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Toxicity of three chemical extracts of black pepper fruits against two stored grain insect pests

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Abstract: *Three extracted fractions from black pepper fruits, Piper nigrum L, in four concentrations were evaluated for their toxicity effects against stored grain pests, red rusty flour beetle Tribolium castaneum Hbst. and their stages, and Sitophilus oryzae L., the adult rice weevil. The obtained results revealed that the etheric fraction caused higher mortality than that of two other solvent fractions, towards all target pests. The adult rice weevil was the most susceptible, followed by the larvae, adult > eggs > pupae of red flour beetle. The mortality percentage reached to 100% at a concentration 2.5% (w/w) after 21 day of treatment of adult rice weevil, while was 100% at conc. 5% (w/w) in same period in case of adult red flour beetle, and the* LC_{50} *between both adult pests were 2.43 and 3.52, respectively. The reduction in* F_1 *progeny in all treatments were greatly significant and was higher than the mortality in low concentrations, ranged from 73% to 100% according to the concentration and insect. By using GC/MS analysis was found a functional groups and compounds in the oil, were determined and identified the main constituents which may cause the mortality, as well as their structures, formulae and percentages. The main components were monoterpenes, Sesquiterpenes, fatty acids and alkaloids, and some of effective constituents were α-pinene, Linalool, α-Copaene, Linoleic acid and Piperin.*

Keywords: *Black pepper, Tribolium, Sitophilus, botanical extracts*

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I. Introduction

Some insects cause serious grains damage by developing inside kernels and feeding, and some other insect species feed and hide inside cracked grains, making their detection very difficult, while some species are fed on damaged grains or fines **[1]**. In addition, the presence of insects in grains can cause cash discount, because the damage affected on the quality, the quantity and the commercial value of the products **[2],** the damage caused by insects affects the quality, the quantity and the commercial value of the products. Many pests of stored products belong to the order Coleoptera and one of the most destructive secondary insect pests of durable stored products is the red flour beetle, *Tribolium castaneum* (Herbst). Stored grain insect pests have been damaging our economy by infesting agricultural stored products and responsible for worldwide loss of 10 - 40% in the stored grains annually **[3]**.

Many plant products have been evaluated for their toxic properties against different stored grain pests especially in form of essential oils **[4]**. A commonly used method for control stored product pests is the application of synthetic contact insecticides and fumigants **[5]**. The synthetic insecticides may cause various problems to the environment and our health, side effects of these insecticides are neurotoxic to man as well as domestic animals, this fact leads us towards the application of natural botanical extracts as insecticides such as crude and essential oils as an alternative mean to the chemical insecticides, the natural insecticides derived from plant extractions were tested by many researchers for their insecticidal properties **[6, 7, 8]**. It was proved that natural insecticides are excellent, mostly safe and have no environmental damage when applied against different insects and pests **[9, 10, 11]**.

Today, attention has been focused to control stored product pests with alternative control agents as extracts of plant leaves, flowers and seeds **[12, 13]**. Additionally, the extracts of some medicinal plants even beneficial for human beings can be used **[14]**. Red flour beetle *Tribolium castaneum* Hbst. and Rice weevil *Sitophilus oryzae* L. are the most abundant and injurious pests of stored grains. They were found in warehouses and flour mills at warm and tropical regions of the world **[15, 16, 17]**. The need for safe and natural management methods as alternative to pesticides is a major concern about health and environment **[18]**. Many investigations were carried out on black pepper fruits revealed its great potency as an efficient insecticide against a wide range of pests **[19, 20]**, besides, it has a known significant economic importance due to its valuable edible spicy taste worldwide.

This study aims to assess the efficacy of different extractions of black pepper fruits against two dangerous stored grain insects; red flour beetle, *Tribolium castaneum* and rice weevil, *Sitophilus oryzae*. GC/MS analysis was performed to record the main chemical constituents in seeds extracts.

II. Materials and methods

2.1. Insect cultures maintenance

The two tested insect pests were collected from warehouses and some infected mills, transferred to the laboratory and reared to be maintained in two separate cultures. They were reared under optimum conditions of 28 ± 1°C and 65 ± 5% RH and fed sterilized wheat for *S. oryzae* and sterilized flour mill for *T. castaneum ad libitum*. The two insect pest cultures were put in suitable clean glass jars with muslin covered mouths to increase the population density for few months. It was produced susceptible strains for treatments and the media were renewed to keep the cultures clean and healthy before the experiments.

2.2. Crude extraction

The clean dried black pepper fruits were grinded with an electric grinder to obtain the fine powder. 500 g of the fine powder were soaked in one liter of petroleum ether put in a 2-liter dark glass jar for 3 days with continuous shaking **[12]**. The solution was filtered with a piece of gauze and then, followed by a filter paper for three times till the clear solution. The obtained solution was evaporated in a rotary evaporator at 50°C and the residue was weighed and kept in a refrigerator at 4°C till use. The extracted crude was dissolved in the same solvent to get a 10% (w/w) stock solution, which diluted to obtain 1.25, 2.5, and 5% solutions. The same procedure was repeated with methylene chloride and absolute Ethanol as ascending arrangement of polarity.

2.3. Bioassay

The toxicity of the three fractions extracts of black pepper fruits was carried out against the two tested insects; *S. oryzae* and *T. castaneum*. Ten grams of flour were mixed with 1.25, 2.5, 5 and 10% concentrations (w/w) of the black pepper fruit petroleum ether extract (the first fraction extract). Each mixture was put in a 50 ml jar and left 24 h for complete dryness of the solvent as a treated medium. Thirty individuals of egg, 3rd instar larvae, pupal and adult stages were separately put in each jar. All jars were triplicated, tightly covered with muslin fixed with rubber band and kept in an incubator at 28 ± 1°C and 65 ± 5% RH. The positive control groups were prepared with the solvent and the media without the crude extract and also triplicated for each concentration. The negative control groups were prepared with the untreated media only. The same procedures (mentioned above) were repeated for the second fraction extract methylene chloride and third fraction extract absolute ethanol extracts. The mortality values were recorded after 2, 3, 5, 7, 10, 14 and 21 days of exposure only for the 3rd larval instar and adult stage of the two tested insects. The numbers of F_1, the first generation, progeny were also recorded after 60 days of media incubation after the isolation of the adults from treated media and the percentage of offspring reduction was calculated.

