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Bioactivity of plant extracts against two stored product insects and use of chromatography and infrared analyses for defining the toxic compounds

Abo Arab, R.B.; G.M.M. Zayed and Abeer, A. Salm
Plant Protection Res. Inst., Agric. Res. Center, Dokki, Giza, Egypt

Abstract

Among five tested plant extracts namely, *Ammi majos, artemesia herba alba, Chenopodium murale, Eugenia aromatica* and *Pimpinella anisum,* two species proved great activity against the experimental pests *Callosobruchus maculatus* (F.) and *Sitophilus oryzae* (L.). These two plant species were *Pimpinella anisum* and *Eugenia aromatica*. Extraction was carried out by using N-hexane and methanol. Synthetic chemical insecticide, pirimiphos-methyl was used as a standard reference against stored product insects. Pirimiphos methyl showed the highest toxicity and percent reduction of progeny for the two tested insect species. Also, *Pimpinella anisum* and *Eugenia aromatica* achieved high detrimental effect on offspring of the two tested insects. Results obtained exhibited some different effects between the two selected plant extracts on the tested insects. Therefore, chemical analyses for the composition of the two selected extracts were achieved by Thin Layer Chromatography (T.L.C.) and infrared spectra. Phytochemical constituents differed according to the solvent used and the plant species. Sterols, triterpenes, carbohydrates, and glycosides were found in high amount of ethanol extracts of *E. aromatica* and *P. anisum*. Tannins, ferric chlorides and flavonoids were found in trace amount of n-hexane extracts of *E. aromatica* and *P. anisum*. It could be concluded that, sterols and triterpenes, saponins, alkaloids, and glycosides are responsible for bioactivity of the studied plants. T.L.C. analysis revealed the occurrence of two fractions of *P. ansimum* of n-hexane and five fractions of ethanol, for the same plant, four fractions of *E. aromatica* with ethanol and three of n-hexane extracts. Results of infrared spectroscopy explain 14 curves with different peaks carrying special wavelength indicating the active phytochemical groups in each curve.

Key words: *Ammi majos , artemesia herba alba , Chenopodium murale , Eugenia aromatic*, Chromatography (T.L.C.)

Introduction

The stored products represent good media for most species of stored product insects. The use of synthetic pesticides in stored products and crop protection resulted in potential hazards for mammals, disturbance of the environment, pest resistance to pesticides and lethal effects on non-target organisms, agroecosystems in addition to direct toxicity to users (**Prakash and Rao, 1987**). Many insects are unable to infest certain plants because of the presence of particular noxious substances (**Fraenkel, 1969**). The use of different plant and mineral oils as protectants for stored grain against insect infestation is an incident method (**Yuntal and Burkholder, 1981; Sukari *et al*., 1992 and Khani *et al*., 2011**). Vegetable oils protect stored legumes and grains from bruchids and weevils attack for long periods of storage (**Schoonhoven, 1978, Shay and Ikan, 1980, Singh *et al*., 1978 and Pereira, 1983**). Large numbers of plants have been screened for their biologically active chemicals and showed a good degree of success as protectants against a number of stored grain insect pests (**Gill and Lewis, 1971, Pandy *et al*., 1986, Jilani *et al*., 1988, Mahgoub and Ahmed, 1996), and Hostettmann, 1999 and Rajalakshmi and Senthil, 2009)**.

In the present study, experiments of comparative toxicity and effects on emergence of progeny of *Sitophilus oryzae* (L.) and *Callosobruchus maculatus* (F.) adults were conducted to evaluate ethanol and hexane extracts of five certain plants. Among the tested plants, the two species, *Pimpinella anisum* and *Eugenia aromatica* were chemically analyzed,therefore some chemical studied include preliminary screening, separation and identification of phytochemical constituents of tested plants, thin layer chromatography (T.L.C.) and infra-red spectra were carried out.

Materials and methods

Insects used:

In the present study, the tested cowpea weevil [*Callosobruchus maculatus* (F.)] and rice weevil [*Sitophilus oryzae* (L.)] were reared for some years at 30 $\pm$ 2^{o}C and 70 $\pm$ 5% R.H., in the Department of Stored Products Pests, Plant Protection Research Institute, Sakha Agricultural Research Station. The cultures were maintained under the same conditions in glass jars each containing 100-200 unsexed adults and 300 g of cowpea or wheat grain seeds. The mouths of jars were covered with muslin, kept in position with rubber bands. The seeds used for both insect cultures and experiments were previously sterilized, to kill any of insect stages, by drying at 50^{o}C for 6 hours. The culture medium was sieved to

collect the beetles and the insects that emerged for experiments.

Preparation of crude extract:

Dried plant powders (Wild mint, *Ammi majos;* Warm wood, *Artemesia herba alba;* Fisseih,*Chenopodium murale;* Clove,*Eugenia aromatica;* Anise, *Pimpinella anisum)* were extracted according to Freedman *et al*. (1979), 250 g of each plant sample was separately soaked in 750 ml of polar solvent (ethyl alcohol) and non-polar solvent (n-hexane) in a large conical flask for 72 hours with shaking for three hours. The contents of the flask were filtered through anhydrous sodium sulphates. The extracts were concentrated by removing the solvent on water bath at 40^{o}C to obtain the crude extract. The obtained extracts were weighed and dissolved in an appropriate volume of pure acetone, and kept in the refrigerator till they were assayed.

The pesticide used:

The organophosphate, pirimiphos-methyl (actellic): O-2-diethyl amin-6-methyl-pyrimidin-4-ylo-O, O dimethyl phosphorothioate, 50% emulsifiable concentrate (EC), was provided by ICI plant protection division agrochemicals.

Bioassay methods:

Seed treatment (mixing with media) method was carried out, where the considerable concentrations (w/v) of each tested material (extracts or chemical insecticide) were diluted with acetone in small glass jars 20 g of seeds was placed. One ml of each concentration was placed in each jar above the surface of seeds using a micropipette. The jar was shaken by hand to mix the grain with extract. The treated seeds were left on jars for a convenient time until the solvent evaporated, each concentration was replicated three times. The jars left without such treatment served as control. Ten pairs of newly emerged adults of *C. maculatus* or *S. oryzae* were transferred to each jar, covered with muslin cloth and kept under laboratory conditions. LC_{50} and LC_{90} values (after 5 days of treatment) were used and the insects were allowed to complete their life cycle to determine the reduction of progeny by the following equation:

$$\text{Percent reduction of progeny} = \frac{\text{MAEC- MAET}}{\text{MAEC}} x100$$

Where: MAEC= Mean number of adults emerged in control and MAET= Mean number of adults emerged in treated.

