

Isolation, Identification and Evaluation of the Effective Phyto-Compounds for The Management of Insects Infesting Stored Groundnuts (*Arachis Hypogaea* L.)

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Abstract

The efficacy of various botanicals was tested against the major pest of groundnuts, the peanut moth *Corcyra cephalonica*, in Lab conditions for one year. Essential oils isolated by Clevengers Apparatus, @1% (v/w) of *Pongamia glabra* (Karanja Oil), *Azadirachta Indica* (Neem Oil), *Brassica campestris* (Mustard Oil), Crown oil (Resin of *Shorea robusta*), *Cymbopogon nardus* (Citronella) oil, *Dalbergia sisso* (Sisso Oil), *Oryza sativa* (Rice bran oil), *Helianthus annuus* (Sunflower Oil), *Ricinus communis* (Castor Oil), *Aegle marmelos* (Bael Oil), *Sesamum indicum* (Til Oil), etc were tested for their bioefficacy against the major pest, of groundnuts, *Corcyra cephalonica*, in Laboratory conditions for one year. Out of all these plant products *Cymbopogon nardus* (Citronella) oil and Crown oil (Resin of *Shorea robusta*) gave absolute pod/Kernel protection for one year. The LD₅₀ values were found out to be - 0.5% v/w. Spectroscopic GC MS analysis of Citronella oil (*Cymbopogon nardus*), lead to the identification of D-Limonene, Neral, Citral and 2,6-octadien-1-ol, 3,7-dimethyl acetate (Z) as essential main constituents. Spectroscopic GC MS analysis of Crown oil (Resin of *Shorea robusta*) are identified as 2-Ethyl-oxetane (RT 2.223), as the major product. It may be due to the aromatic nature of these phyto-products. Hence these phyto-products may be used as fumigants or insect repellants against *Corcyra cephalonica* and can be included in the package of practices to save stored groundnut in storage.

Keywords: Clevengers Apparatus, *Corcyra Cephalonica*, Bioefficacy, Spectroscopic, Phyto-Products Etc.

1. Introduction

Groundnut, *Arachis hypogaea* (L.) [1] is one of the major oilseed crop of India and popularly known as peanut. *Corcyra cephalonica* is a serious pest of stored groundnuts under hot dry conditions. India has a rich flora of economic importance especially higher plants having pesticidal properties (Prakash and Rao, 1997; Prakash et al., 1986, 89.) [2] To control their natural enemies, some plants have developed the ability to synthesize products that are derived from their secondary metabolism with some specific properties against insects. (Isman, 2006) The use of

naturally occurring plant materials to protect agricultural products against a variety of insect pests is an age old practice in some parts of the world (Peter 1985) [3]. Extracts from different plants have been shown to possess insecticidal properties against a wide range of insect pests [4] (Golob et al. 1982, Delobel and Malonga, 1987). About 2000 species of plants have been reported to possess biopesticidal properties in their bio-active components (Ahmed et al. 1984) [5]. The existing information on insecticidal, repellent and feeding deterrent

properties of plant components was reviewed by McIndoo (1945) [6], Jacobson (1958,1975). However relatively a few of these components are actually used in crop protection today. Research efforts are currently focused on the use of plant products such as plant powders, extracts and oils which are cheaper, safer and eco-friendly (Adediran and Ajayi,1996) [7]. Botanical insecticides tend to have a broad spectrum activity (Talukder and Howse, 2000). They are safe and relatively specific in their mode of action, easy to process and use (Sighamony.et.al.1984, Rajapakse and Van Embden 1997) [8]. There is a need to explore these plant products for their biopesticidal properties against the pest *Corcyra cephalonica*. Hence the present study was set up to examine the bioefficacy of certain phyoproducs against *Corcyra cephalonica* for their use in management of the losses caused by this serious pest on shelled groundnuts.