2.4. Data analysis

The correction of the mortality percentages were carried out by using **[21]**. The percentage of offspring reduction was calculated according to the equation of **[22]**. The mortality percentages were probit analyzed by using ANOVA test using a computer program Costate, and mean values were adjusted according to Duncan's Multiple Range test **[23]** at 0.01 and 0.05 significance levels and LSD with Statistical Software version 6.3.0.3, and also by using LDP-line to estimate the LC_{50}, LC_{90}, slope values and toxicity index of the tested black pepper fruit extracts **[24]**.

2.5. GC/MS analysis

The chemical constituents of the three black pepper fruit crude extracts were identified by Gas chromatography-mass spectrometry (GC/MS) at the Central Pesticides Laboratory, Dokki, Giza, Egypt. The compounds identification was done by comparing NIST, WILEY libraries and with the authentic spectra **[25]** and the data of peaks with those reported in literature. The constituent percents were computed using GC peak areas on BP-I column without applying correction factors.

III. Results

3.1. Toxicological findings

3.1.1. Red flour beetle

The data in Table (1) showed the increase of the recorded mortality values with the increase of the applied concentrations and exposure time in most treatments. The mortality percentage of adult flour beetle *T.*

castaneum was 100% with 5% (w/w) petroleum ether black pepper fruits extract after 21 days of exposure (LSD = 14.8, $p < 0.05$). The mortality percentage was 100% for 10% (w/w) after 14 days exposure time (LSD = 11.9, $p < 0.01$). The reduction in F_1 progeny reached 100% with all concentrations. In case of treatment with methylene chloride and ethanolic extracts, the mortality percentages were 66.6, 80, 92.2, 100%, and 60, 76.6, 84.4, 96.6% at concentrations of 1.25, 2.5, 5, 10% (w/w), respectively, after 21 days of treatment. The reduction in F_1 progeny was ranged from 73.5% to 100% with the increase of concentration and exposure time in all treatments. The LC_{50} value was 3.52 in case of methylene chloride with a toxicity index of 100% as in Table (2) and Fig. (1).

The mortality values in case of the 3rd larval instar of flour beetles *T. castaneum*, recorded in Table (1). The toxicity caused by using petroleum ether, methylene chloride and ethanolic extracts resulted in mortality percentages of 83.3, 96.6, 100, 100%; 53.3, 75.5, 87.7, 94.4% and 51.1, 65.5, 73.3, 87.7% with concentrations of 1.25, 2.5, 5, 10% (w/w), respectively, after 14 and 21 days of exposure. There were high significant differences (LSD = 15.3) and the LC_{50} was 2.67 and toxicity index 100% as shown in Table (2) and Fig. (1).

The pupal mortality percentages of the red flour beetles *T. castaneum* were increased with the increase of concentration. The recorded data in Table (3) showed that the mortality ratios obtained by using petroleum ether, methylene chloride and ethanolic extracts were 40, 62.2, 73.3, 85.5%; 24.4, 43.3, 66.6, 82.2% and 21.1, 35.5, 52.2, 65.5% at concentrations of 1.25, 2.5, 5, 10% (w/w), respectively. There were high significant differences (LSD 23.6) and the LC_{50} was 2.9 as shown in Table (2) and Fig. (1). The unhatched eggs of the red flour beetles *T. castaneum* treated with the three previous extracts showed that the mortality percentages of eggs were 73.3, 86.6, 90, 96.6%; 66.6, 75.5, 83.3, 90% and 60, 73.3, 80, 86.6% at concentrations of 1.25, 2.5, 5, 10% (w/w), respectively, with high significant differences (LSD = 15.6) as shown in Table (3).

Table 1. Toxicity of pepper fruits extracts against adult and 3rd larval instar of *T. castaneum*.

Extract	Conc. (%) (w/w)	Stage	Mortality after indicated days							No. of F_1 progeny	% of F_1 progeny reduction
			2	3	5	7	10	14	21		
Petroleum ether	1.25%	adult	10±0	16.6±3.3	23.3±3.3	41.1±3.3	60±5.7	73.3±3.3	86.6±6.6	0	100%
		larva	6.6±3.3	20±5.7	26.6±3.3	43.3±6.6	56.6±3.3	70±5.7	83.3±8.8		
	2.5%	adult	13.3±3.3	23.3±3.3	36.6±3.3	46.6±6.6	66.6±6.6	76.6±3.3	93.3±3.3	0	100%
		larva	16.6±8.8	33.3±8.8	53.3±6.6	61.1±8.8	73.3±8.8	86.6±3.3	96.6±3.3		
	5%	adult	23.3±3.3	36.6±5.8	50±5.7	63.3±6.6	80±5.7	90±0	100±0	0	100%
		larva	21.1±6.6	40±5.7	66.6±6.6	76.6±3.3	83.3±6.6	93.3±3.3	100±0		
	10%	adult	40±5.7	53.3±3.3	70±5.7	83.3±3.3	93.3±3.3	100±0	100±0	0	100%
		larva	36.6±3.3	63.3±6.6	80±5.7	86.6±6.6	96.6±3.3	100±0	100±0		
Methylene chloride	1.25%	adult	0±0	3.3±3.3	10±5.7	15.5±8.8	26.6±3.3	46.6±6.6	66.6±6.6	38	79.4%
		larva	1.7.±1.7	6.6±3.3	13.3±3.3	16.6±3.3	23.3±3.3	36.6±3.3	53.3±6.6		
	2.5%	adult	6.6±3.3	10±0	16.6±3.3	26.6±6.6	40±5.7	63.3±8.8	80±5.7	11	94.1%
		larva	11.1±6.6	20±0	33.3±6.6	50±5.7	56.6±3.3	60±5.7	75.5±3.3		
	5%	adult	13.3±3.3	23.3±3.3	30±5.7	36.6±3.3	53.3±6.6	75.5±6.6	92.2±3.3	0	100%
		larva	16.6±3.3	21.1±6.6	36.6±8.8	53.3±6.6	60±0	73.3±8.8	87.7±6.6		
	10%	adult	26.6±6.6	33.3±3.3	46.6±6.6	66.6±3.3	80±5.7	93.3±3.3	100±0	0	100%
		larva	20±5.7	26.6±3.3	40±5.7	56.6±8.8	64.6±6.6	80±5.7	94.4±3.3		
Ethanol	1.25%	adult	1.7±1.7	3.3±3.3	10±0	16.6±3.3	25.3±6.6	43.3±6.6	60±5.7	49	73.5%
		larva	2.2±3.3	11.1±6.6	20±5.7	26.6±6.6	30±5.7	35.5±3.3	51.1±5.7		
	2.5%	adult	6.6±3.3	10±0	16.6±6.6	23.3±3.3	33.3±3.3	50±5.7	76.6±3.3	24	87.%
		larva	5.6±6.6	16.6±3.3	25.5±6.6	33.3±3.3	40±5.7	53.3±6.6	65.5±8.8		
	5%	adult	11.1±6.6	16.6±3.3	23.3±3.3	36.6±6.6	43.3±6.6	56.6±3.3	84.4±6.6	5	97.3%
		larva	10±0	20±0	30±5.7	43.3±6.6	56.6±3.3	63.3±8.8	73.3±6.6		
	10%	adult	20±5.7	33.3±3.3	43.3±6.6	55.3±6.6	68.1±8.8	80±5.7	96.6±3.3	0	100%
		larva	16.6±3.3	23.3±6.6	33.3±3.3	52.2±8.8	63.3±6.6	71.2±3.3	87.7±3.3		
Significant differences between extracts	LSD (5%)	adult	7.9	8.88	10.5	10.5	12.56	11.9	14.86		
		larva	12.24	13.47	17.77	16.38	13.76	17.31	15.39		
	Sig.	adult	***	***	***	***	***	***	*		
		larva	***	***	***	***	***	***	***		
Control			0±0							185	