Emerging adults were counted for five weeks beginning from the first emergence

Preliminary screening on the phytochemical constituents in studied bioactive plant extracts:

The chemical analysis (by chromatograph gas and infrared analysis) was done at the laboratories of Faculty of Agriculture, Cairo University

Sterols and triterpenes:

Sterols and triterpenes were determined according to the method of **Wall *et al*. (1964)**. Some drops of chloroform were added to one ml of plant extract, then few drops of sulphuric acid were added. When the resulting yellow colour changes into red, this means the presence of sterols and triterpenes.

Phenolic glycosides:

Balbaa (1981) determined phenolic glycosides by the following procedure: some drops of sulphuric acid were added to 1 ml of plant extract. A red colour was produced which disappeared on the addition of distilled water.

Alkaloids:

Alkaloids were estimated by the method described by **Romo (1966)**. One ml from the extract was rendered slightly. 2 ml diluted hydrochloric acid was added. 1 ml of the solution in the test tube was transferred. About five drops of iodine potassium iodide (Wagner's reagent) was added, shacked after the addition of each drop, or 2 drops of sulfuric acid. After some time, a precipitate is formed indicating the presence of alkaloids.

Flavonoids:

Solution of sodium hydroxide (10%) was added to ten ml of crude extract until the appearance of a yellow colour. This yellow colour indicates the probable presence of flavonoids (**Claus, 1961)**.

Saponin glycosides:

Saponin glycosides were calculated according to the method mentioned by **Wall *et al*. (1964)**. Haemolylic effect on red blood corpuscles: place 5 ml of 5% suspension of red blood corpuscles on normal saline solution into each of two tubes. To one test tube solution, add 5 ml of normal saline solution, to the other tube, add 5 ml of plant extract in which 0.05 g sodium chloride has been previously dissolved to render it isotonic with normal saline solution. Shake gently each of the two tubes. The liquid in the first tube will remain opaque i.e. contain a suspension of the red blood corpuscles. In the second tube, a clear red liquid will be haemolyzed indicating the presence of saponin glycosides.

Tannins test:

Tannins were tested by the method described by **Claus (1961)**. A ferric chloride solution 5% was added to the plant extract, drop by drop and the produced colour was observed. Condensed tannins (catechol or chloroglucinol) usually give a green

colour, while hydrolyzable tannins (Pyrogallol) give a blue black colour.

Carbohydrates and/or glycosides:

Karawya *et al*. (1975) determined carbohydrates and/or glycosides by the following technique: one g of each sample was completely extracted with alcohol. About 5 ml of each alcoholic extract was mixed with 0.5 ml alcoholic α-naphthaol (20% w/v) followed by 5 ml concentrated sulphuric acid, poured carefully on the walls of the test tube to form a lower layer below the alcohol i.e. layer. The formation of a bluish violet zone at the junction of the two layers indicates the presence of carbohydrates and/or glycosides.

Separation and identification of n-hexane and ethanol crude extracts of *Pimpinella anisum* and *Eugenia aromalica*:

Thin layer Chromatography

A layer of Silica Gel GF 254 of 1.5 mm thickness was spread on 20 x 20 cm glass plates. Fifteen grams silica in about 30 ml water was prepared and spread over the plate with applicator. The plates were allowed to stand for 2 hours at room temperature then activated in an oven for two hrs at 100°C. Marks were made near the edge of each plate at a distance of 2 cm to define the spoling line. The n-hexane and ethanol crude extracts of *Pimpinella anisum* and *Eugenia aromatica* were developed on TLC plates using different solvent systems such as: chloroform: methanol (90: 10), chloroform: methanol (85: 15), chloroform: methanol (75: 25) and chloroform: methanol: water (65: 25: 4) to determine the suitable solvent system. The developed solvent system poured in jar chamber to depth of 10 mm and the spotted plates were placed in the chamber, so that the bottom edge was in contact with the solvent, and the lid was then replaced. When the solvent was developed to high of 15 cm, the plates were removed and the solvent was allowed to evaporate.

Fractionation of n-hexane and ethanol crude extracts of *Pimpinella anisum* and *Eugenia aromatica*:

About 0.4 g of crude extract was applied to the plate in the form of a line at a distance of about 2 cm from the lower edge of the plate. The application was done using a micropipette by gently moving its end on the plate in the form of straight line. Four plates were exposed each one separately to one of U.V spectrophotometer to visualize the bands of separated fractions and to determine their RF values.

Spectroscopic analysis

The infrared absorption spectrum of the isolated fractions dissolved in methanol was carried out. Infrared namely, FTIR UNIT BRUKER-Vectro 22 was used for this purpose. Interpretation of IR spectra was performed according to **Lambert *et al*. (1976)**.

Methods of statistical analysis

Statistical analysis were used as the method of Duncan multiple range test (**Duncan, 1955**).

Results and discussion

1. *Callosobruchus maculatus*

1.1. Toxicity

Data obtained in Table (1) showed that based on LC_{50} and LC_{90} values *P. anisum* and *E. aromatica* were the strongest plant extracts against *C. maculatus* with LC_{50} and LC_{90} values of 6.8 & 8.7 an 15.3 & 40.5 and 14.9 & 6.5 and 52.5 & 37.2 mg/kg media for ethanol and N-hexane solvents.

