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Efficacy of certain organic extracts at three concentrations on tomato plants infected with the root-knot nematode with reference to GC-MS analysis

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Abstract

A greenhouse experiment was conducted to determine the efficacy of cumin, garlic, and black pepper as organic extracts at three concentrations against root-knot nematode,*Meloidogyne arenaria* . Garlic extract had the highest potential effects in reducing the number of galls, egg masses, and second- stage juveniles/250 g of soil by 63.97, 65.1, and 58.19%, respectively. Cumin extract was the most effective extract in reducing the number of eggs/g roots (60.91% reduction eggs/g root system)**.** Pepper extract showed higher shoot fresh and dry weights (61.95 and 16.21 g/plant, respectively) without significant differences from other treatments. Ethanol and petroleum ether extracts were the most effective extracts in reducing the number of galls, egg masses, eggs per g, and juveniles/250 g soil. A positive correlation between concentration and the reduction of gall formation, egg masses, eggs per g, and juveniles. Ethanol extract from garlic gave the highest potential effect in reducing the number of galls, egg masses, and second-stage juveniles/250 g of soil, with percentages of reduction of 67.67, 67.9, and 65.53%, respectively. Ethanol extract of cumin was the most effective extract, as it reduced the number of eggs per gram root by 65.84%. GC-MS analysis of crude ethanolic extracts of *Allium sativum*, *Cuminum cyminum*, and *Piper nigrum* revealed that the major compounds that were found in *Allium sativum* are trisulfide, di-2-propenyl with a peak area of 38.18%, disulfide, di-2-propenyl 9.95%, 4-(Methylthio) butyric acid 9.12%, propanal, 2 methyl-3-phenyl 7.86%, and tetrasulfide, di-2-propenyl 4.39%. The major compounds that were found in *Cuminun cyminum* Propanal, were 2-Methyl-3-Phenyl with a peak area of 29.44%, 4,5,6-trimethoxy-1H-indole-2-carboxylic acid 23.39%, 1-Ispropylidene-3-N-Butyl-2-Cyclobutene 16.48%, 2,3,3-Trimethyl-3H-indole 5.54%, and gamma-Terpinene 2.26%. Piperine with a peak area of 40.39%, 10-Hydroxy-10-(Phenylethynyl) Anthrone 5.57%, trans-Caryophyllene, 1,3-Dimethyl-4-azaphenanthrene 5.36%, Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-5.50%, 1H-Indole-2-carboxylic acid, and 6-(4-ethoxyphenyl) 3.09%, were the major five compounds that were found in *P. nigrum*. Thus, the ethanol extract of garlic had the highest potential as an alternative nematicide against *M. arenaria*.

Introduction

Vegetable crops, especially tomatoes, are susceptible and heavily infested by such plant parasitic nematodes; however, the root-knot nematodes, *Meloidogyne* spp., are major pests of vegetable crops in intensive agriculture (Tzortzakakis and Gowen, 1996; Eddaoudi *et al.*, 1997; Verdejo-Lucas *et al.*, 2002 and Karssen and Moens, 2006). Damage caused by *Meloidogyne arenaria* is especially serious on vegetable crops in tropical and subtropical countries (Sikora and Fernandez, 2005). The modern way of nematode management is totally based on the nematicides, however, these nematicides are not only toxic to the root-knot nematodes but also accumulate in plants and cause environmental pollution as well as the depletion of the stratospheric zone (Wheeler and Starr, 1987). Hence, there is a needless need for eco-friendly nematode management. The demand for organically produced products is increasing all over the world due to growing concerns about food safety and environmental pollution. Organic farming is a system that provides healthy food and other products through natural ecological cycles and methods that care for the environment and have fair relations with all involved (IFOAM, 2007). In organic production systems, farmers rely on preventive, cultural, biological control, and integrated methods for disease management. In this regard, plant disease control can be achieved by crop rotation, intercropping, organic manuring, and the use of resistance cultivars and biocontrol agents.

The ability of plant parts or products to reduce crop damage caused by root-knot nematodes, *Meloidogyne* spp., is well documented (Begum *et al.*, 2003; Youssef and Ali, 1998; Musabyimana and Saxena, 1999, and Jesse *et al.*, 2006). The use of botanical products to control nematodes has received considerable attention in recent years. Their ability, minimum toxicity, safety to the environment, and effectiveness in controlling the nematodes make using plant parts indispensable to nematode control (Jesse and Jada, 2004).

Many compounds that have nematicidal activity, such as thienyls, alkaloids, phenols, sesquiterpenes, diterpenes, pentacyclic triterpenoids, and polyacetylenes, have been found in healthy plant tissue (Kogiso *et al.*, 1976; Gommers and Barker, 1988; Matsuda *et al.*, 1989 and Qamar *et al.*, 2005). The effective microorganisms (EM) appliance is notorious for augmenting the microbial diversity of soil and plants, improving soil quality, and increasing yield and crop quality (Kishore, 2000). Therefore, such materials can be touted as attractive alternatives to synthetic pesticides for pest control.

Materials and methods

1. Preparation of organic extracts:

Plant samples of pepper fruits, *Piper nigrum (*Piperaceae), garlic cloves peel, *Allium sativum* (Lilaceae), and cumin fruits, *Cuminum cyminum* (Apiaceae), were collected from local markets and ground in an electric blender to a fine powder. Each plant powder was extracted in a Soxhlet apparatus with the following solvents: ethanol, methanol, petroleum ether (60-80 ˚c) or chloroform till exhaustion. The solvent was evaporated under reduced pressure in a rotary evaporator. Dried crude extracts were preserved, in tightly colored brown bottles and stored in a refrigerator until used.

2. Nematode inoculum:

Root-knot nematode *Meloidogyne arenaria* (Kofoid and White) Chitwood eggs were isolated from infested roots of *Solanum* (*Solanum melongena* L). Gall roots were washed from the adhering soil particles by running water and cut out into small pieces, then homogenized in a blender for 10 seconds in 0.5% sodium hypochlorite (NaOCl) solution to dissolve the gelatinous matrix to get free nematode eggs from the mass matrix (Hussey and Barker, 1973). The suspension was passed through a 200-mesh sieve nested upon a 400-mesh sieve. Then, eggs were washed with a slow stream of tap water to rinse off residuals before inoculation.