* = significant difference, ** = high significant difference and *** = very high significant difference.

3.1.2. Rice weevil

The toxicity effects of black pepper fruit extracts on the adult rice weevil *S. oryzae* were recorded in Table (4), which showed that the petroleum ether extract caused the most high mortality percentages. The maximum mortality was 95.5% at a concentration of 1.25% (w/w) after 21 days of treatment, 100% at 2.5% (w/w) after 21 days, 100% at 5% (w/w) after 14 days and 100% at 10% (w/w) after 10 days of treatment. There were very high significant differences (LSD = 12.8) as well as the reduction in F_1 progeny ranged from 98.1-100% according to the concentration. The mortality percentages resulted from methylene chloride fraction was 100% at a concentration of 10% (w/w) after 14 days of treatment. Whereas, the mortality percentages at concentrations of 1.25, 2.5 and 5% (w/w) were 83.3, 94.4 and 100% after 21 days of treatment, respectively. There were high significant differences (LSD = 8.4) and the F_1 progeny reduction was varied between 90% and 100% according to the concentration as shown in Table (4). The toxicity of ethanolic extract caused mortality percentages of 66.6, 83.3, 92.2 and 100% at concentrations of 1.25, 2.5, 5 and 10% (w/w), respectively. The reduction in F_1 progeny ranged from 81.4% to 99.7% according to the concentration with high significant differences and the LC_{50} was 2.43 as in Table (2) and Fig. (1).

Table 2. LC_{50} values of black pepper fruits extracts against the target insects

Extract	LC_{50}	LC_{90}	Slope	Toxicity index (%)
Eth. 1	2.437	14.915	1.629	100
Ptr. 2	2.679	17.419	1.576	90.967
Mth. 3	2.914	15.263	1782	83.631
Mth. 4	3.521	23.534	1.553	76.1

Eth. 1 = Ethanolic extract on adult rice weevil, Ptr. 2 = Etheric extract on flour beetle larva, Mth. 3 = Methylene chloride extract on flour beetle pupa, Mth. 4 = Methylene chloride extract on adult flour beetle.

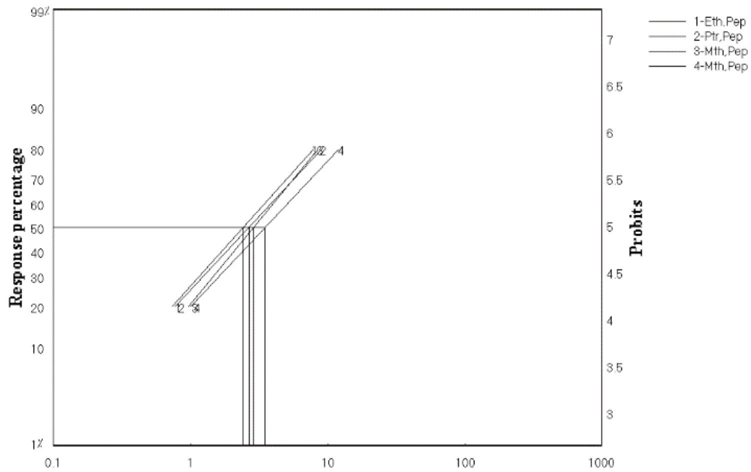

Fig. 1. Toxicity line of black pepper fruits extract against the target insects.

Table 3. Toxicity of pepper fruits extracts against pupae and eggs of *T. castaneum*.

Extract	Conc. (%) w/w	Stage	Survival Number	Pupae & eggs mortality
Petroleum ether	1.25%	Pupae	36	40±5.7
		Eggs	66	73.3±6.6
	2.5%	Pupae	56	62.2±8.8
		Eggs	78	86.6±3.3
	5%	Pupae	66	73.3±6.6
		Eggs	81	90±5.7
	10%	Pupae	77	85.5±3.3
		Eggs	87	96.6±6.6
Methylene chloride	1.25%	Pupae	22	24.4±3.3
		Eggs	60	66.6±8.8
	2.5%	Pupae	39	43.3±3.3
		Eggs	68	75.5±6.6
	5%	Pupae	60	66.6±3.3
		Eggs	75	83.3±8.8
	10%	Pupae	74	82.2±3.3
		Eggs	81	90±5.7
Ethanol	1.25%	Pupae	19	21.1±3.3
		Eggs	54	60±5.7
	2.5%	Pupae	32	35.5±8.8
		Eggs	66	73.3±3.3
	5%	Pupae	47	52.2±6.6
		Eggs	72	80±5.7
	10%	Pupae	59	65.5±8.8
		Eggs	78	86.6±3.3
Significant difference between extracts	Significance	Pupae		***
		Eggs		***
	LSD 5%	Pupae		23.66
		Eggs		15.63
	Control		**0±0**	

*** = very high significant difference.

Table 4. Toxicity of pepper fruits extracts against adult *S. oryzae*.