2. Materials and Methods

First the promising plants were screened against the moth *Corcyra cephalonica* and their bioefficacy was tested. The effectiveness of certain plant products (table) were tested under artificial infestation in laboratory conditions for nine months. Hundred grams of groundnut pods/Kernels were taken in glass bottles in three replicated sets for each test plant products. Oil formulations extracted by Clevenger's apparatus [9] (Hydrodistillation method) were applied @ 0.1% v/w to the pods/Kernels. The disease-free plants were chosen from the of university's botanical garden to extract bioactive compounds. The plants and their leaves were collected, chopped into pieces, and allowed to dry at room temperature before the cut leaves of the chosen plants were inserted into the 5000 ml Flask of the Clevenger's Apparatus for extraction of the volatile essential oils [10]. Fresh whole plants were subjected to hydro distillation in a modified Clevenger type apparatus to extract the volatile components. The essential oils obtained were each around 5g (2.78%) after drying over 1g anhydrous Sodium Sulphate. Once the drying process was complete, the mixture was filtered to remove the sodium sulfate with a funnel with filter paper. Ten pairs of freshly emerged live *Corcyra cephalonica* insects of equal number of

both sexes were released into each bottle 24 hours after treatment. The adult male & female population of the test insect was counted and recorded at intervals of 24h, 48h, 72h, 96h, 30 days, 60 days, 90 days, 120 days, 180 and 210 days of release. The final adult male and female population of test was recorded after 270 days of release.

Adulticidal effect of extracts: To further test the various extracts, young adults of cultivated beetle were used on 90mm width Petri dishes. The experiment was carried out in 90 mm width petridish with Whatman's filter paper that had been soaked in 3 ml of the test solution. In a petridish, 12 insects (6 male and 6 females) younger than 24 hours are placed for each concentration and control group. Dead and living organisms are counted 12, 24, 48, and 96 hours (Refer Table 1 &Figure 1 & 2).



Figure 1 Samples of Plant Extracts



Figure 2 Clevengers Apparatus

Table 1 Population Reduction

S.No	Treatment	Concentra tion of Treatment	Insect population after 270 days			Mean Insect population	% Reduction of insect population over Control
			R1	R2	R3		
1.	Arachis hypogea (Groundnut Oil)	0.1% v/w	21	30	23	24.66	64.77
2.	Mentha spicata (Mint Oil)	0.1% v/w	27	17	19	21.0	70.00
3.	Pongamia glabra (Karanja Oil)	0.1% v/w	21	30	23	22.66	68.57
4.	Brassica campestris(Mustard Oil)	0.1% v/w	41	37	38	36.67	47.61
5.	Azadirachta indica (Neem Oil)	0.1% v/w	11	10	21	19.67	71.9
6.	Aegle marmelos (Bael Oil)	0.1% v/w	27	34	21	28	60.0
7.	Sesamum indicum (Til Oil)	0.1% v/w	28	17	13	20.34	70.94
8.	Crown oil (Shorea robusta)	0.1% v/w	NIL	NIL	NIL	NIL	100.00
9.	Oryza sativa (Rice bran oil)	0.1% v/w	24	22	26	24	65.71
10.	Helianthus annus (Sunflower Oil)	0.1% v/w	45	47	41	40.34	42.37
11.	Ricinus communis (Castor Oil)	0.1% v/w	31	41	45	34.34	50.94
12.	Dalbergia sisso (Sisso Oil)	0.1% v/w	36	42	41	32.67	53.32
13.	Linum usitatissimum (Linseed Oil)	0.1% v/w	42	44	38	45.67	34.75
14.	Citronella oil (Cymbopogon nardus)	0.1% v/w	NIL	NIL	NIL	NIL	100.00
	Control		72	68	70	70	

The percent reduction of insect population over control has been calculated using the formula as follows:

$$\text{The \% Population reduction} = \left(\frac{\text{Population in control} - \text{population in treatment}}{\text{Population in control}} \right) \times 100$$

The data thus recorded were analyzed statistically and present in the following tables. That active