1.2. Reduction of progeny

Data summarized in Table (1) revealed that ethanol *P. anisum* and *E. aromatica* plant extracts had the strongest adversal action on adult offsprings among the used extracts, since they reduced the emerging progeny of *C. maculatus* at LC_{50} level to 50.6 and 48.5%, respectively when compared with the control. The two extracts had an effect equal statistically to that of pirimiphos-methyl, where there is no significant difference between their effects and that of pirimiphos-methyl. *C. murale* and *A. majos* had the lowest effects on progeny of *C. maculatus* at LC_{50} level. *A. herba alba* had the second grade among the used ethanol extracts with 42.6% reduction of progeny. At LC_{90} level the ethanol extracts had the same order with LC_{50} level. At LC_{90} level, there were significant differences between control and the ethanol extracts. Plant extracts with n-hexane at LC_{50} and LC_{90} levels caused significant reductions in progeny of *C. maculatus* when compared with the control. *E. aromatica* was the best extract if compared with pirimiphos-methyl. Based on the reduction percentage of progeny of *C. maculatus*, n-hexane extracts at LC_{50} level had the following descending order, *E. aromatica, P. anisum, A. herba alba, A. majos and C. murale* while at LC_{90} level the descending order was as follows: *E. aromatica, P. anisum, A. herba alba, A. majos* and *C. murale.* Results in Table (1) showed that, when the concentration increased the emerged progeny decreased. Also, results showed that n-hexane plant extracts had often lower effects than those of the ethanol extract at LC_{50} and LC_{90} levels. The different effects between n-hexane and ethanol extracts may be due to the type and the amount of extracted chemical compositions influenced by the solvent.

Table 1. Comparative toxicity and reduction of progeny (F_1) of *C. maculatus* adults from cowpea seeds treated with LC_{50} and LC_{90} values after five days of treatments.

Tested materials	Ethanol treatment					
	LC_{50} mg/kg	Mean adult emerged	% reduction	LC_{90}	Mean adult emerged	% Reduction
Ammi majos	26.5	143.3 bc	40.7	105.8	68.3 b	72.0
A. herba alba	16.6	139.3 c	42.6	61.7	43.3 c	82.0
C. murale	31.5	154.6 b	36.3	112.2	74.7 b	68.6
E. aromatica	8.7	123.3 d	48.5	40.5	32.3 d	86.6
P. anisum	6.8	119.0 d	50.6	15.3	18.0 e	91.6
Pirimiphos-methyl	0.0021	112.7 d	53.6	0.0044	8.0 f	96.6
Control	-	243.09 a	--	-	243.09 a	
	N-hexan treatment					
Ammi majos	25.3	150.0 c	38.3	83.4	71.7 c	70.5
A. herba alba	19.5	133.3 d	44.5	89.8	31.7 d	86.6
C. murale	37.8	165.0 b	32.5	125.4	106.7 b	56.3
E. aromatica	6.5	122.7 de	49.6	37.2	15.3 e	93.6
P. anisum	14.9	128.3 d	46.7	52.3	30.0 d	87.6
Pirimiphos-methyl	0.0021	112.7 e	53.6	0.0044	8.0 e	96.6
Control	-	243.09 a			243.09 a	

2. *Sitophilus oryzae*

2.1. Toxicity

Data in Table (2) showed that *P. anisum* and *E. aromatica* extracts by ethanol or n-hexane had the highest toxic activity compared to the remained extracts.

2.2. Reduction of progeny

Results obtained in Table (2) indicated that exposure of *S. oryzae* adults to wheat grains treated with concentrations of LC_{50} and LC_{90} after 5 days of treatments of ethanol extracts produced a significant reduction of progeny in comparison with the control treatment. According to the reduction percentage of progeny of *S. oryzae*, ethanol plant extracts at both concentrations had the same order with that of *C. maculatus*. Also, according to mentioned criterion, n-hexane extracts at LC_{50} and LC_{90} levels had the same order. The different effects on progeny of *S. oryzae* produced by ethanol extracts or n-hexane may be due to the isolated chemical composition of the different plants by a solvent and also to their different concentrations.

Plant materials have repellent, toxic and antifeeding effects which have been identified in large number of plant species (**Fraenkel, 1969 and Jilani and Malek, 1973).** It is noteworthy that the percent of reduction increased with the increasing level of concentrations.

Table 2. Comparative toxicity and reduction of progeny (F_1) of *S. oryyzae* adults from cowpea seeds treated with LC_{50} and LC_{90} values after five days of treatments.

Tested materials	Ethanol treatment					
	LC_{50} mg/kg	Mean adult emerged	% R	LC_{90}	Mean adult emerged	% R
Ammi majos	21.75	60.7 b	41.3	75.11	21.0 c	78.5
Artemesia herba alba	13.55	56.0 c	45.6	62.8	17.7 cd	83.0
Chenopodium murale	28.86	62.7 b	39.4	99.24	31.7 b	69.0
Eugenia aromatica	6.95	55.0 c	46.6	43.27	11.7 d	88.5
Pimpinella anisum	5.29	52.0 d	50.5	15.20	1.67 e	98.4
Pirimiphos-methyl	0.0012	51.0 d	50.6	0.0051	0.01 e	100
Control		103.3 a			103.3 a	-
			N-hexan treatment			
Ammi majos	33.5	63.3 bc	38.5	103.4	31.3 ab	69.8
Artemesia herba alba	13.4	62.0 b	40.0	47.87	22.0 ab	78.6
Chenopodium murale	26.2	66.3 b	36.6	106.8	36.7 ab	64.5
Eugenia aromatica	3.52	53.3 cd	48.3	19.8	4.3 b	96.0
Pimpinella anisum	12.0	58.3 bcd	43.6	44.7	18.3 ab	82.3
Pirimiphos-methyl	0.0012	51.0 d	50.6	0.0051	0.01 b	100
Control		103.3 a			103.3 a	

The abovementioned findings are in agreement with those of **Bhaduri *et al*. (1985)** who found that extracts of *Ipomoea carnea* (leaves), *Parthenium hysterophorus* (whole plant), *Tridax procumebens* (flowers) and *Embellia ribes* (seeds) were significantly effective in reducing the progeny of *C. maculatus*.