Extract	Conc. (%) (w/w)	Mortality after indicated days							No. of F_1 progeny	% of F_1 progeny reduction
		2	3	5	7	10	14	21		
Petroleum ether	1.25%	35.5±3.3	43.3±6.6	56.6±3.3	65.5±6.6	73.3±8.8	86.6±3.3	95.5±3.3	3	98.1%
	2.5%	50±5.7	55.5±8.8	67.7±6.6	76.6±8.8	84.4±6.6	90±5.7	100±0	1	99.9%
	5%	61.1±8.8	73.3±8.8	80±5.7	84.4±6.6	91.1±3.3	100±0	100±0	0	100%
	10%	76.6±8.8	81.1±6.6	86.6±8.8	93.3±3.3	100±0	100±0	100±0	0	100%
Methylene chloride	1.25%	32.2±5.7	40±5.7	54.4±8.8	65.5±8.8	72.2±6.6	77.7±8.8	83.3±6.6	16	90.7%
	2.5%	41.1±3.3	53.3±8.8	65.5±6.6	70±5.7	80±5.7	86.6±3.3	94.4±3.3	4	97.6%
	5%	54.4±3.3	66.6±3.3	73.3±6.6	74.4±6.6	85.5±8.8	93.3±6.6	100±0	1	99.4%
	10%	70±5.7	77.7±3.3	80±5.7	86.6±8.8	92.2±6.6	100±0	100±0	0	100%
Ethanol	1.25%	16.6±3.3	18.8±5.7	21.1±6.6	25.5±3.3	46.6±3.3	54.4±8.8	66.6±3.3	33	80.9%
	2.5%	37.7±3.3	43.3±6.6	52.2±5.7	57.7±4.4	64.4±6.6	75.5±3.3	83.3±6.6	18	89.5%
	5%	42.2±5.7	45.5±3.3	60±5.7	73.3±3.3	83.3±6.6	90±5.7	92.2±3.3	6	96.6%
	10%	63.3±8.8	73.3±6.6	76.6±6.6	80±5.7	90±5.7	97.7±3.3	100±0	1	99.4%
Significant differences between extracts	LSD (5%)	17.31	17.98	17.31	17.98	15.12	12.87	8.42		
	Sig.	***	***	***	***	***	***	***		
	Control	0±0							173	

* = significant difference, ** = high significant difference and *** = very high significant difference.

3.2. GC/MS analysis

The analysis by GC/MS Chromatography revealed that the chemical constituents of the petroleum ether fraction of the black pepper fruits, *Piper nigrum* L, were characterized and identified by comparing their peaks and mass spectra with those of their analogous reported by NIST and WILEY libraries and with the authentic spectra. The analysis comprised the determination of the peak name, the amount in percentage, the formula, the structures of the components and the classification into their classes. The results of the analysis showed that many functional groups and components were found and classified to a number of classes, Monoterpenes, Sesquiterpenes, Acetogenins (fatty acids or derivatives), alkaloids, and miscellaneous components. Terpenes are the primary components of many plant essential oils, which derived biosynthetically from units of isoprene and have a basic molecular formula of C_5H_8 and their multiples linked isoprene units $(C_5H_8)_n$. Monoterpenes composed of two isoprene units with a formula of $C_{10}H_{16}$, Sesquiterpenes consist of three isoprene units and the formula is $C_{15}H_{24}$, Diterpenes consist of four isoprene units and formula is $C_{20}H_{32}$ and Triterpenes contain six isoprene units with a formula $C_{30}H_{48}$.

The identification of the petroleum ether fraction of black pepper fruit extracts by using GC/MS analysis (Fig. 2), revealed that mainly twenty three peaks representing the main twenty three compounds found were characterized and classified into ten monoterpenes, six sesquiterpenes, five acetogenins (fatty derivatives), one alkaloid compound, and one miscellaneous compound, as recorded in Table (5). The mass spectra and percentages of major compounds of the main obtained constituents were α-pinene (1.5%), sabinene (3.21%), γ-terpinene (1.92%), 3-carene (4.89%), linalool (0.76%), carvone (0.5%), α-copaene (8.5%), α-zingiberene (13.36%), α-humulene (1.73%), α-elemene (3.94%), δ-cadinene (4.82%), aromadendrene (2.07%), linoleic acid (2.79%), docosane (2.92%) and piperin (2.71%), all data were shown in Table (5) and Figs. (3-17).

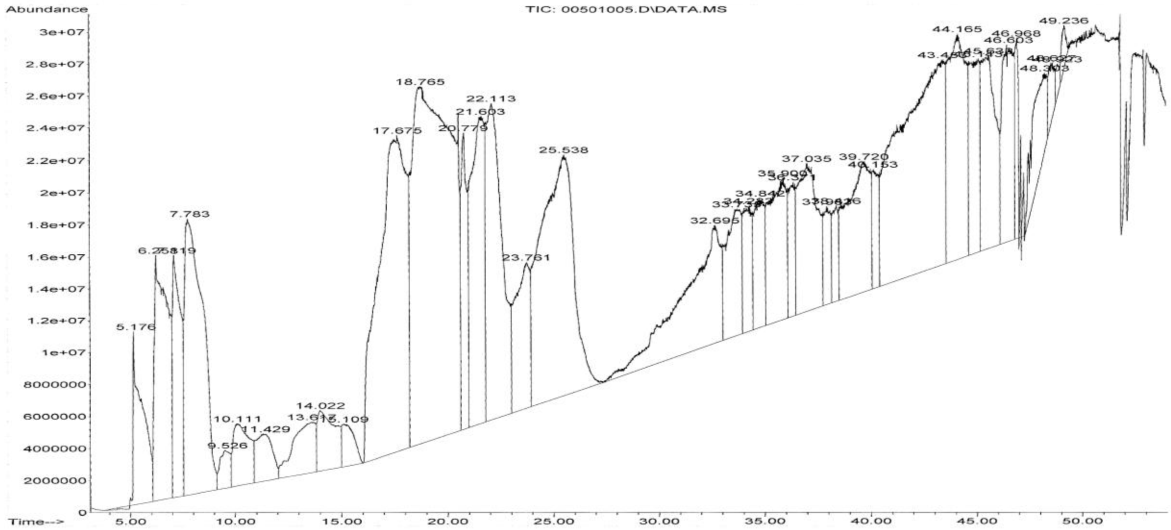

Fig. 2. GC/MS analysis of black pepper fruits extract.