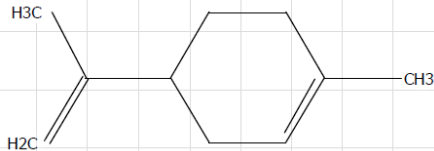
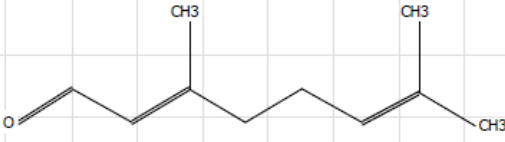
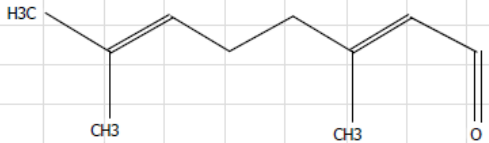
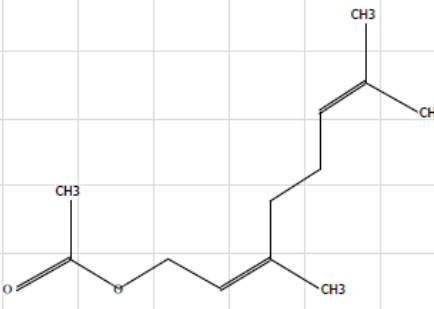
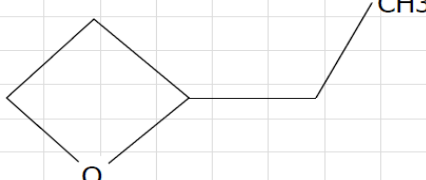
ingredients of the most effective oil formulations were further subjected to GC-MS to determine the chemical structure of the major compounds.

2.1.GCMS analysis

A GC-MS apparatus (model MSGC-11) with an HP-3 capillary column (50 mm 90.521 mm, film thickness 0.25 mm) was used to examine the extract. For analysis, a 1 ml extract was cautiously added to a GC-MS. Peaks and retention time were present in the

resulting chromatogram. A peak represents a compound's m/z ratio, and the altitude of the peak indicates the amount of the chemical. The solution's retention indices (RI) and mass fragmentation pattern were used to determine its chemical makeup. (Refer Table 2 & Figure 3). The major compounds identified in the GC MS study of the isolated Citronella oil (*Cymbopogon nardus*) are as follows-

Table 2 Structure of the Oil

S.No.	Name of the Oil	Major component	Structure	Remark
1	Citronella oil (<i>Cymbopogon nardus</i>)	D-Limonene		Pleasant citrus aroma, Insect Repellents, Deodorizing Properties
2		Neral		Aromatic Deterrence, antimicrobial, antifungal, and anti-inflammatory.
3		Citral		Fresh, lemony aroma.
4		2,6-octadien-1-ol, 3,7-dimethyl acetate (Z)		Found in the essential oils of: Lavender (<i>Lavandula</i> spp.) Bergamot (<i>Citrus bergamia</i>) Clary sage (<i>Salvia sclarea</i>)
5		2-Ethyl-oxetane		Ether-like smell.

D-Limonene is one of the major products (RT 4.007).

Compound Label	Name	RT	Algorithm
Cpd 7: D-Limonene; C ₁₀ H ₁₆ ; 4.007	D-Limonene	4.007	Find by Integration