Petroleum ether and methanol extracts of aerial parts of *Lantana camara* at 5% concentration caused complete feeding deterrent action and reduction of progeny of *C. maculatus* (**Saxena *et al*., 1992)**. Similar remarkable reduction in the average number of progeny emerged from wheat grains and cowpea seeds treated with LC_{25}, LC_{50}, LC_{75} of *Petreselinum sativum* oil while no adult emergence was detected at LC_{90} (**Mahgoub *et al*., 1998**). However, **Makanjuola (1989)** indicated that all extracts of neem leaves and seeds significantly reduced adult emergence of *C. maculatus* on treated cowpeas. **Ferial (1985)**, reported that citrus oil of navel orange, sweet orange and grapefruit at rates of 0.25, 0.50, 0.75, 1.00% significantly reduced the total emerging adults of *Callosobruchus maculates* emerged (F_1 progeny) compared to untreated seeds (control).

In this respect, **Rahuman *et al*. (2008)** isolated and identified mosquito larvicidal compound from petroleum ether extract of *Abutilon indicum* (Linn.) sweet. **Khani *et al*. (2011)** tested the chemical composition of extracts from black pepper, *Piper nigrum* L. and physic nut, *Jatropha curcas* L. against rice weevil, *S. oryzae* under laboratory conditions. The chemical composition of the extracts was identified by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The major extracted components of *P. nigrum* were piperine (74.34%) and caryophyllene (18.53%), and for *J. curcas* were oleic acid (40.67%, linoleic acid (34.17%) and plasmatic acid (18.03%). They found that the mortality of adults increased with increasing concentration of extracts also. They concluded that the tested extracts had repellent and antifeedant activities against *S. oryzae* adults. Furthermore, F_1 adults were suppressed at the lowest concentration (2µl/g) and no F_1 progeny was produced in all treatments.

It is well known that chemical pesticides have the property of poisoning even at low concentrations. Therefore, the chemical insecticide pirimiphos-methyl had the highest adversal effect against the two tested insects in this study. These results are in good agreement with those of **Jotwani and Sircar (1965), Ivbijaro (1983), Pereira (1983), Akou Edi (1984), Benjilali *et al*. (1984), Alzouma and Boubacar (1987), Arnason *et al*. (1989), Jacobson (1989), Morallo-Rejesus *et al*. (1990), Shaaya *et al*. (1991), El-Aidy and Helal (1997), Helal (1998), and Abo Arab *et al*. (1998).** In this respect, **Guirguis *et al*. (1991)** who studied the toxic action of 12 citrus oils extracted from peels of citrus fruits and one conventional insecticide pirimiphos-methyl to adults of *S. oryzae* using residue thin film technique. They found that pirimphos-methyl was the most toxic compound in comparison with tested citrus oils. Although, pirimiphos-methyl had a quick effect with low concentrations, but it has many disadvantages, since it pollutes the environment, disorganizes the ecosystem and develops the resistance of the treated insect pests. In contrast, plant materials have many advantages, because they are relatively safe on humans and environment and they are available with low cost.

3. Preliminary screening, separation and identification of phytochemical constituents of tested plants

Data concerning the preliminary screening, separation and identification of phytochemical components of n-hexane and ethanol extracts of the *E. aromatica* and *P. anisum* are presented in Table (3). Results show the important role of plant species and parts used, as well as the solvent of extraction in determining the phytochemical constituents of the tested plant extracts. In general, sterols, triterpenes, saponins, alkaloids, carbohydrates and glycosides were found in high amounts of ethanol extract of *E. aromatica, P. anisum* and n-hexane extract of *E. aromatica*, followed by tannins, ferric chloride and phenolic glycosides of n-hexane extract of *E. aromatica* and ethanol extract of *P. anisum*. Tannins, ferric chloride and flavonoids were found in trace amounts of n-hexane extracts of *E. aromatica* and *P. anisum*. These data indicate that sterols and triterpenes, saponins, alkaloids and glycosides may be responsible for bioactivity on the studied pests. **Mahajan *et al*. (1985)** indicated that out of the 28 phenolic compounds tested, there was a greatest nematicidal activity against *Meloidogyne. incognita* due to transcinnamic acid followed by pyrogallol, naphthoic acid and ethyl gallote. **Rahuman *et al*. (2008)** isolated and identified mosquito larvicidal compound from petroleum ether extract of *Abutilon indicum* (Linn.) sweet. They identified chemical composition of black pepper, *Piper nigrum* L. and physic nut, *Jatropha curcas* L by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The major extracted components of *P. nigrum* were piperine (74.34%) and caryophellene (18.53%) and for *J. curcas* were oleic acid (40.67%, linoleic acid (34.17%) and plasmatic acid (18.03%) (**Khani *et al*., 2011**).

Sukul (1994) indicated that the effective of pure compounds obtained from plants are glycosides, quinones, unsaturated and saturated hydrocarbons, heterocyclic compounds, organic acids, aromatic compounds, esters, sulphur compounds and terpenes. Among the terpenes, monoterpenes showed strong pest properties.

4. Thin layer chromatography (T.L.C.)

T.L.C. results of crude constituents of ethanol and n-hexane *E. aromatica* and *P. anisum* are shown in Table (4) and Figure (1) from which the following results can be detected:

Table 3. Preliminary phytochemical screening of ethanol and n-hexane crude extracts of *Eugenia aromatica* and *Pimpinella anisum*.

Constituents	Ethanol extracts		n -hexane extracts	
	E. aromatica	*P. anisum*	*E. aromatica*	*P. anisum*
Sterols and triterpens	+++	+	++	++
Tannins	+	++	-	+
Ferric chloride	+	++	-	+
Phenolic glycosids	+	++	++	+
Saponins	+++	++++	+	-
Alkaloids	++	+++	++++	++
Flavonoids	+	+	-	+
Carbohydrates and/or glycosids	++	+++	+++	+++

Table 4. Rf values of the ethanol and n-hexane crude constituents isolated from *Eugenia aromatica* and *Pimpinella anisum* with different solvent systems.