3. Egg masses staining and counting:

Egg masses of *M. arenaria* were stained by placing them in an aqueous solution of Phloxine B (0.15 g per liter of tap water) for

20–30 minutes. Root systems were rinsed in tap water to remove residual stains on the roots. Phloxine B primarily stains the gelatinous egg sac and naked viable eggs (Barker**,** 1985).

4. Nematode extraction:

A soil sample of 250 g was successively wet sieved through 100, 200, and 325 mesh sieves. The active nematode present in the fine sieve was extracted by the Baermann-plate technique (Goodey, 1963). The final volume of the nematode extract solution was adjusted to a known volume, and the second-stage juveniles (J_2) of *M. arenaria* in each sample were counted microscopically using a counting slide.

5. Greenhouse experiment:

Plastic pots (15 cm in diam. and 20 cm in depth) were filled with a 2.5 kg mixture of autoclaved sand and peat moss (3:1, V: V). One tomato seedling (*Lycopersicon esculentum* mill CV. Fardos), 30 days old were transplanted in each pot and watered every two days and fertilized every week. After one week from transplanting time, each pot was inoculated with a suspension containing approximately 5000 eggs and newly hatching second-stage juveniles in three holes around the plant stem, simultaneously with two control groups: untreated inoculated control with *M. arenaria* and non-inoculated control. All pots were arranged in a completely randomized design (CRD) with three pots per treatment in a greenhouse. After 60 days of inoculation, plants were carefully removed, and soil particles adhering to the roots were washed thoroughly by running tap water. The fresh and dry weights of shoots and roots were determined in addition to shoot length. Also, egg masses, number of galls per root system, number of eggs per g root, and number of juveniles per 250 g soil were evaluated. Efficacy of some organic extracts, i.e., ethanol, methanol, petroleum ether, and chloroform. Each organic extract as a soil drench 20 ml solution of all concentrations for each extract. Each concentration from each organic extract was suspended in distilled water with Triton X-100 added at a concentration of 0.01%; additional 20 ml of distilled water with 0.01% Triton X-100 for two controls. All treatments were compared with the synthetic nematicide cadusafos (0.12 g/kg), the previous extract, and the synthetic nematicide added at the same time of inoculation.

The reduction of nematode parameters was calculated according to Raddy *et al.* (2013):

$$\% \text{ Reduction} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

6. GC-MS analysis:

An Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector with a direct capillary interface and fused silica capillary column PAS-5 ms (30 mm×0.25 um film thickness) was used for analysis. Samples were injected under the following conditions: Helium was used as carrier gas at approximately 1 ml/min. pulsed spitless mode. The solvent delay was 3 min., and the injection size was 1.0 ul. The mass spectrophotometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from m/z 50 to 500. The ion source temperature was 230˚C, and the quadruple temperature was 150˚C. The electron multiplier voltage (EM voltage) was maintained at 1250v above auto-tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60˚C for 3 min. Then elevated to 280 ˚C at a rate of 8˚C/min and 10 min. held at 280˚C. The Wiley and NIST 05 mass spectral database were used in the identification of the separated peaks.

7. Statistical analysis:

The data was subjected to the analysis of variance using SAS software (SAS, 2000). A comparison of means was made by using the least significant difference (LSD) at the 5% level of probability.

Results and discussion

Data within Table (1) showed that the three tested plant extracts, four solvents, and three concentrations significantly ($p \leq 0.05$) reduced the numbers of galls, egg masses, eggs per gram, and juveniles compared to the untreated inoculated control. Application of the tested

extracts of cumin, garlic, and pepper resulted in a reduction of gall formation by averages ranging from 51.34 to 63.97% compared to the untreated inoculated control. The most effective extract in the reduction of galls was garlic extract (63.97%), followed by cumin and pepper extracts (56.29 and 51.34%, respectively). Also, the most effective extract in the reduction of egg masses was garlic extract (65.10%), followed by cumin and pepper extracts (56.92 and 52.14%, respectively). On the other hand, cumin extract was the most effective extract in reducing the number of eggs/g roots (60.91%), followed by garlic and pepper extracts (55.03 and 49.47%, respectively). On the other hand, garlic extract was the most effective extract in reducing the 2^{nd} stage J_2/250 g soil, followed by cumin and pepper extracts. Meanwhile, the application of the synthetic nematicide cadusafos 10% G at the recommended dose reduced galling formation, egg masses, eggs per g root, and second-stage juveniles/250 g soil by 96.46, 95.57, 95.91, 95.35, and 93.95%, respectively.

Table (1): Effects of different plant extracts, solvent type, and extract concentrations (%) against *Meloidogyne arenaria* associated with tomato plants under greenhouse conditions.

Treatments	No. of galls/ root system	Reduct-ion%	No. egg masses per root system	Reduct-ion%	eggs/g root	Reduct-ion%	Mean juvenils 2^{nd}/250 g of soil	Reduct-ion%
Plants								
Untreated inoculated control	1136.33^{a}	0.00	1099.33^{a}	0.00	817.67^{a}	0.00	936.67^{a}	0.00
Cumin	496.64^{b}	56.29	473.58^{b}	56.92	319.64^{d}	60.91	418.44^{c}	55.33
Garlic	409.39^{c}	63.97	383.69^{c}	65.10	367.72^{c}	55.03	391.58^{c}	58.19
Pepper	552.89^{b}	51.34	526.11^{b}	52.14	413.14^{b}	49.47	485.78^{b}	48.14
Cadusafos	50.33^{d}	95.57	45.00^{d}	95.91	38.00^{e}	95.35	56.67^{d}	93.95
Solvents used in extraction								
Untreated inoculated control	1136.33^{a}	0.00	1099.33^{a}	0.00	817.67^{a}	0.00	936.67^{a}	0.00
Ethanol	432.04^{d}	61.98	409.70^{c}	62.73	331.59^{c}	59.45	360.11^{c}	61.55
Petroleum ether	460.67^{cd}	59.46	437.41^{bc}	60.21	355.33^{bc}	56.54	442.15^{b}	52.80
Methanol	533.93^{b}	53.01	505.19^{b}	54.05	398.48^{b}	51.27	478.85^{b}	48.88
Chloroform	518.59^{bc}	54.36	492.22^{b}	55.23	381.93^{b}	53.29	446.63^{b}	52.32
Cadusafos	50.33^{e}	95.57	45.00^{d}	95.91	38.00^{d}	95.35	56.67^{d}	93.95
Concentrations of extractable constituents								
Untreated inoculated control	1136.33^{a}	0.00	1099.33^{a}	0.00	817.67^{a}	0.00	936.67^{a}	0.00
1%	674.75^{b}	40.62	646.17^{b}	41.22	478.53^{b}	41.48	524.69^{b}	43.98
2%	497.25^{c}	56.24	471.00^{c}	57.16	395.94^{c}	51.58	449.11^{c}	52.05
4%	286.92^{d}	74.75	266.22^{d}	75.78	226.03^{d}	72.36	322.00^{d}	65.62
Cadusafos	50.33^{e}	95.57	45.00^{e}	95.91	38.00^{e}	95.35	56.67^{e}	93.95

Values in each column followed by the same letter (s) are not significantly different according to LSD at 0.05% level.