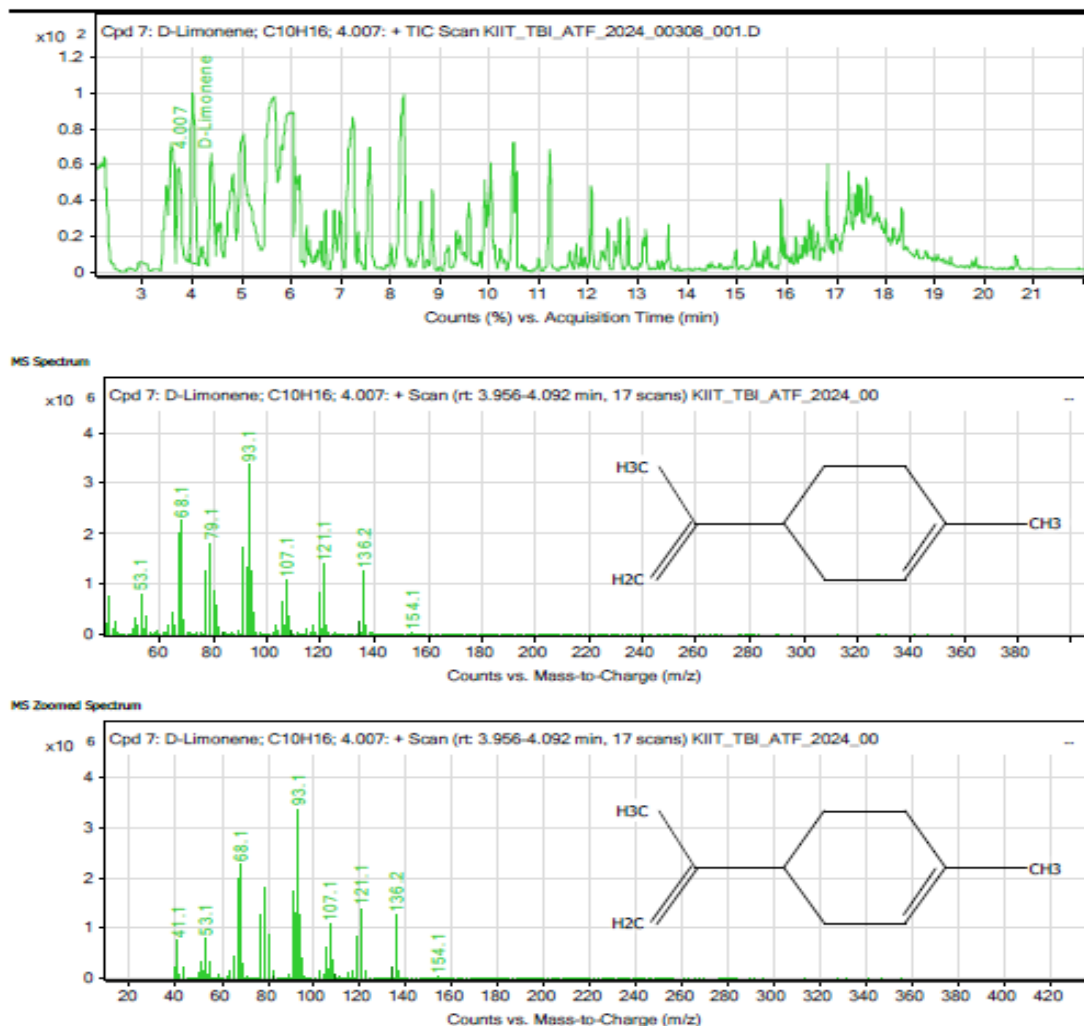


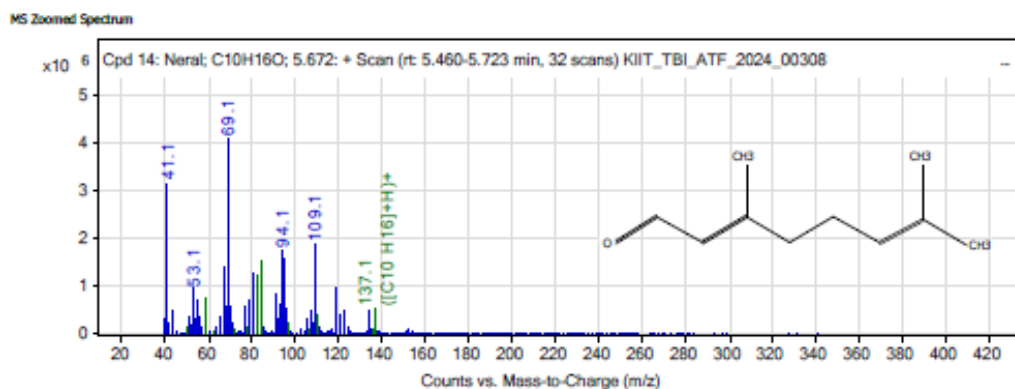
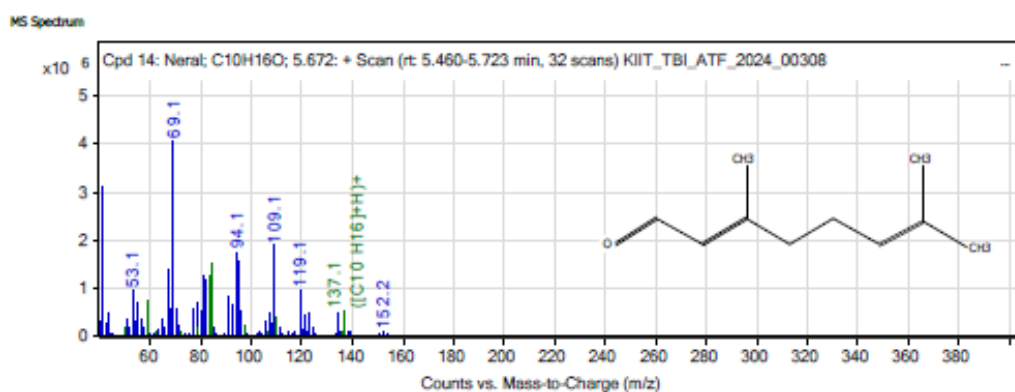
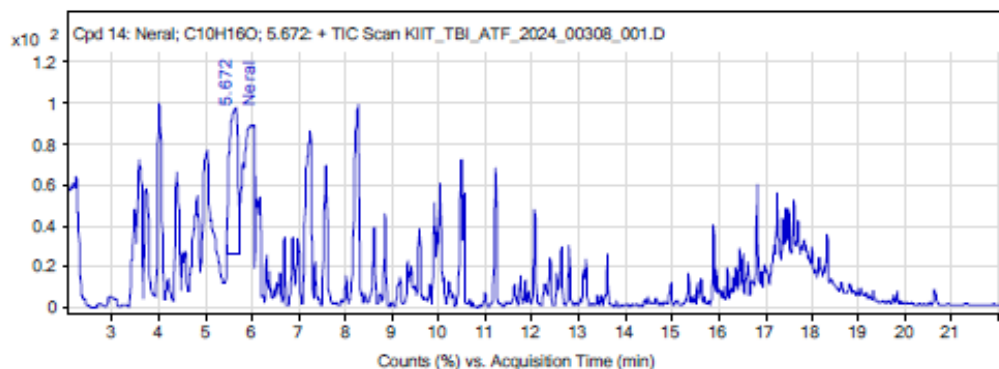
Figure 3 GCMS spectra of Limonene