Isolated fractions	Retard fatctor values of Ethanol		Retard factor values of n-hexane	
	E. aromatica 90: 10 Ch: M	*P. anisum* 90: 10 Ch: M	*E. aromatica* 65: 25: 4 Ch: M: W	*P. anisum* 65: 25: 4 Ch: M: W
F_1	0.221	0.107	0.214	0.321
F_2	0.379	0.214	0.643	0.786
F_3	0.552	0.429	0.857	-
F_4	0.862	0.679	-	-
F_5	-	0.964	-	-

Ch: Chloroform M: Methanol W: Water F = Fraction

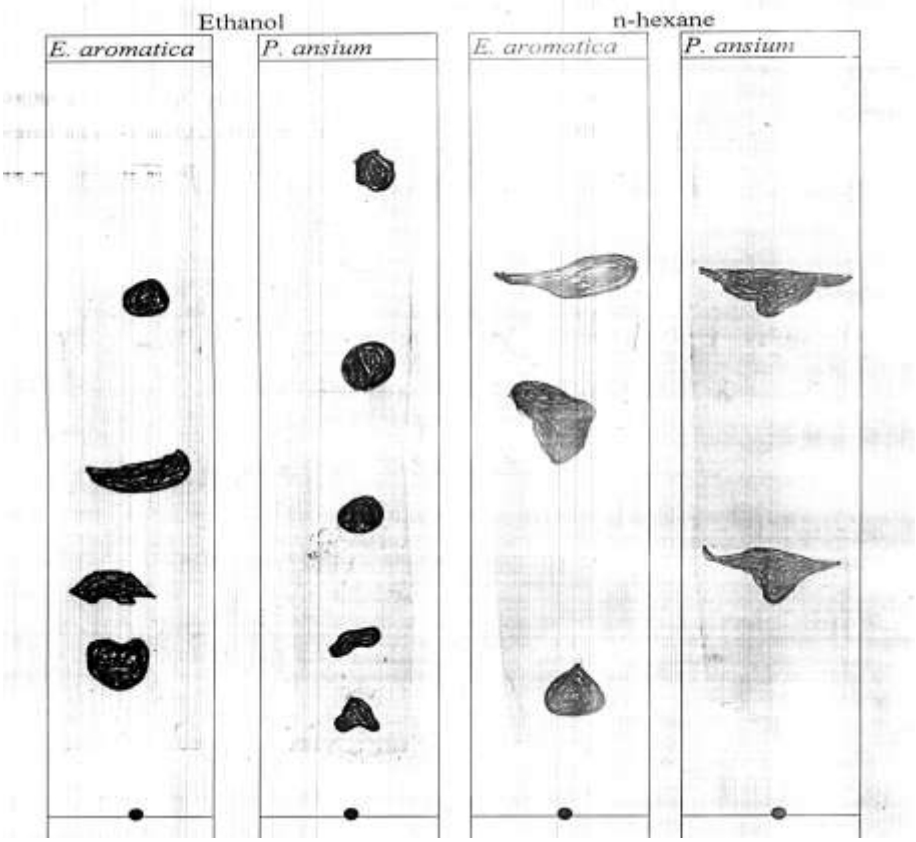

Fig. (1): Silica gel T.L.C. of ethanol and n-hexane crude extracts of *E. aromatica* and *P. anisum* plate (20 x 20 cm) silica gel using migrated chloroform: methanol and chloroform: methanol: water (90:10) and (65:25:4) as solvent system.

The solvent system chloroform: methanol: water (65:25:4) showed the presence of three fractions with FR values of 0.214, 0.643 and 0.857 with n-hexane extracts of *E. aromatica*, where the same solvent system showed the presence of two fractions at RF

values of 0.321 and 0.786 in case of n-hexane extracts of *P. anisum*.

In the solvent system of chloroform: methanol (90: 10), 4 spots were indicated with Rf values of 0.221, 0.379, 0.552 and 0.862 in case of ethanol extract of *E. aromatica*. Also, it gave five similar constituents with 0.107, 0.214, 0.429, 0.679 and 0.964 Rf values in the same solvent system ethanol extract of *P. anisum*. The aforementioned results indicated that Rf value of fraction which possessed low Rf values increased with methanol increasing in the solvent system. **Conley (1972)** reported that the compounds could be separated according to their polarity, less polar compounds having higher Rf values and more polar compounds having smaller Rf values.

5.Infrared spectroscopy (I.R.S)

The infrared spectral analysis shown in Table (5) and Figs. (2-15) for the purified compounds according to **Alpert *et al.* (1970) and Silverstein *et al.* (1974)** could be represented as follows:

1- Broad bands associated O.H stretching vibrations and intramolecular hydrogen bonding of OH groups appeared in 3600-3500 cm^{-1} region. On the other hand, free OH stretching vibration bands are observed at 3635-3610 cm^{-1} region.,
2- The C-H stretching in 3000-2800 cm^{-1} region to indicate the presence of the polycyclic six membered rings. These bands assigned to R-Co-NH_2 bands in 1650-1700 cm^{-1} region. On the other hand, C = N stretching vibration bands are observed at 1680-1630 cm^{-1} region because of carbon bonding in the compound.
3- Strong sharp bands at 1650-1600 cm^{-1} region and the band at 1630-1550 cm^{-1} showed that both-O-NO_2 and -N-NO_2 group may be present. On the other hand, -C-NO_2 bands are observed at 1570-1500 cm^{-1} region because of nitrogen bonding in the compound.

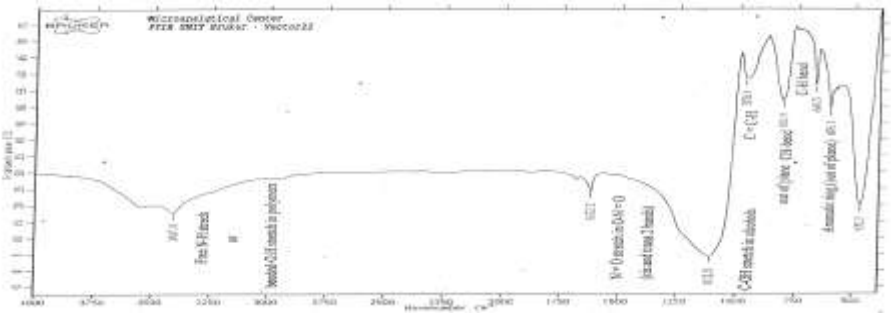

Fig. (2): Infrared spectrum of fraction 1 of ethanol extract of *E. aromatica*.