The estimated percentage reduction of gall formation was 61.98, 59.64, 54.36, and 53.01% for ethanol, petroleum ether, chloroform, and methanol extracts, respectively. Also, ethanol extract was the most effective in the reduction of egg masses/root system, as it reduced the number of egg masses/root system by

62.73%, followed by petroleum ether, chloroform, and methanol extracts (60.2, 55.22, and 54.05%, respectively). The same trend was observed with the effect on the number of eggs per g root; ethanol extract was the most effective in reducing the number of eggs per g root and J_2/250 g soil (59.45 and 61.55%, respectively), followed by petroleum ether, chloroform, and methanol extracts. Concerning the effect of concentration of extracts, there was a positive correlation between concentration and reduction of gall formation, egg masses/root system, eggs/g root, and juveniles/250 g soil. The most effective extract in the reduction of the number of galls/root system, number of egg masses/root system, and the number of J_2/250g soil was garlic extract with percentages of reduction of 63.97, 65.1, and 58.19%, respectively, while the most effective extract in the reduction of the number of eggs/g root was cumin extract (60.91%).

Data in Table (2) indicated that all the tested plant extracts significantly ($p \leq 0.05$) increased the shoot fresh and dry weights compared to the untreated inoculated control but were less significant ($p \leq 0.05$) than the non-inoculated control), pepper extract showed higher shoot fresh and dry weights (61.95 and 16.21 g/plant), respectively, with significant differences with other treatments. Also, the same results were obtained with shoot length; all treatments significantly ($p \leq 0.05$) increased shoot length compared to untreated inoculated control but were less significant ($p \leq 0.05$) compared to non-inoculated control. Cumin extract gave the highest shoot length (58.8 cm/plant). Also, all the plant extracts significantly ($p \leq 0.05$) increased fresh and dry root weights of tomato compared to untreated inoculated control, without significant differences compared to non-inoculated control. Cumin extract was the best extract in this respect.

Results in the same table revealed that all the tested solvents used in extraction significantly ($p \leq 0.05$) increased the growth parameters compared to the untreated inoculated control but were less significant ($p \leq 0.05$) than the non-inoculated control. No significant differences were observed between the tested solvent extractions in increasing tested growth parameters.

Data in Table (3) show that all different plant extracts significantly ($p \leq 0.05$) reduced the numbers of galls, egg masses/root systems, eggs per g, and juveniles compared to untreated inoculated control. Application of the tested extract of cumin, garlic, and pepper resulted in the reduction of gall formation by averages ranging from 45.88 to 67.67% compared to the untreated inoculated control. The highest effect was obtained from the ethanol extract of garlic (67.67%) with significant differences compared with all treatments, followed by the petroleum ether extract of garlic (65.47%). Conversely, the methanol extract of pepper was the lowest treatment, which reduced the number of galls by 45.88%. Also, the same results were found with the egg masses/root system; the highest effect was obtained from the ethanol extract of garlic (67.9%), with significant differences compared with all treatments, followed by the petroleum ether extract of garlic (66.67%). Again, methanol extract of pepper was the lowest treatment, which reduced the number of galls by 46.53%.

Table (2): Effects of different plant extracts, solvent type, and extract concentrations on some growth parameters of tomato plants infected with *M. arenaria* under greenhouse conditions.

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot length (cm)	Root fresh weight (g)	Root dry weight (g)
Plants					
Non-inoculated control	67.89^{a}	18.98^{a}	65.17^{a}	18.48^{a}	4.28^{a}
Untreated inoculated control	40.76^{c}	10.13^{d}	47.58^{c}	13.42^{b}	2.98^{c}
Cumin	57.49^{b}	14.57^{bc}	58.80^{b}	17.12^{a}	3.52^{b}
Garlic	56.53^{b}	14.55^{bc}	56.93^{b}	16.71^{a}	3.45^{bc}
Pepper	61.95^{b}	16.21^{b}	57.56^{b}	16.50^{a}	3.32^{bc}
Cadusafos 10% G	56.82^{b}	13.40^{c}	58.74^{b}	16.71^{a}	3.52^{b}
Solvents used in extraction					
Non-inoculated control	67.89^{a}	18.98^{a}	65.17^{a}	18.48^{a}	4.28^{a}
Untreated inoculated control	40.76^{c}	10.13^{d}	47.58^{c}	13.42^{c}	2.98^{c}
Ethanol	58.30^{b}	14.84^{bc}	57.23^{b}	16.71^{ab}	3.48^{b}
Petroleum ether	58.85^{b}	15.16^{b}	57.74^{b}	16.18^{b}	3.34^{bc}
Methanol	58.48^{b}	15.25^{b}	57.28^{b}	17.18^{b}	3.50^{b}
Chloroform	59.00^{b}	15.19^{b}	58.81^{b}	17.03^{b}	3.40^{bc}
Cadusafos 10% G	56.82^{b}	13.40^{c}	58.74^{b}	16.71^{b}	3.52^{b}
Concentrations of extractable constituents					
Non-inoculated control	67.89^{a}	18.98^{a}	65.17^{a}	18.48^{a}	4.28^{a}
Untreated inoculated control	40.76^{c}	10.13^{e}	47.58^{c}	13.42^{c}	2.98^{c}
1%	56.65^{b}	13.93^{cd}	57.07^{b}	16.04^{b}	3.34^{bc}
2%	58.64^{b}	15.53^{bc}	57.74^{b}	17.07^{ab}	3.47^{b}
4%	60.68^{b}	15.87^{b}	58.48^{b}	17.22^{ab}	3.49^{b}
LSD	5.10	1.65	5.47	1.92	0.45

Values in each column followed by the same letter (s) are not significantly different according to LSD at 0.05% level.