Table 3 MS Spectrum Peak List

m/z	z	Abund
67.1		1998696.25
68.1		2279747.75
77.1		1262120.13
79.1		1811141.13
91.1		1735272.88
92.1		1326500.88
93.1		3366274.25
94.1		1263497.13
121.1	1	1390161
136.2	1	1270134.5

Neral is one of the major products (RT 5.672).

Compound Label	Name	RT	Algorithm
Cpd 14: Neral; C10H16O; 5.672	Neral	5.672	Find by Integration

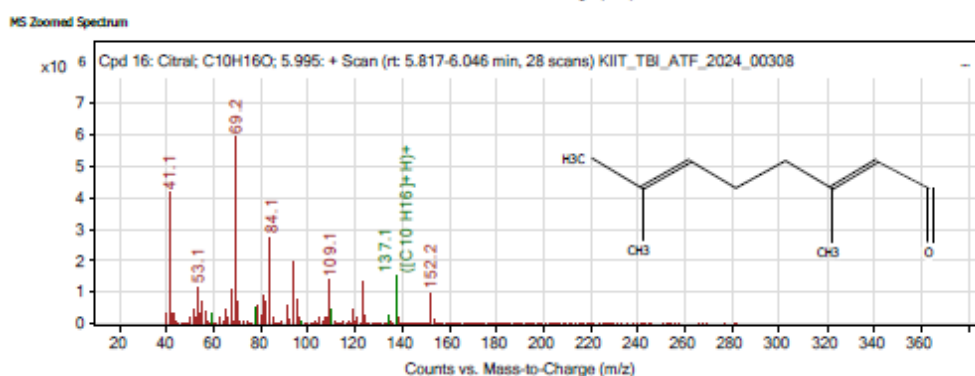
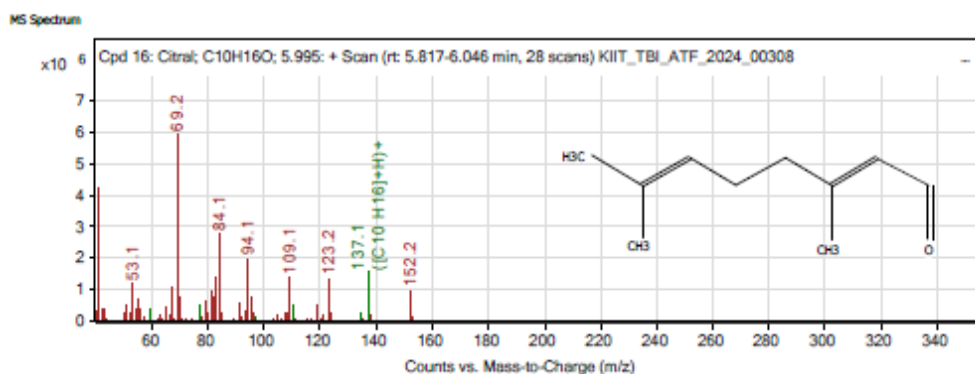
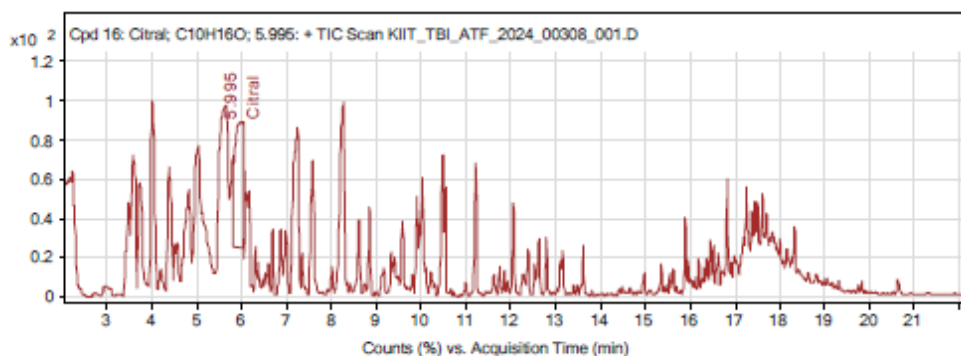


