests to volatiles of *Allium sativum* (Liliaceae). *Bulletin of Entomological Research* **86**: 29–31.
- GUO, L., DESNEUX, N., SONODA, S., LIANG, P., HAN, P. & GAO, X-W. 2013. Sublethal and transgenerational effects of chlorantraniliprole on biological traits of the diamondback moth, *Plutella xylostella* L. *Crop Protection* **48**: 29–34.
- HASAN, H.A., RASHEED, R.A.M., ABD RAZIK, B.M. & RASOOL, H.B.A. 2012. Chemical composition and antimicrobial activity of the crude extracts isolated from *Zingiber officinale* by different solvents. *Pharmaceutica Analytica Acta* **3**: 1000184.
- HO, S.H., KOH, L., MA, Y., HUANG, Y. & SIM, K.Y. 1996. The oil of garlic, *Allium sativum* L. (Amaryllidaceae), as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *Postharvest Biology and Technology* **9**: 41–48.
- HOUGHTON, P.J. 1996. Compounds with anti-HIV activity from plants. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**: 601–604.
- ILHAN, A., GUREL, A., ARMUTCU, F., KAMISLI, S. & IRAZ, M. 2005. Antiepileptogenic and antioxidant effects of *Nigella sativa* oil against pentylenetetrazol-induced kindling in mice. *Neuropharmacology* **49**: 456–464.
- ISHAAYA, I., YABLONSKI, S. & HOROWITZ, A.R. 1995. Comparative toxicity of two ecdystroids, RH-2485 and RH-5992 on susceptible and pyrethroid resistant strains of the Egyptian cotton leafworm, *Spodoptera littoralis*. *Phytoparasitica* **23**: 139–145.
- ISMAN, M.B. 2000. Plant essential oils for pest and disease management. *Crop Protection* **19**: 603–608.
- ISMAN, M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* **51**: 45–66.
- JOHNSON, O.O., AYOLA, G.A. & ADENIPEKUN, T. 2013. Antimicrobial activity and the chemical composition of the volatile oil blend from *Allium sativum* (garlic clove) and *Citrus reticulata* (tangerine fruit). *International Journal of Pharmaceutical Science and Drug Research* **5**: 187–193.
- KHALIL, S.I.Y. & ISMAIL A.A. 2001. Efficiency of some wild plant extracts against the black cutworm, *Agrotis ipsilon* (Hufn.). *Journal of Agricultural Science Mansoura University* **26**: 2315–2329.
- KONO, Y. & FRIDOVICH, I. 1982. Superoxide radical inhibits catalase. *Journal of Biological Chemistry* **25**: 5751–5754.
- KRISHNAN, N. & KODRÍK, D. 2006. Antioxidant enzymes in *Spodoptera littoralis* (Boisduval): are they enhanced to protect gut tissues during oxidative stress? *Journal of Insect Physiology* **52**: 11–20.
- LÓPEZ, J.D. Jr., LATHEEF, M.A. & HOFFMANN, W.C. 2010. Effect of emamectin benzoate on mortality, proboscis extension, gustation and reproduction of the corn earworm, *Helicoverpa zea*. *Journal of Insect Science* **10**: 1–16.
- MOAWAD, S.S. & EBADAH, I.M.A. 2007. Impact of some natural plant oils on some biological aspects of potato tuber moth *Phthorimaea operculella*, (Zeller) (Lepidoptera: Gelechiidae). *Research Journal of Agriculture and Biological Sciences* **3**: 119–123.
- MOUSTAFA M.A.M., KÁKAI, A., AWAD, M. & FÓNAGY, A. 2016. Sublethal effects of spinosad and emamectin benzoate on larval development and reproductive activities of the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae). *Crop Protection* **90**: 197–204.
- NAWROT, J. & HARMATHA, J. 1994. Natural products as antifeedants against stored product insects. *Postharvest News and Information* **5**: 17N–21N.
- OKONKWO, C.O. & OHAERI, O.C. 2013. Insecticidal potentials of some selected plants. *Journal of Chemical and Pharmaceutical Research* **5**: 370–376.