Table 5. Main components identified by GC/MS analysis of petroleum ether black pepper fruits extract.

No.	Peak Name	Formula	M.W.	RT (min)	Area (%)
1	**(R)-α-pinene**	$C_{10}H_{16}$	136.2340	5.176	1.51
2	**Sabinene**	$C_{10}H_{16}$	136.2340	6.254	3.21
3	**γ-Terpinene**	$C_{10}H_{16}$	136.2340	7.120	1.92
4	**3-Carene**	$C_{10}H_{16}$	136.2340	7.782	4.89
5	**Terpinolene**	$C_{10}H_{16}$	136.2340	9.530	0.36
6	**2-Carene**	$C_{10}H_{16}$	136.2340	10.108	0.98
7	**Linalool**	$C_{10}H_{18}O$	154.2493	11.432	0.76
8	**trans-Sabinenhydrate**	$C_{10}H_{18}O$	154.2493	13.614	1.08
9	**trans-Dihydrocarvone**	$C_{10}H_{16}O$	152.2334	14.021	1.00
10	**Carvone**	$C_{10}H_{14}O$	150.2176	15.108	0.50
11	**α-Copaene**	$C_{15}H_{24}$	204.3511	17.671	8.50
12	**α-Zingiberene**	$C_{15}H_{24}$	204.3511	18.766	13.36
13	**α-Humulene**	$C_{15}H_{24}$	204.3511	20.778	1.73
14	**α-Elemene**	$C_{15}H_{24}$	204.3511	21.602	3.94
15	**δ-Cadinene**	$C_{15}H_{24}$	204.3511	22.111	4.82
16	**(+)-Aromadendrene**	$C_{15}H_{24}$	204.3511	23.758	2.07
17	**(2E,4Z)-N-Isobutyl-2,4-decadienamide**	$C_{14}H_{25}$	223.354	32.697	4.31
18	**1-Eicosene**	$C_{20}H_{40}$	280.5316	36.372	0.84
19	**Linoleic acid**	$C_{18}H_{32}O_2$	280.4455	37.034	2.79
20	**Eicosene**	$C_{20}H_{42}$	282.5475	37.985	0.66
21	**Docosane**	$C_{22}H_{46}$	310.6006	39.717	2.92
22	**Tricosane**	$C_{23}H_{48}$	324.6272	40.150	0.72
23	**Piperin**	$C_{17}H_{19}NO_3$	285.3377	45.634	2.71

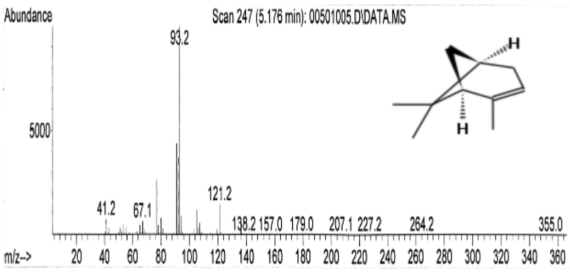

Fig. 3. Mass spectrum of (R)-α-pinene.

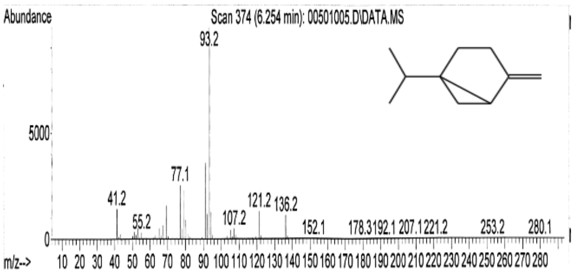

Fig. 4. Mass spectrum of sabinene.

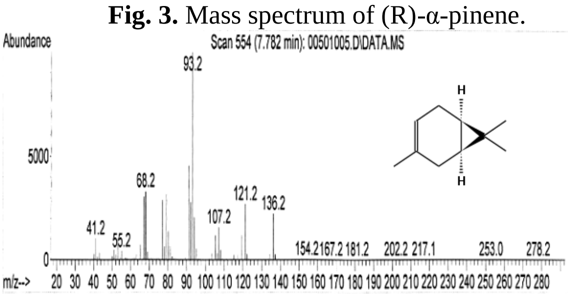

Fig. 5. Mass spectrum of γ-terpinene.

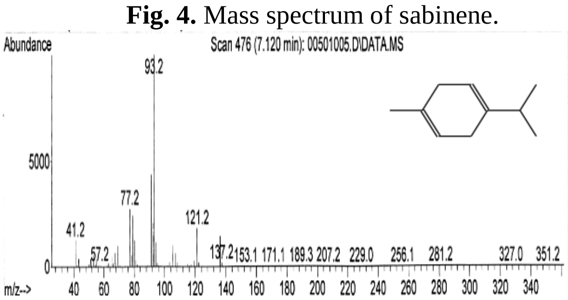

Fig. 6. Mass spectrum of 3-carene.

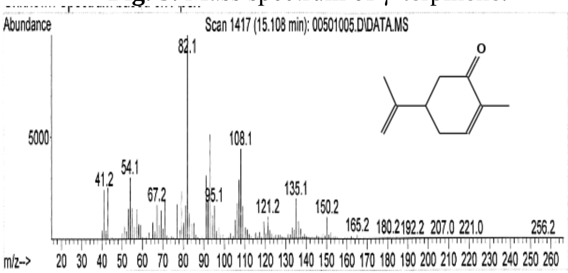

Fig. 7. Mass spectrum of linalool.