Table (3): Effect of the nematicidal activity of different extracts of cumin, garlic, and pepper by some different solvents in tomato plants infected with *Meloidogyne arenaria* under greenhouse conditions.

Interaction		No. of galls/root system	Reduction%	No. egg mass per root system	Reduction %	eggs/g root	Reduction%	Mean juveniles 2nd/250 g of soil	Reduction%
Untreated inoculated control		1136.33^{a}	0.00	1099.33^{a}	0.00	817.67^{a}	0.00	936.67^{a}	0.00
Cumin	**Ethanol**	445.89^{f}	60.76	421.56^{f}	61.65	279.33^{i}	65.84	330.00^{h}	64.77
	Petroleum ether	471.44^{e}	58.51	452.67^{e}	58.82	303.67^{i}	62.86	426.00^{f}	54.52
	Methanol	547.89^{c}	51.78	522.22^{c}	52.50	327.89^{h}	59.90	440.89^{ef}	52.93
	Chloroform	521.33^{d}	54.12	497.89^{d}	54.71	367.67^{f}	55.03	476.89^{d}	49.09
Garlic	**Ethanol**	367.33^{i}	67.67	352.89^{g}	67.90	340.00^{gh}	58.42	322.89^{h}	65.53
	Petroleum ether	392.33^{h}	65.47	366.44^{g}	66.67	349.00^{g}	57.32	372.11^{g}	60.27
	Methanol	438.89^{f}	61.38	405.56^{f}	63.11	390.22^{d}	52.28	444.00^{e}	52.60
	Chloroform	439.00^{f}	61.37	409.89^{f}	62.71	391.67^{d}	52.10	427.33^{ef}	54.38
pepper	**Ethanol**	482.89^{e}	57.50	454.67^{e}	58.64	375.44^{df}	54.08	427.44^{ef}	54.37
	Petroleum ether	518.22^{d}	54.40	493.11^{d}	55.14	413.33^{c}	49.45	528.33^{c}	43.59
	Methanol	615.00^{b}	45.88	587.78^{b}	46.53	477.33^{b}	41.62	551.67^{b}	41.10
	Chloroform	595.44^{b}	47.60	568.89^{b}	48.25	386.44^{d}	52.74	435.67^{ef}	53.49
Cadusafos		50.33^{j}	95.57	45.00^{h}	95.91	38.00^{j}	95.35	56.67^{i}	93.95

Values in each column followed by the same letter (s) are not significantly different according to LSD at 0.05% level.

Results in Table (3) indicate that application of the tested plant extracts resulted in a reduction of the number of eggs per g root by averages ranging from 41.62 to 65.84%. Ethanol extract of cumin was the most effective treatment, as it reduced the number of eggs per g root by 65.84%, followed by petroleum ether extract of cumin (62.86%), while the lowest effect was obtained by the methanol extract of pepper, as it reduced the number of eggs per gram root by 41.62%. Also, the application of the tested extracts significantly ($p \leq 0.05$) reduced the number of second-stage juveniles in the soil compared to the untreated inoculated control. The highest effects were obtained from the ethanol extract of garlic (65.53%), followed by the ethanol extract of cumin (64.77%). On the other hand, the methanol extract of pepper was the least effective extract. The most effective plant extract in the reduction of the number of galls/root systems, and the number of egg masses/root systems, and the number of J_2/250 g soil was the ethanol extract of garlic with percentages of reduction of 67.67, 67.9, and 65.53%, respectively, while the most effective plant extract in the reduction of the number of eggs/g root was the ethanol extract of cumin (65.84%). The nematicidal activity of the plant extracts in this part of the study may be attributed to the different active constituents extracted by different organic solvents.

The potential of using plant extracts in controlling plant parasitic nematodes has been recorded by many authors (Adegbite and Adesiyan, 2005; Opareke *et al.,* 2005; Oka *et al.*, 2006; Orisajo *et al.,* 2007; Abbasi *et al.,* 2008; Ntalli *et al.,* 2010, and Aoudia *et al.,* 2012). Furthermore, the use of plants and plant products is one of the most promising methods for nematode control. They are cheap, easy to apply, produce no pollution hazards, and have the capacity to improve soil health (Zasada *et al*., 2010) structurally and nutritionally. Also, nematicidal phytochemicals are generally safe for the environment (Chitwood, 2002). Additionally, such agents often act at multiple and novel target sites, thereby reducing the potential of plant-parasitic nematodes becoming resistant to them (Isman, 2000 and 2006).

1. GC-MS analysis of crude ethanolic extractives of *Allium sativum*:

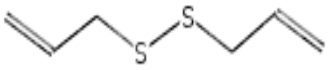
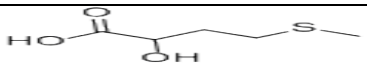
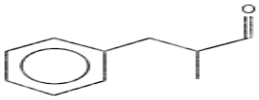
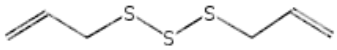
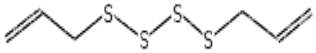
GC-MS analysis of crude ethanolic extracts of *A. sativum* revealed the presence of forty-seven (47) peaks as shown in Table (4) and illustrated in Figure (1). The spectra of the compounds were matched with NIST and Willey Library. Their structure was identified by the percentage similarity values. They were confirmed by the study of the classical fragmentation patterns, base peaks, and molecular ion peaks of the compounds. The major compounds, as shown in Table (4), were found to be Trisulfide, di-2-propenyl, with a peak area of 38.18%; Disufide, di-2-propenyl, 9.95 %, 4-(methylthio) butyric acid 9.12%; Propanal, 2 methyl-3-phenyl 7.86%, and Tetrasulfide, di-2-propenyl 4.39%.