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
41.1				3141769.75		
67.1				1386761.25		
69.1				4079399.75		
81.1				1244124		
82.1				1143467.13		

Citral is one of the major products (RT 5.995).

Compound Label	Name	RT	Algorithm
Cpd 16: Citral; C ₁₀ H ₁₆ O; 5.995	Citral	5.995	Find by Integration

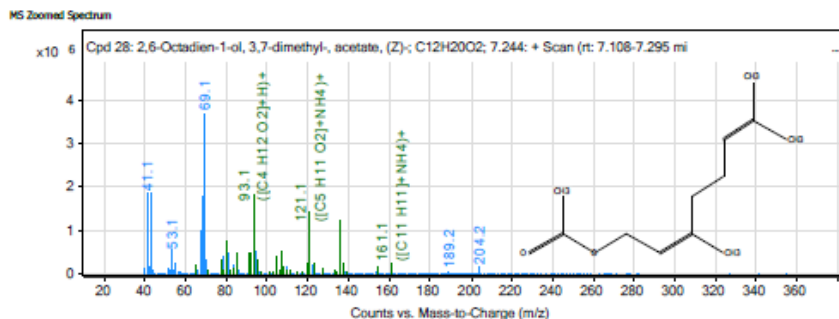
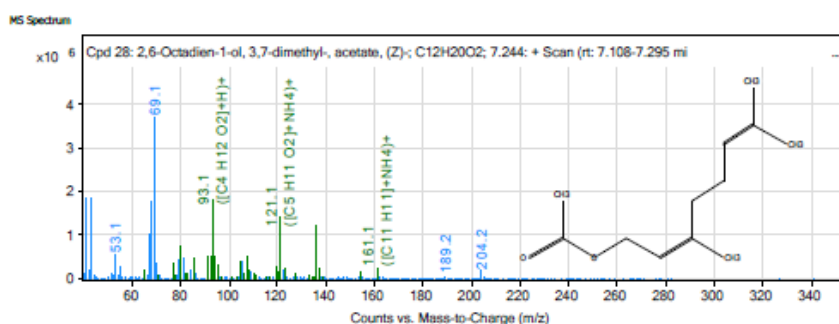
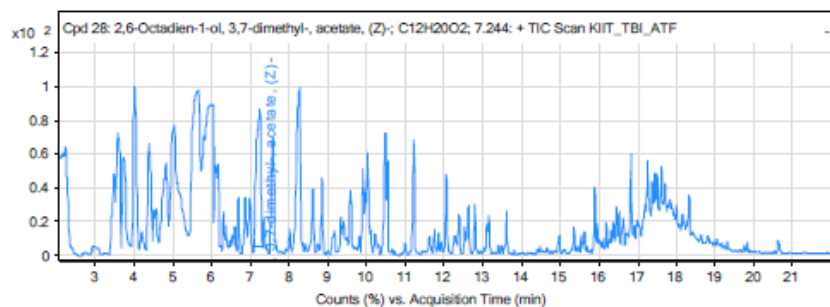


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
41.1				4188797.25		
53.1				1169810.38		
67.1				1096767.63		
69.2				5946461		
83.1				1372431.75		
84.1			1	2754443.25		
94.1				1971483		
109.1				1383663.88		
123.2				1341724.75		
137.1	136.1	-7331.35	1	1537158.63	C ₁₀ H ₁₆	(M+H) ⁺

2,6-octadien-1-ol,3,7-dimethyl acetate (Z) is one of the major products (RT 7.244).