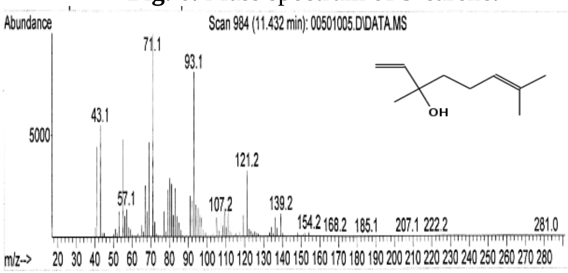

Fig. 8. Mass spectrum of carvone.

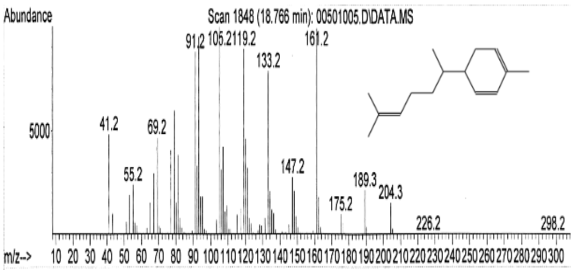

Fig. 9. Mass spectrum of α-copaene.

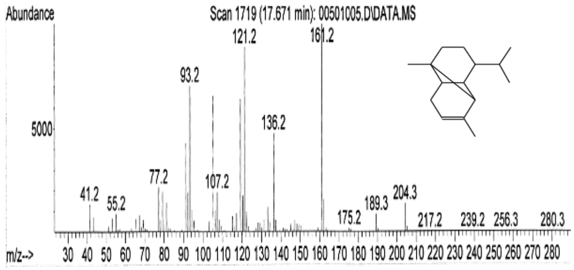

Fig. 10. Mass spectrum of α-zingiberene.

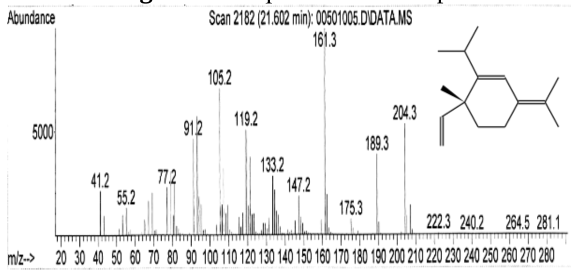

Fig. 11. Mass spectrum of α-humulene.

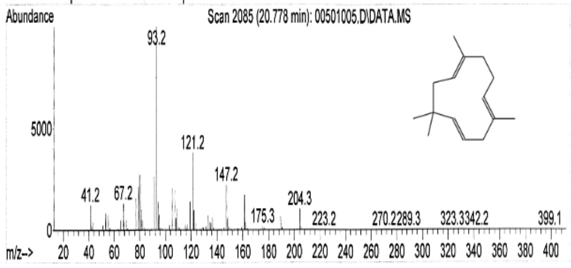

Fig. 12. Mass spectrum of α-elemene.

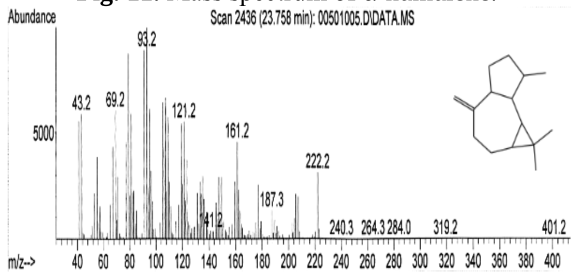

Fig. 13. Mass spectrum of δ-cadinene.

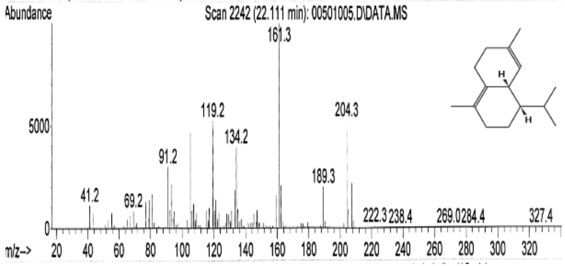

Fig. 14. Mass spectrum of (+)-aromadendrene.

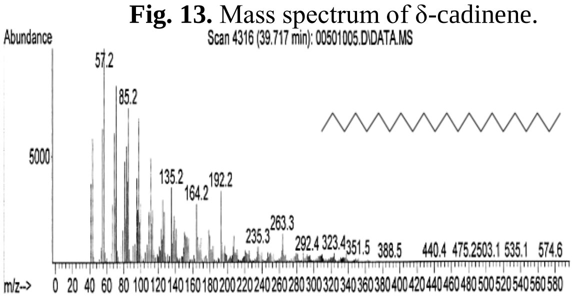

Fig. 15. Mass spectrum of linoleic acid.

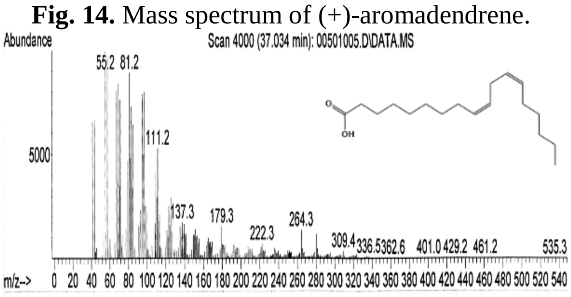

Fig. 16. Mass spectrum of docosane.

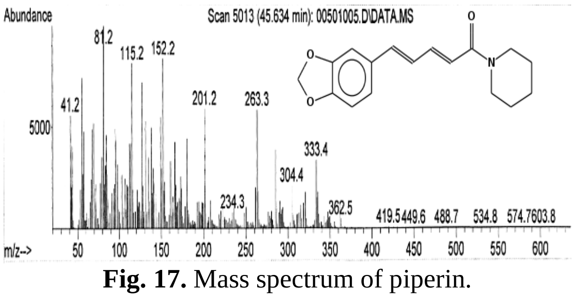

Fig. 17. Mass spectrum of piperin.

IV. Discussion

The mortality percents in this research showed that the increase of mortality was dependent on concentration and exposure time in all tests. The mortality results of the adult red flour beetle and adult rice weevil caused by treatment of petroleum ether extract were higher than that of other solvents, and the rice weevil was the most susceptible followed by the larvae, adult, eggs, and pupae of red flour beetle. The reduction in F_1 progeny was higher than the mortality especially in low concentrations. These high mortality results in most treatments were due to presence of insecticidal activities of some components in the oil, such as, α-pinene, linalool, carvone, α-copaene, linoleic acid and piperin.