It was reported that dimethyl disulfide, dipropyl disulfide, and dial-lyl disulfide were all biocidal (Auger *et al.,* 2004). Several liliaceous crops, such as *A. sativum* L., *A. cepa* L., and *A. fistulosum* L., contain sulphur compounds that are hydrolyzed to form a variety of isochiocyanates with broad insecticidal, nematicidal, fungicidal, antibiotic, and phytotoxic effects (Choi *et al.,* 2007). Results in Table (5) agreed with Pyun and Shin (2005) and Douiri *et al.* (2013), who reported that garlic essential oil content near 0.32% ± 0.2 of the clove's fresh weight, and the principal chemical components are trisulfide di-2propenyl, disulfide di-2propenyl, trisulfide methyl 2propenyl, and diallyl disulfide.

Table (4): GC-MS analysis of crude ethanolic extractives of *Allium sativum*.

Peak N0.	Compound	Rt (min)	Area (%)
1	1-Propene, 3,3-thiobis-	3.61	3.27
2	Disulfide, methyl 2-propenyl	4.91	1.93
3	n-Propyl ally sulfide	5.18	1.03
4	Thiourea, N, N –dimethyl-	5.72	2.51
5	Dimethyl trisulfide	5.96	1.47
6	Benzene, 1-methyl-4-(1-methylethyl)	7.04	0.87
7	1,4 Cyclohexadiene 1-methyl-4-(1-methylethyl-	7.81	1.42
8	Disufide, di-2-propenyl	8.03	9.95
9	Diallyldisulphide	8.51	0.34
10	Disulfide, di-2-propenyl	8.64	1.22
11	1-Oxo-4,6-diazacycclooctane-5-thion	8.76	1.08
12	2-Hydroxy-4-(methylthio)butyric acid	9.57	9.12
13	1,3,5-Trithiane	10.05	0.53
14	3,4- Dihydro-3-vinyl-1,2-dithiin	10.68	0.39
15	(3-chlorophenyl) acetylene	10.92	0.26
16	2-vinyl- [4 H]-1,3-dithiin	11.19	0.63
17	Propanal,2 methyl-3-phenyl	11.70	7.86
18	2H-1-Benzopyran, 3,4-dihydro-2-methyl	12.38	0.09
19	Trisulfide, di-2-propenyl	12.84	38.18
20	2-Prophenylthioacetonitrile	13.05	0.93
21	1-Propene, 3,3 –thiobis	13.32	1.20
22	1, 2, 4, 6- Tetrathiepane	14.07	1.38
23	Disulfide, methyl 2-propenyl	14.29	1.52
24	Heptanoic acid, 3-oxo-, methyl ester	14.58	0.93
25	Ethanol, 2- [(2-chloroethyl) dithio]	14.92	0.22
26	2-Prophenylthioacetonitrile	14.45	0.29
27	1H-Benzocycloheptene, 2, 4a, 5, 6,7, 8, 9, 9a-Octahdro-3, 5, 5 trimethyl-9-methylene	15.85	0.34
28	2-Thiazolidinethione	16.43	0.37
29	Tetrasulfide, di-2-propenyl	16.98	4.39
30	3a(1H)- Azulenol, 2, 3,4 ,5,8,8a-hexahydro-6,8a-Dimethyl-3-(1-methylethyl-6,8a-dimethyl-3-(1-Methylethyl)	17.90	0.16
31	2-Butenoic acid, 3-[(dimethoxyphosphinyl) oxy]	18.70	0.28
32	Silane, trimethyl (3-methylbutoxy)	18.87	0.36
33	Dimethyl 2-methoxyhexane-1,6-dioat	18.99	0.67
34	5-allyl-4,5-dihydro-4,4-dimethyl-2-phenyl-1,3-Thiazol-5-thiol	20.12	0.53
35	Methyl 4-nitrohexanoate	20.49	0.11
36	Cyclohexasiloxane, dodecamethyl	20.96	0.18
37	Butanol, 1- [2, 2, 3, 3-tetramethyl-1-(3-methyl-1-Penynyl) cyclopropyl]	21.30	0.21
38	6H- Furo [2,3:4,5] oxoazolo[3,2-a] pyrimidin-6- one2, 3, 3a,9a-tetrahydro-3-hydroxy-2-(hydroxymethyl)-7-methyl	21.82	0.15
39	Silane, trimethy (3-meyhylbutoxy)	22.22	0.58
40	n-Hexadecanoic acid	23.19	0.84
41	Octadecanoic acid	23.77	0.05
42	Cyclooctaneacetic acid, 2-oxo-	25.29	0.07
43	2-Acetyl-4-nitrocyclooctanone	26.22	0.36
44	Benzeneacetonitrile, alpha-acetyl	26.51	0.12
45	2-Hydroxy-4-(4-methoxyphenyl) quinolone	27.04	0.44
46	Di-(2-ethylhexyl) phthalate	29.54	1.09
47	1,8-Bis (3,4-dicyanophenyl) anthracene	37.42	0.12

Table (5): The chemical properties of the major compounds isolated from ethanolic extractives of *Allium sativum* L. using GC/MS analysis.

Compound	Structure	Mw	Formula	Area%	RT
Disufide, di-2-propenyl		164.274	$C_6H_{10}S_2$	9.95	8.3
2-Hydroxy-4-(methylthio)butyric acid		150.2	$C_5H_{10}O_3S$	9.12	9.57
Propanal,2 methyl-3-phenyl		148.201	$C_{10}H_{12}O$	7.86	11.7
Trisulfide, di-2-propenyl		178.339	$C_6H_{10}S_3$	38.18	12.84
Tetrasulfide, di-2-propenyl		210.404	$C_6H_{10}S_4$	4.39	16.98