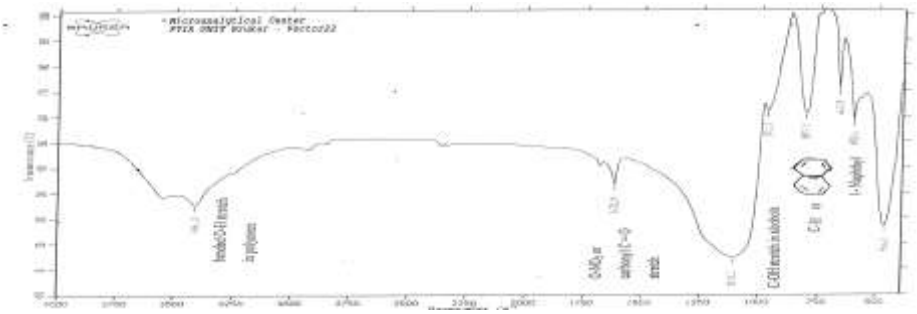

Fig. (3): Infrared spectrum of fraction 2 of ethanol extract of *E. aromatica*.

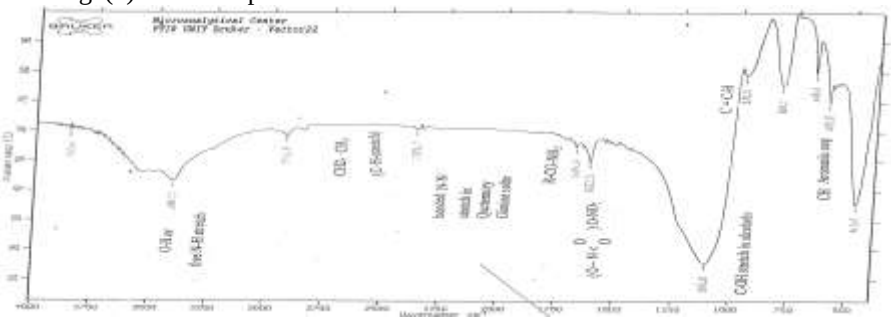

Fig. (4): Infrared spectrum of fraction 3 of ethanol extract of *E. aromatica*.

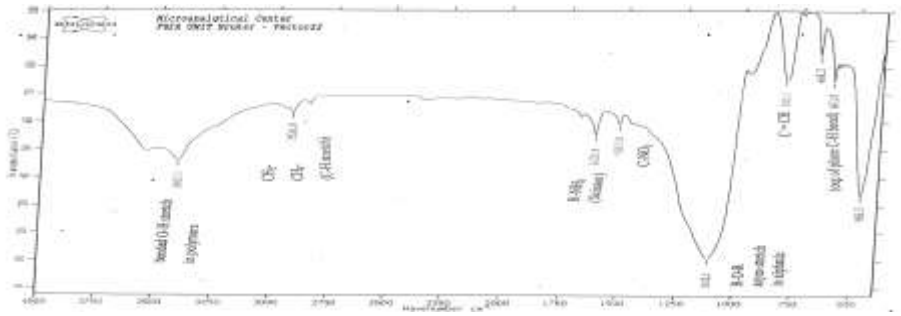

Fig. (5): Infrared spectrum of fraction 4 of ethanol extract of *E. aromatica*.

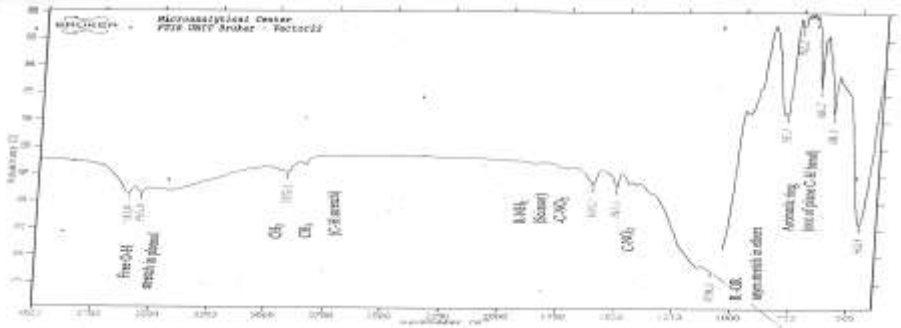

Fig. (6): Infrared spectrum of fraction 1 of n-hexane extract of *E. aromatica*.

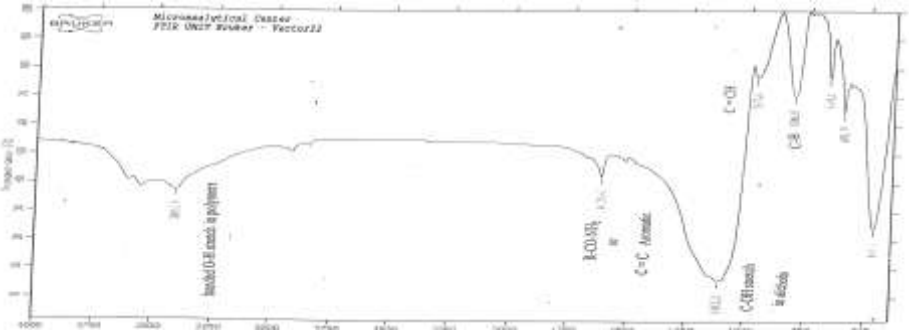

Fig. (7): Infrared spectrum of fraction 2 of n-hexane extract of *E. aromatica*

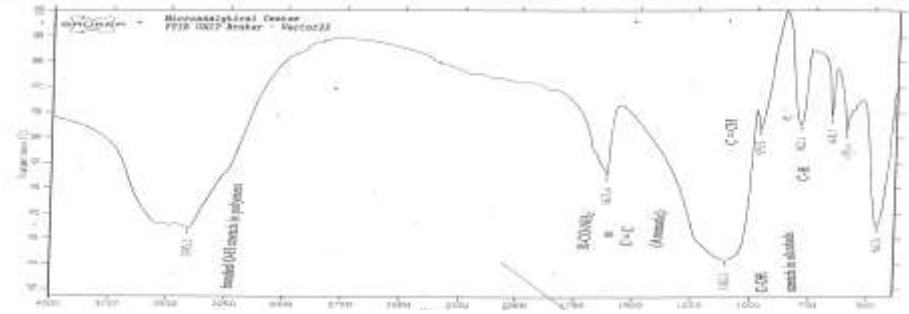

Fig. (8): Infrared spectrum of fraction 3 of n-hexane extract of *E. aromatica*.