The obtained results from this research were nearly agreed with those about the toxicity of garlic oil extract against the eggs, larvae and adults of *T. castaneum* and adults of *Sitophilus zeamais* **[26]**. They found that *T. castaneum* egg mortality increased with oil concentration, and the eggs were the most susceptible stage, followed by adults, and older larvae, while the adult red flour beetle was more susceptible to garlic oil than *S. zeamais* adults, and were failed to produce F_1 progeny.

Also acetonic extracts of black pepper, dill, and cumin against cowpea beetles were investigated, and revealed that the mortality, repellency, reduction in F_1 progeny, oviposition rates and hatchability rations were exhibited different levels of mortality and the black pepper was the most effectiveness **[27]**. The petroleum ether extract of cumin seeds and 99.8% N_2 were tested for their toxic effect against the rice weevil and red flour beetle, the results showed a high adult mortality values at the highest concentration 10% of cumin seed extract in 2 weeks after treatment, while very low mortality with *T. castaneum* and suppression in F_1 progeny was higher than mortality **[28]**.

The effect of petroleum ether extracts of black pepper fruits, dill seeds, and clove fruits alone and under phosphine treatment were studied against red flour beetle *T. castaneum*, and found a low adult mortality after 7 days of treatment with reduction in F_1 progeny higher than mortality percent, Whereas the toxicity effect of extracts under phosphine treatment was higher than for each extract alone **[29]**. The essential oil of *Piper nigrum* was investigated for its repellent, insecticidal and developmental inhibitory activities against adult and larvae of *T. castaneum*, and found that the mortality increased with concentration increasing, and the larval survival and adult emergence decreased with increasing the concentration **[30]**. The insecticidal activities of black pepper fruits powder and red pepper fruits powder were determined against *S. granaries*, the results showed the black pepper caused mortality higher than red pepper, and both extracts caused a complete reduction in F_1 progeny **[20]**.

The seed powder of black pepper fruits was studied to evaluate their effects on some biological aspects and mortality of *Trogoderma granarium*, and results showed that a high mortality, high reduction in F_1 progeny and high potential of repellency, and caused a prolonged period of F_1 adult emergence of the insect life cycle **[31]**. The insecticidal activity of essential oils were evaluated and identified by GC/MS of black pepper against adult *S. oryzae* and 3rd instars larvae of rice moth, and results revealed that the *S. oryzae* is the most susceptible **[19]**.

The obtained results of the present research of identification by using GC/MS analysis were in the same directions and greatly harmonized with those results of a research, they reported more than 80 components **[32]**. Also another agreement research, they studied 42 components of black Pepper extract **[33]**. The main compounds of the essential oils of black pepper fruits were demonstrated and identified, and the resulted constituents by GC/MS analysis revealed that the main components were germacrene D 11.01%, limonene 10.26%, β-pinene 10.02%, α-phellandrene 8.56%, β-caryophyllene 7.29%, α-pinene 6.40% and cis-β-ocimene 3.19% **[34]**.

The essential oils of *Piper nigrum* were analyzed and identified, and found thirty components which the main constituents were β-caryophyllene 23.49%, 3-carene 22.20%, D-limonene 18.68%, β-pinene 8.92% and α-pinene 4.03 %, Eugenol 7.39%, D-limonene 6.70%, Zingiberene 6.68% and Cubenol 3.64% **[35]**. The total percents of the essential oils of the two varieties of black pepper were estimated by using GC/MS and found that the total monoterpenes 65.421% and sesquiterpene 34.579% **[36]**.

V. Conclusion

According to obtained results, *P. nigrum* oils showed strong potential toxicity effects on two serious and notorious insect pests of stored grains, red flour beetle *T. castaneum* and rice weevil *S. oryzae*, and could use these oils to protect the stored grains, which are non-toxic in handling and use. So that the studied oils have a high potential to suppress, struggle, lethal and toxic effects on all developmental stages and causing a prolonged life cycles of these serious pests and considered a promising alternative source and safe natural insecticides or bio-insecticides.

References

[1] Satya, V.; Jindal, S. and Vir, S. 1996. Field infestation of *Caryedon serratus* Olivier (Coleoptera: Bruchidae) on the pods and seeds of *Acacia nilotica* in the Thar desert of India. Journal of Tropical Forest Science, 9(2): 189-193.

[2] Santos, J. P.; Maia, J. D. G. and Cruz, I. 1990. Damage to germination of seed corn caused by maize weevil (*Sitophilus zeamais*) and Angoumois grain moth (*Sitotroga cerealella*). *Pesquisa agropecuaria brasileira*, 25(12): 1687-1692.

[3] Matthews, G. A. 1993. Insecticide Application in the Stores. In Matthews, G.A. and Hislop, E.C. (eds.). Application Technology for Crop Protection. CAB, London, pp. 305-315.

[4] Ngamo, T. L. S.; Goudoum, A.; Ngassoum, M. B.; Mapongmetsen, P. M.; Lognay, G.; Malaisse, F. and Hance, T. 2007. Chronic toxicity of essential oils of 3 local aromatic plants towards *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae). Afr. J. Agri. Res., 2: 164-167.

[5] Chaubey, M. K. 2008. Fumigant toxicity of essential oils from some common spices against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). J. Oleo. Sci., 57: 171-179.

[6] Tooba, H.; Usman, N. F. and Abbas, T. 2005. Screening of plant leaves as grain protectants against *Tribolium castaneum* during storage. Pak. J. Bot., 37(1): 149-53.

[7] Iqbal, J.; Jilani, G. and Aslam, M. 2010. Growth inhibiting effects of plant extracts against the grain moth, *Sitotroga cerealella* (Oliv.) (Gelechiidae: Lepidoptera). Pak. J. Zool., 42(5): 597-601.

[8] Sarwar, M. and Sattar, M. 2012. Appraisal of different plant products against *Trogoderma granarium* Everts to protect stored wheat a laboratory comparison. Nucleus, 49(1): 65-69.

[9] Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51, 45-66.