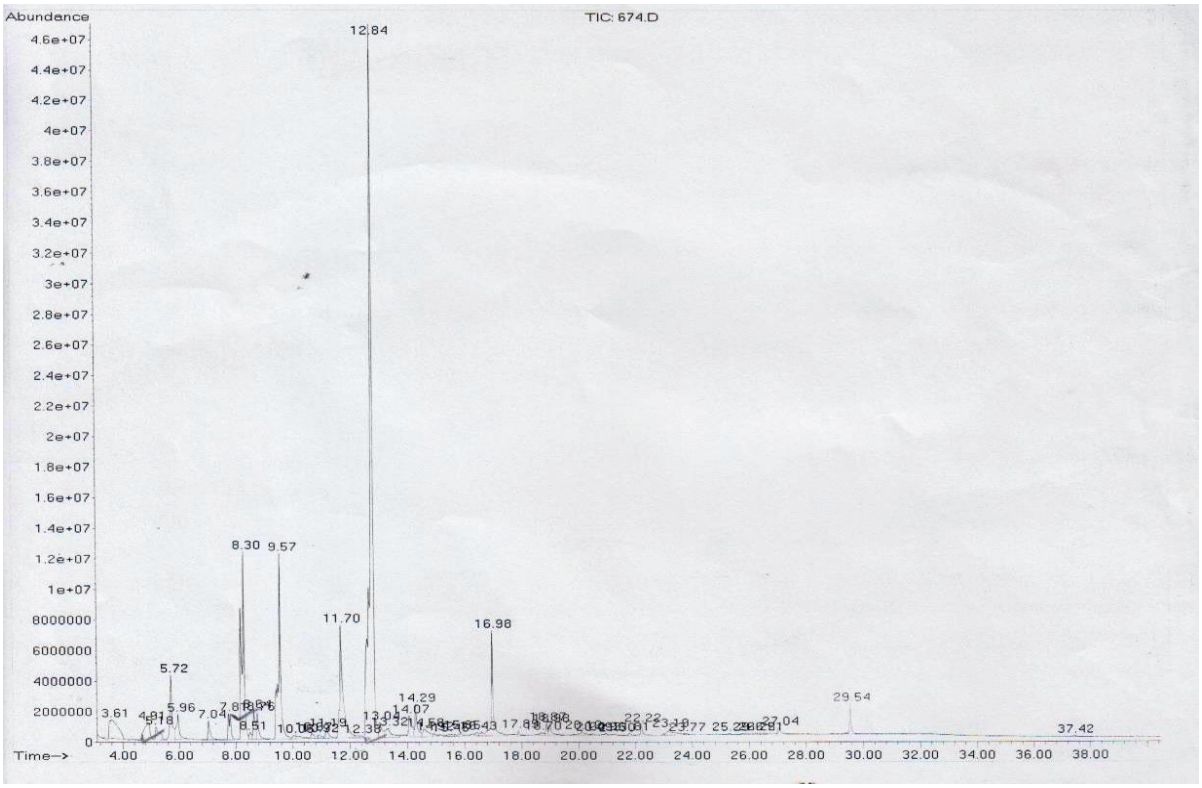


Figure (1): GC-MS analysis of crude ethanolic extractives of *Allium sativum.*

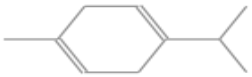
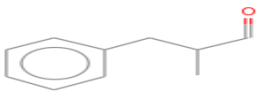
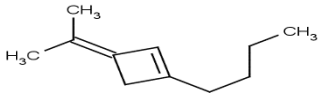
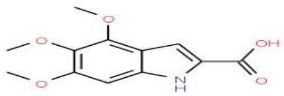
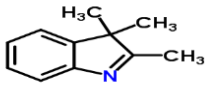
2. GC-MS analysis of crude ethanolic extractives of *Cuminun cyminum*:

GC-MS analysis of crude ethanolic extracts of *C. cyminum* revealed the presence of forty-two (42) peaks as shown in Table (6) and illustrated in Figure (2). The major compounds, as shown in Table (7) were found to be Propanal, 2-Methyl-3-Phenyl with a peak area of 29.44%, 4,5,6-trimethoxy-1H-indole-2-carboxylic acid 23.39%, 1-Ispropylidene-3-N-Butyl-2-Cyclobutene 16.48%, 2,3,3-Trimethyl-3H-indole 5.54% and gamma-Terpinene 2.26%.

Table (6): GC-MS analysis of crude ethanolic extractives of C*uminum cyminum.*

Peak N0.	Compound	Rt (Min)	Area (%)
1	Bicyclo [3, 1,0] hexane,4-methylene-1-(1-methylethyl)	5.86	0.11
2	Benzene, methyl (1-methylethyl)	6.79	1.39
3	1,4- Cyclohexadiene, 1-methyl-4-(1-methylethyl)	7.47	2.26
4	1,3,3- Trimethylcyclohex-1-ene-4-Carboxaldehyde	10.43	1.70
5	Propanal, 2-Methyl-3-Phenyl	11.28	29.44
6	p-menth-1-en-7-al	12.02	0.40
7	1-Isopropylpylidene-3-n-butyl-2-cyclobutene	12.14	16.48
8	Benzenemethonal, 4-(1-methylethyl)	13.21	0.8
9	Naphthalene, 1,2,3,4,4a,5,6,8a-Octahydro-7-Methylene-1-(1-Methyletyl)	13.80	1.86
10	trans-Caryophyllene	14.53	0.36
11	Alpha-Cedrane	14.77	0.15
12	Trans-beta- Farnesene	15.12	0.57
13	Carbofuran-3-hydroxy-7-phenol	15.29	0.28
14	1,3-Cyclohexadiene, 5-(1, 5-dimethyl-4-hexenyl)-2-methyl	15.45	0.96
15	1H-Cyclopro[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	15.88	0.58
16	Cyclohexane, 1, 5-diethenyl-3-methyl	17.31	0.12
17	Carotol	17.48	0.79
18	3, 4-Dimethylbenzamide, N-2-methylpropyl	17.72	0.18
19	1, 2, 4-triazolo [3, 4-b] [1, 3] benzothiazine-5-one	20.01	0.33
20	Bicyclo [3. 1. 0] hex-2-one, 2-methyl-5(1-methylethyl)	20.28	0.08
21	2-Methyl-4- [1, 2, 2-trimethylbicyclo [3. 1. 0] hex-3-yl] but-2-enal	20.76	0.95
22	Naphthalene-4a, 8a-dicarboxylic acid, 1 ,4, 4a, 8, 8a-hexahydro-, dimethyl ester	23.64	0.42
23	1, 4-Methanonaphthalen-9-ol, 1, 2, 3, 4-tetrahydro-	23.91	0.12
24	2-Dichlormethylthiophene	24.04	0.31
25	7- (1-methyl-ethenyl)-1-hydroxy-1,4-dimethyl-1, 2, 4, 5- [3H, 6H] octahydroazulene	24.73	0.52
26	2-Propen-1-one, 1, 3-diphenyl	25.38	0.35
27	Methanol, 1- [2- [4- (1-methylethyl) phenyl]-4-nitro-1,3-dioxan-5-yl]	25.63	0.21
28	Cyclopentene-1-decanoic acid-hydroxy-3-oxo-2-pentyl-, methyl ester	26.37	1.10
29	4,5,6-trimethoxy-1H-indole-2-carboxylic acid	26.56	23.39
30	2, 4- Dimethyloxanilic acid N-veratrylidenehydrazide	27.50	0.15
31	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4, 5, 6, 7-tetrahydro-isopropyl ester	28.46	0.10
32	3, 4- Dimethoxybenzaldehydeoxime	28.70	0.24
33	5, 6, 8, 9-tetramethoxy-2-methylpepero (3, 4, 5-JK)-9, 10-dthydrophenanthracene	28.84	0.18
34	2-Sec-Butyl-4,6-dinitrophenyl 3-methylcrotonate	29.20	1.66
35	Pent-2-enoic acid, 6-(4-cyano-phenyl)-naphthalen-2-yl ester	30.03	0.43
36	Benzo [h] quinolone, 2, 4-dimethyl	30.43	0.58
37	1-methoxy-2, 5, 6-trimethyl-1, 2, 3, 6-tetrahydro-1, 2, 6-phosphadiazine-1, 3-dione	30.82	0.43
38	8- Amino-2-hydroxymethyl-6-methoxyquinoline	30.95	0.93
39	2,3,3-Trimethyl-3H-indole	31.21	5.54
40	2-(phenyl)-6-(tert-butyl) pyrimidin-4 (3H)- one	31.95	0.58
41	4-H-3-(p-methylanilino) 1-benzothiopyran-4-one 1-oxide	35.26	0.45
42	2 H-1-Benzopyran-6-ol,3, 4-dihydro-2, 5, 7, 8-tetramethyl-2-(4, 8, 12-trimethytridecyl)	36.43	0.58