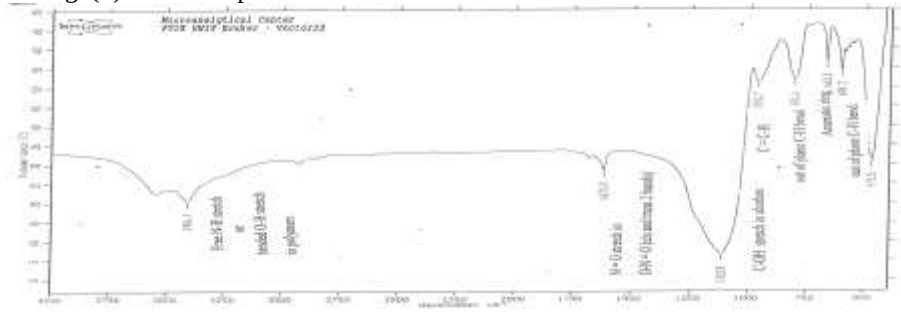

Fig. (9): Infrared spectrum of fraction 1 of ethanol extract of *P. anisum*.

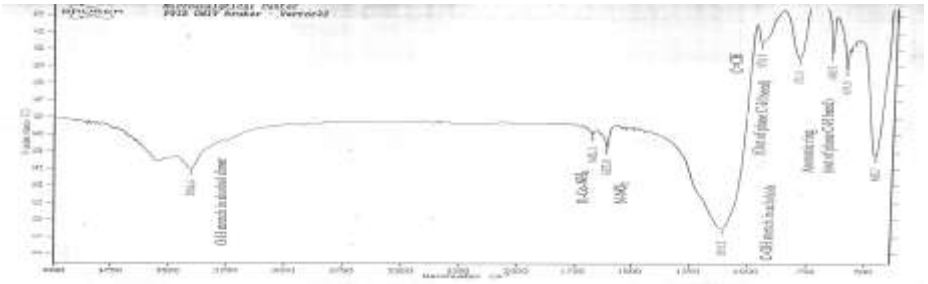

Fig. (10): Infrared spectrum of fraction 2 of ethanol extract of *P. anisum*.

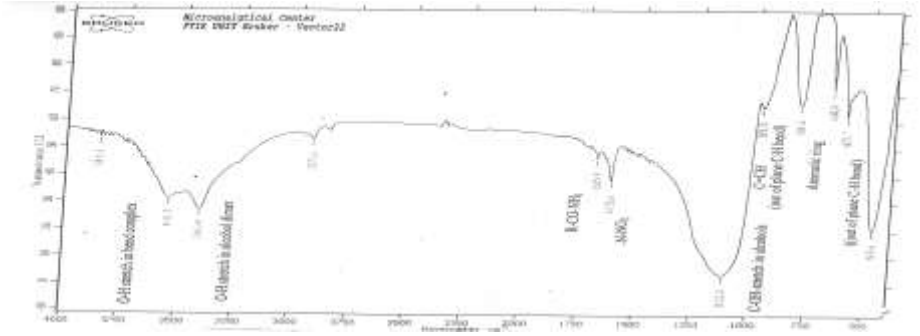

Fig. (11): Infrared spectrum of fraction 3 of ethanol extract of *P. anisum*.

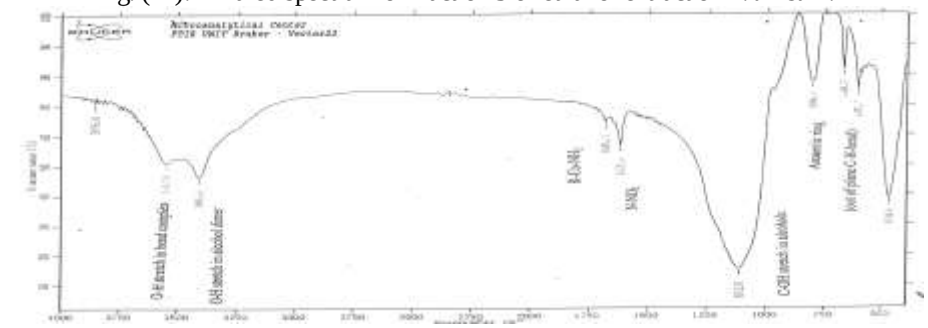

Fig. (12): Infrared spectrum of fraction 4 of ethanol extract of *P. anisum*.

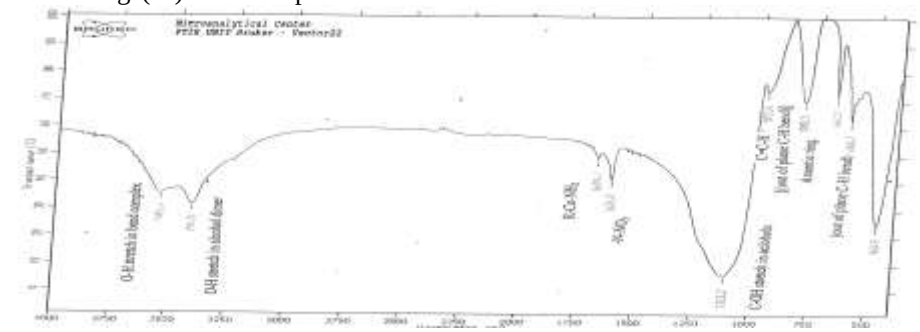

Fig. (13): Infrared spectrum of fraction 5 of ethanol extract of *P. anisum*.