[10] Tripathi, A. K.; Upadhyay, S.; Bhuiyan, M. and Bhattacharya, P. R. 2009. A review on the prospects of essential oils as biopesticides in insect pest-management. J. Pharmacognosy Phytotherapy, 1(15): 52-63.

[11] Sarwar, M.; Ashfaq, M.; Ahmad, A. and Randhawa, M. A. M. 2013. Assessing the potential of assorted plant powders on survival of caloglyphus grain mite (Acari: Acaridae) in wheat grain. Int. J. Agric. Sci. Bioresour. Eng. Res., 2(1): 1-6.

[12] Su, H. C. F. 1985. Laboratory study on effects of *Anethum graveolens* seeds on four species of stored product insects. J. Econ. Entomol., 78: 451-453.

[13] Halawa, Z. A.; EL-Sappagh, I. A. and EL-Assal, M. M. 2009. Toxicity of Toothpick Seed (*Ammi visnaga* L.) extracts against the lesser grain borer, *Rhizopertha dominica*, F. (Bostrychidae: Coloeoptera) Egypt. J. Agric. Res., 88(2): 485-493.

[14] Nawaz, A. 1999. Appraisal of weight losses in stored grains by *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae) and its mortality by plant extracts. M.Sc. (Hons) Thesis. Department of Entomology, NWFP Agricultural University, Peshawar. pp. 39.

[15] Semple, R. L. 1986. Problems relating to pest control and use of pesticides in grain storage: The current situation in ASEAN and future requirements. Pesticides and Humid Tropical Grain Storage System: ACIAR Proc., 45-47.

[16] Rees, D. P. 2004. (ed.): Insects of Stored Products. Manson Publishing, Ltd., UK.

[17] Beckett, S. J.; Fields, P. G. and Subramanyam, Bh. 2007. Disinfestation of Stored Products and Associated Structures using Heat. In: Heat Treatments for Postharvest Pest Control (Tang J., Mitcham E., Wang S. and Lurie S., eds.), CAB International, UK, pp. 182-237.

[18] Kostyukovsky, M.; Rafaeli, A. and Gileadi, C. 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. Pest Management, 58(11): 1101-106.

[19] Khani, M.; Awang, R. M. and Omar, D. 2012. Insecticidal effects of peppermint and black pepper essential oils against rice weevil, *Sitophilus oryzae* L. and rice moth, *Corcyra cephalonica* (St.). J. Med. Plants., 11(43): 97-110.

[20] Ashouri, Sh. and Shayesteh N. 2009. Insecticidal activities of black pepper and red pepper in powder form on adults of *Rhyzopertha dominica* (F.) and *Sitophilus granarius* (L.). Pak. Entomol., 31(2): 122-127.

[21] Abbott, W. J. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-276.

[22] El-Lakwah, F.A.; Darwish, A. A. and Halawa, Z. A. 1996. Toxic effect of extracts and powders of some plants against the cowpea beetle (*Callosobruchus maculatus*, F.). Annals-of-Agricultural-Science, Moshtohor, 34(4): 1849-1859.

[23] Duncan, D. B. 1951. A significance test for differences between ranked treatments in an analysis of variance. Virginia J. Sci., 2: 171-189.

[24] Finney, D. J. 1971. Probit analysis. A Statistical Treatment of The Sigmoid Response Curve. 7th Ed., Cambridge Univ. Press, England.

[25] Adams, R. P. 1995. Textbook of identification of essential oil components gas chromatography/mass spectroscopy. 2nd ed. 46: 1460.

[26] Ho, S. H.; Koh, L.; Ma, Y.; Huang, Y. and 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(1): 41-48.

[27] El-Lakwah F. A. and Mohamed R. A. 1998. Toxicity effect of some plant extracts against the cowpea beetle, *Callosobruchus maculatus* (F.). Annals of Agric. Sc., Moshtohor, 36(4): 2627-2635.

[28] El-Lakwah, F. A.; Mohamed, R. A. and Abd El-Aziz, A. E. 2001. Toxicity and Joint Action of Cumin Seeds Extract with Certain Controlled Atmospheres against Stored product Insects. Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Fresno, CA. 29 Oct. - 3 Nov. 2000, Executive Print. Serv., Clovis, CA, U.S.A. pp. 133-147.

[29] Mohamed, R. A. 2001. Efficacy of some plant extracts alone and under phosphine treatment against the red flour beetle *Tribolium castaneum* (Herbst). Annals of Agric. Sc., Moshtohor, 39(3): 1767-1777.

[30] Upadhyay, R. K. and Jaiswal, G. 2007. Evaluation of biological activities of *Piper nigrum* oil against *Tribolium castaneum.* Bulletin of Insectology, 60(1): 57-61. ISSN 1721-8861.

[31] Mhemed, A. J. 2011. The Efficacy of four seed powders on some biological aspects and mortality of Khapra beetle. Iraqi Journal of Agricultural Sciences, 42(6): 112-123.

[32] Gopalakrishnan, M.; Menon, N.; Padmakumari, K. P.; Jayalakshmi, A. and Narayanan, C. S. 1993. GC analysis and odor profiles of four new Indian Genotypes of *Piper nigrum* L. Journal of Essential Oil Research, 5: 247-253.

[33] Zachariah, J. 1995. Essential oil and its major constituents in selected black pepper accessions. Plant Physiology and Biochemistry, New Delhi, 22(2): 151-153.

[34] Jirovetza, L.; Buchbauera, G.; Ngassoumb, M. B. and Geisslerc, M. 2002. Aroma compound analysis of *Piper nigrum* and *Piper guineense* essential oils from Cameroon using solid-phase microextraction–gas chromatography, solid-phase microextraction–gas chromatography-mass spectrometry and olfactometry. Journal of Chromatography A, (976): 265-275.

[35] Liu, L.; Song, G. and Hu, Y. 2007. GC–MS Analysis of the Essential Oils of *Piper nigrum* L. and *Piper longum* L. Chromatographia, 66: (9/10):785-790.

[36] Aziz, S.; Naher, Sh.; Abukawsar, M. D. and Roy, S. K. 2012. Comparative studies on physicochemical properties and gc-ms analysis of essential oil of the two varieties of the black pepper (*Piper nigrum* Linn.). Int. J. Pharm. Phytopharmacol. Res., 2(2): 67-70.