Table (7): The chemical properties of the major compounds isolated from ethanolic extractives of *Cuminum cyminum* using GC/MS analysis.

Compound	Structure	Mw	Formula	Area %	RT
1,4-Cyclohexadiene, 1-metyl-4-(1-methylethyl)		$C_{10}H_{16}$	136.234	2.26	7.47
Propanal, 2-methyl-3-phenyl-		$C_{10}H_{12}O$	148.201	29.44	11.28
1- Ispropylidene-3-N-butyl-2-cyclobutene		$C_{11}H_{18}$	150.261	16.48	12.14
4,5,6-trimethoxy-1H-indole-2-carboxylic acid		$C_{12}H_{13}NO_5$	251.24	23.39	25.56
2,3,3-Trimethyl-3H-indole		$C_{11}H_{13}N$	159.228	5.54	31.21

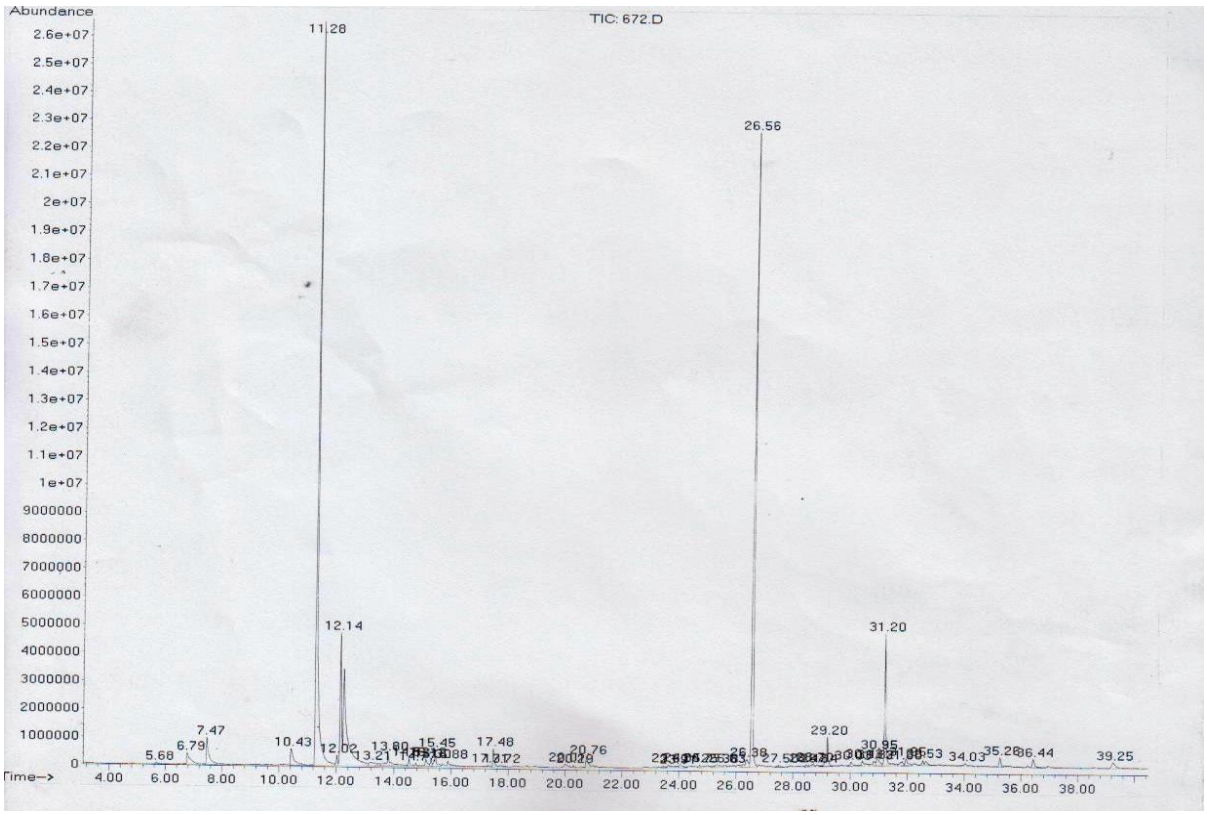


Figure (2): GC-MS analysis of crude ethanolic extractives of *Cuminum cyminum.*

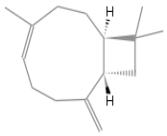
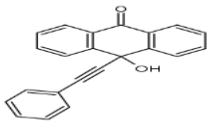
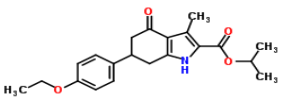
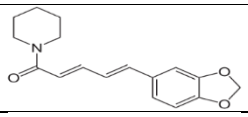
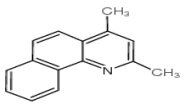
3. GC-MS analysis of crude ethanolic extractives of *Piper nigrum*:

GC-MS analysis of crude ethanolic extracts of *P. nigrum* revealed the presence of the major compound peaks as shown in Table (8) and illustrated in Figure (3). The major five compounds, as shown in Table (9) were found to bepiperine with a peak area of 40.39%, 10-Hydroxy-10-(Phenylethynyl) Anthrone 5.57%, Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene 5.50%, trans-Caryophyllene,5.36 %, 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl) 3.09%.