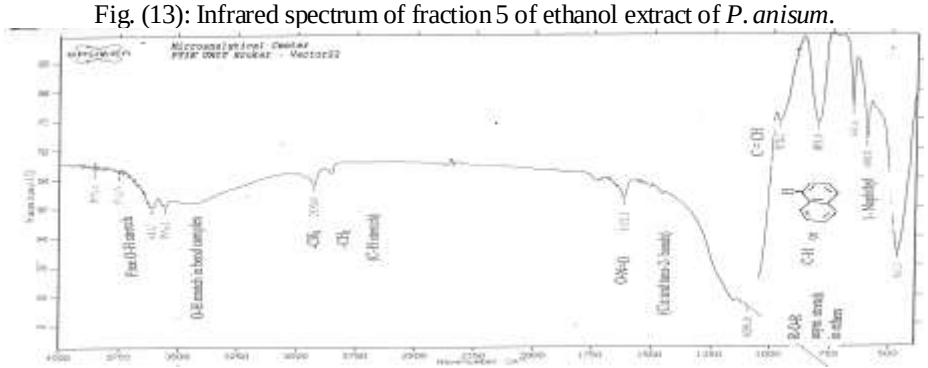

Fig. (14): Infrared spectrum of fraction 1 of n-hexane extract of *P. anisum*.

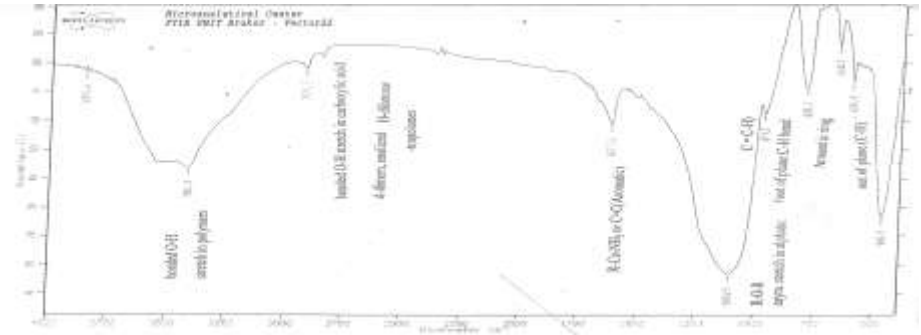

Fig. (15): Infrared spectrum of fraction 2 of n-hexane extract of *P. anisum*.

Table 5. Infrared (IR) wave number of group assignment of compounds isolated from *E. aromatica* and *P. anisum.*

Wave number (cm^{-1})	Assignment
3600-3500	O-H stretch bended complex
3635-3610	Free O-H stretching
3000-2800	C-H stretching
1650-1700	R-CO-NH_2
1680-1630	C = N stretching
1650-1600	-O-NO_2
1630-1550	-N-NO_2
1570-1500	-C-NO_2
1270-1070	R-O-R asym. stretching ether
1200-1000	C-OH stretch in alcohols
980-690	C = C-H
870-670	Out of plans C-H band
700-550	-C-S stretching
720-718	-(CH_2)

4- A strong sharp bands at 1270-1070 cm^2 region corresponding to the R-O-R asym. Stretching in ether, in addition to C-OH stretching in alcohols at 1200-1000 cm^{-1} region and the band at 980-690 showed that C = C-H groups my be present and at least one of the latter being adjacent to carbonyl group occurred at 870-670 cm^{-1}. This indicates the presence of out of plants C-H bands.

5- Stretching vibration sharp band assigned to the C-S linkage occurred in the region of 700-550 cm^{-1} in the compound, in addition to -(CH_2)- group band at 720-718 cm^{-1}. These groups are present in thiazole rings.

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التأثير الإبادى لمستخلصات نباتية ضد اثنين من آفات الحبوب المخزونة ، واستخدام التحليل الكروماتوجرافى والاشعة تحت الحمراء لتحديد المركبات السامة فى المستخلصات

رأفت بدر أبو عرب ، جمال محمد محمود زايد ، عبير عبد السلام سالم

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقى– الجيزة – مصر

تم اختبار خمس مستخلصات نباتية لمذيبى الإيثانول ، والهكسان العادى وهى نباتات الخلة البرية والشيح البلدى والزربيح والقرنفل والينسون بالإضافة إلى مبيد البيريمفوس ميثيل (مبيد الإكتيليك) كأحد المبيدات الموصى بها وذلك على حشرتى خنفساء اللوبيا وسوسة الإرز. أعطى مستخلص نبات الينسون ونبات القرنفل نتائج مرضية ضد الحشرات المختبرة. كما أظهرت النتائج تفوق المبيد الكيميائى البيريميفوس ميثيل على جميع المستخلصات النباتية حيث أعطى أقوى تأثير إبادى وأقل عدد فى الذرية الناتجة (الجيل الأول من الحشرتين). كما أوضحت النتائج أيضا أن المستخلصين اللذين تم اختبارهما حققا خفضا كبيرا فى ذرية الحشرتين موضوع الدراسة. نظرا لتباين تأثير المستخلصين على الحشرتين المختبرتين تم عمل تحليل كيميائى لمعرفة تركيب هذين المستخلصين وذلك باستخدام كروماتوجرافى الفيلم الرقيق (TLC) والأشعة تحت الحمراء وقد اختلف التركيب الكيميائى بين المستخلصين وقد يرجع ذلك لنوع المذيب وكذلك لنوع النباتات. أعطى مستخلص الإيثانول كمية عالية من الاستيرولات والترای تربينز والكربوهيدرات والجليكوسيدات لنبات القرنفل ونبات الينسون. أما التانينات وكلوريدات الحديد والفلافيندات فقد وجدت بكميات ضئيلة جدا فى مستخلص الهكسان العادى للنباتات التى تم تحليلها. يمكن القول أن مركبات الاستيرولات والترای تربنز والصابونينز والقلويدات والجليكوسيدات ربما تكون هى المسئولة عن التأثير الإبادى لهذه النباتات على الحشرتين موضوع الدراسة. أسفر التحليل الكروماتوجرافى عن وجود مكونين فى نبات الينسون لمستخلص الهكسان وخمس مكونات مع الإيثانول وبالنسبة لنبات القرنفل ثبت وجود أربعة مكونات مع الإيثانول وثلاثة مع الهكسان العادى. أظهرت نتائج تحليل الأشعة تحت الحمراء وجود أربعة عشر منحنيا ذات قمم مختلفة عند أطوال موجية معينة تشير إلى وجود مجاميع من المركبات الكيماوية فى كل منحنى والتى ربما يرجع إليها التأثير الإبادى للمستخلص.