- OLIVER, B. 1959. Nigeria's useful plants. *Nigerian Field* **24**: 160–182.
- ONYENEKWE, P.C. & HASHIMOTO, S. 1999. The composition of the essential oil of dried Nigerian ginger (*Zingiber officinale* Roscoe). *European Food Research and Technology* **209**: 407–410.
- OSMAN, S.M. 1999. A biochemical and toxicological study of the effect of some plant extracts on the black cutworm. *Agrotis ipsilon*. Ph.D. thesis, Ain Shams University Cairo, Egypt.
- PANJI, C., GRIMM, C., RAY, V.W., WITTE, L. & PROKSCHS, P. 1991. Insecticidal constituents from four species of the Zingiberaceae. *Phytochemistry* **14**: 415–419.
- RAJENDRAN, S. & SRIRANJINI, V. 2008. Plant products as fumigants for stored-product insect control. *Journal of Stored Products Research* **44**: 126–135.
- REHMAN, J.U., WANG, X.G., JOHNSON, M.W., DAANE, K.M., JILANI, G., KHAN, M.A. & ZALOM, F.G. 2009. Effects of *Peganum harmala* (Zygophyllaceae) seed extract on the olive fruit fly (Diptera: Tephritidae) and its larval parasitoid *Psyttalia oncolor* (Hymenoptera: Braconidae). *Journal of Economic Entomology* **102**: 2233–2240.
- ROSS, Z.M., MASLIN, D.J. & HILL, D.J. 2000. The effect of steam distilled garlic oil on lactic acid and other enteric bacteria. 4th Symposium on European Microbiological Societies. *FEMS Microbiology* **12(G43)**: 137.
- ROZMAN, V., KALINOVIC, I. & KORUNIC, Z. 2007. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored product insects. *Journal of Stored Products Research* **43**: 349–355.
- SALEM, M.Z.M., ALI, H.M., EL-SHANHOREY, N.A. & ABDEL-MEGEED, A. 2013. Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: antibacterial and antioxidant activities, total phenolic and flavonoid contents. *Asian Pacific Journal of Tropical Medicine* **6**: 785–791.
DOI: 10.1016/S1995-7645(13)60139-X
- SAS, 2001. *User Guide: Statistics* (Release 8.02), SAS Institute, Cary, NC, U.S.A.
- SEKIWA, Y.K., KUBOTA, K. & KOBAYASHI, A. 2000. Isolation of novel glucosides related to gingerdiol from ginger and their antioxidative activities. *Journal of Agricultural and Food Chemistry* **48**: 373–377.
- SHARABY, A. & EL-NOJIBIAN, A. 2015. Evaluation of

- some plant essential oils against the black cutworm *Agrotis ipsilon*. *Global Journal of Advanced Research* **2**: 701–711.
- SMET, H., VAN-MELLAERT, H., RANS, M. & DE-LOOF, A. 1986. The effect of mortality and reproduction of beta-asarone vapours on two insect species of stored grain: *Ephestia kuehniella* (Lepidoptera) and *Tribolium confusum* Duval (Coleoptera). *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **51**: 1197–1204.
- WANG, G., HUANG, X., WEI, H. & FADAMIRO, H.Y. 2011. Sublethal effects of larval exposure to indoxacarb on reproductive activities of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Pest Biochemistry and Physiology* **101**: 227–231.
- WHO. 1999. *Monographs on Selected Medicinal Plants*. Volume 1. World Health Organisation, Geneva, Switzerland. 16-19.
- YIN, X-H., WU, Q-J., LI, X-F., ZHANG, Y-J. & XU, B-Y. 2008. Sublethal effects of spinosad on *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Crop Protection* **27**: 1385–1391.
- YUKIHIRO, K., MAKOTO, I., JIRO, Y., NAOKI, K., NAOTO, U., ISAO, S., NAGATOSHII & KAZUHISA, O. 2002. Pharmacokinetic study of allixin, a phytoalexin produced by garlic. *Chemical & Pharmaceutical Bulletin* **50**: 352–363.