Table (8): GC-MS analysis of crude ethanolic extractives of *Piper nigrum.*

Peak N0.	Compound	Rt (min)	Area (%)
1	1H-Cyclopenta [1,3] cyclopropa [1,2] benzene, 3a,3b,4,5,6,7-hexahydro-3,7-dimethyl-4-(1-ethylethyl)-	13.80	1.12
2	Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene- [1R-(1R*,4Z,9S*)]	14.54	5.50
3	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,7α,7aβ,7bα)]	15.97	0.21
4	1H-Pyrazole-1-acetamide, 4-iodo-N-(phenylmethyl)-	17.48	0.63
5	Naphthalene, 1,2,3,5,6,7,8,8a- Octahydro-1,8-di Methyl-7-(1-methylethenyl)	18.37	2.84
6	1H-Indole-2-carboxylic acid,6-(4-ethoxyphenyl) -3-methyl-4-oxo-4, 5, 6, 7-tetrahydro, isopropyl ester	26.19	3.09
7	1, 1, 1, 3, 5, 5 ,5-heptamethyltrisiloxane	30.57	3.28
8	10-Hydroxy-10- (Phenylethynyl) Anthrone	30.76	5.57
10	1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine	33.45	44.39
11	1,3- Dimethyl-4-azaphenanthrene	34.68	5.36

Table (9): The chemical properties of the major compounds isolated from ethanolic extractives of *Piper nigrum* using GC/MS analysis.

Compound	Structure	Formula	Mw.	Area %	RT
Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)]		$C_{15}H_{24}$	204.351	5.50	14.54
10-Hydroxy-10-(phenylethynyl) anthrone		$C_{22}H_{14}O_2$	310.345	5.57	30.76
1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)		$C_{21}H_{25}NO_4$	355.427	3.09	26.19
1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine		$C_{17}H_{19}NO_3$	285.34	44.39	33.45
1,3- Dimethyl-4-azaphenanthrene		$C_{15}H_{13}N$	207.27	5.36	34.68

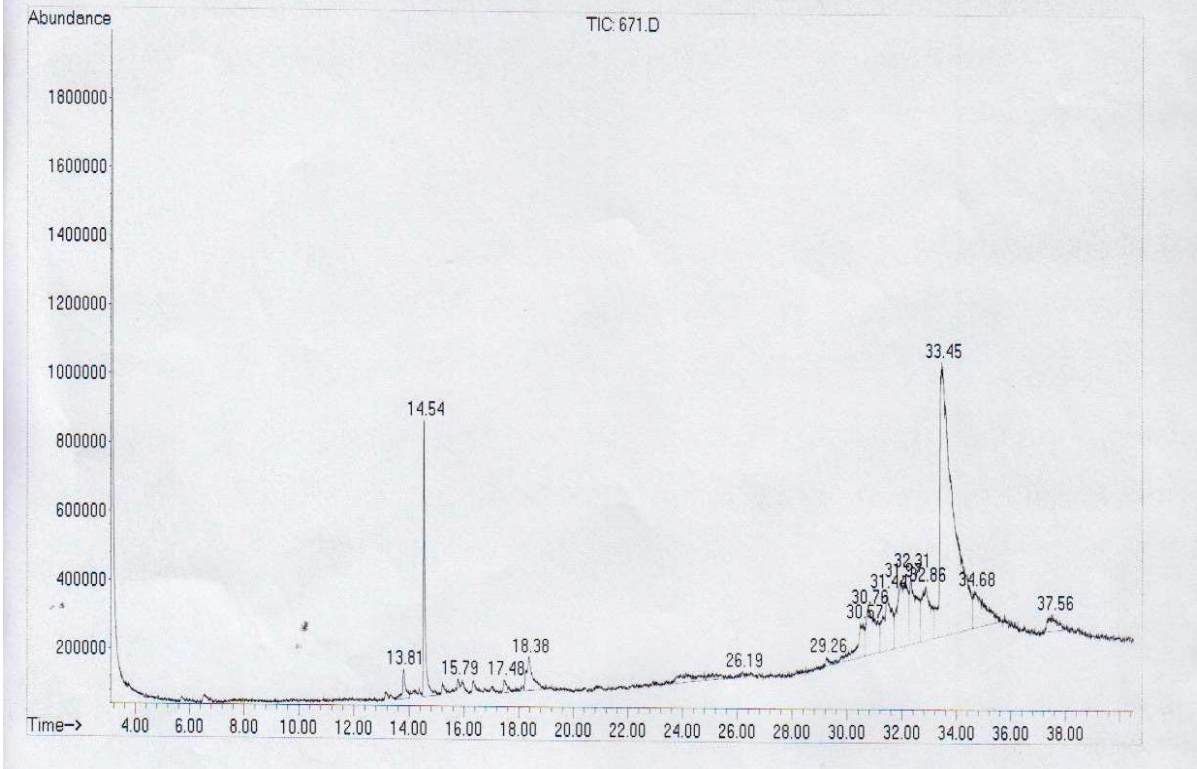


Figure (3): GC-MS analysis of crude ethanolic extractives of *Piper nigrum.*

The nematicidal principles of plants, based on materials such as isothiocyanates, thiophenics, glucosides, alkaloids, phenolics, thianins, and fatty acids, have been identified and reviewed (Fawole and Fatoki 2000; Ntalli *et al.*, 2010; Cavoski *et al*., 2012 and Aoudia *et al.,* 2012). The nematicidal effect of the tested extracts may be attributed to higher contents of certain oxygenated compounds, which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membranes of nematode cells and their functional groups interfering with enzyme protein structure (Knoblock *et al.*, 1989).

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