Compound Label	Name	RT	Algorithm
Cpd 28: 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-; C ₁₂ H ₂₀ O ₂ ; 7.244	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	7.244	Find by Integration



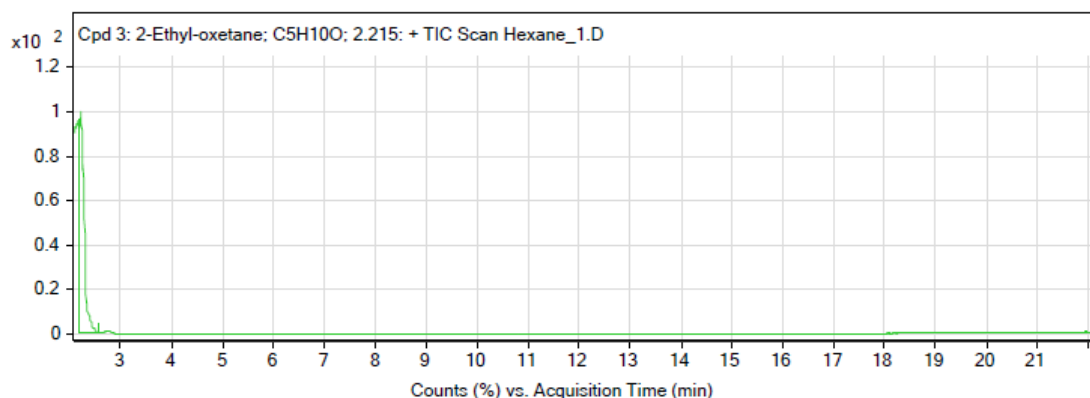
MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
41.1				1854846.75		
43.1			1	1853784.5		
53.1				533093.38		
67.1				995897.19		
68.1				1780784		
69.1				3690500.25		
80.1	80.1	7.56		734706.19	C ₂ H ₆ O ₂	(M+NH ₄) ⁺
93.1	93.1	10.81		1798012.38	C ₄ H ₁₂ O ₂	(M+H) ⁺
121.1	103.1	-148911.27	1	1419201.13	C ₅ H ₁₁ O ₂	(M+NH ₄) ⁺
136.1				1230713.88	C ₆ H ₁₄ O ₂	(M+NH ₄) ⁺

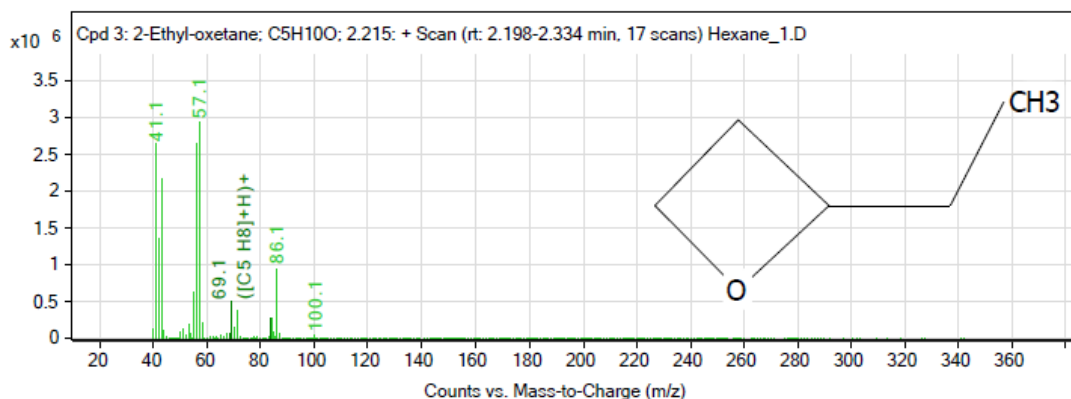
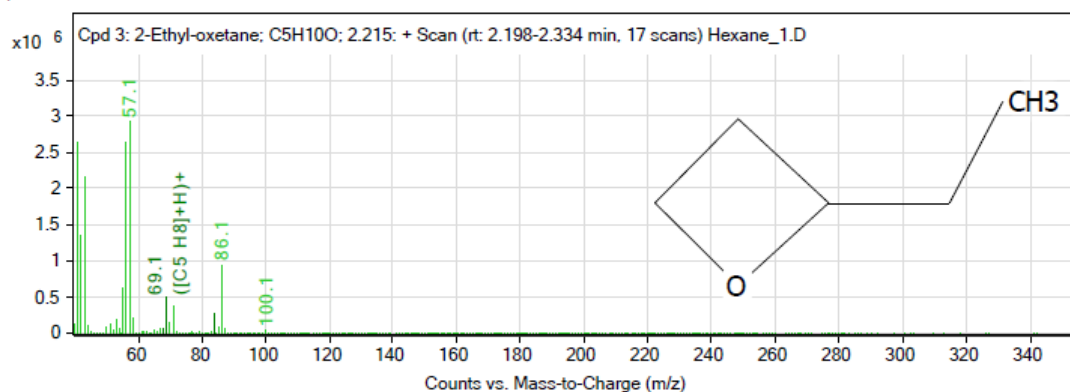
The major compound identified in the GC-MS study of the isolated Crown oil are as follows-

MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
41.1				2635557.5		
42.1				1352945.38		
43.1			1	2163007		
55.1				613889.56		
56.1				2641819.75		
57.1			1	2926010.25		
69.1	69.1	-1.79		489190.5	C5 H8	(M+H)+
71.1				358559.94		
84.1	84.1	9.23		261556.2	C5 H6	(M+NH4)+
86.1			1	944073.5		



MS Spectrum



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
41.1				2635557.5		
42.1				1352945.38		
43.1			1	2163007		
55.1				613889.56		
56.1				2641819.75		
57.1			1	2926010.25		
69.1	69.1	-1.79		489190.5	C5 H8	(M+H)+
71.1				358559.94		
84.1	84.1	9.23		261556.2	C5 H6	(M+NH4)+
86.1			1	944073.5		

3. Results and Discussion

Spectroscopic GC-MS analysis of Citronella oil (*Cymbopogon nardus*), lead to the identification of D-Limonene, Neral, Citral and 2,6-octadien-1-ol,3,7-dimethyl acetate (Z) as major constituents. Spectroscopic GC-MS analysis of active component of Crown oil (*Shorea robusta*) are identified as 2-Ethyl-oxetane as the major component. It may be due to the aromatic nature of these compounds. The investigation on the effectiveness of Citronella oil & Crown oil extract as a green pesticide against the pest *Corcyra cephalonica* revealed that the extracts have potent pesticidal effects on the groundnut seed beetle. The extract significantly decreased egg-hatching rates and induced mortality among adult beetles, according to the laboratory's present investigation. The extract worked better at higher concentrations, indicating a dose-dependent response. To create commercial formulations, the ideal concentration for optimum performance without phytotoxicity was determined as 0.1% v/w. The protective capability of treated seeds was demonstrated by their reduced degrees of damage as compared to untreated controls. This suggests that it could be used as a natural substitute for artificial insecticides. Citronella oil (*Cymbopogon nardus*) and Crown oil leaf extracts are an eco-friendly choice because they are plant-based. The presence of bioactive substances in both plant leaves, such as D-Limonene, Neral, Citral and 2,6-octadien-1-ol,3,7-dimethyl acetate (Z), 2-Ethyl-oxetane and Carene, which are known to have insecticidal and repellent qualities, may cause the pesticide effect. These substances probably cause death and decreased reproduction by interfering with the beetle's neurological system, breathing system, or eating habits. Because the extract is economical,

biodegradable, and unlikely to cause bugs to develop resistance, it is consistent with sustainable pest management techniques. The plant is affordable for farmers with low resources because it is widely accessible and its extraction procedure is not too complicated. The leaf extract is a safer option for ecological and agricultural systems because it may present less danger to non-target creatures. To confirm its commercial use as a biopesticide, the study may suggest more research to standardize extraction techniques, adjust dosages, and assess its long-term effects in field settings. This finding encourages more research and development for practical application while highlighting the potential of the studied leaf extract as a green insecticide. Hence these phytoproducts may be used as fumigant or insect repellent against the moth *Corcyra cephalonica* and can be added in the package of practices to save stored shelled groundnut in storage.

Conclusion

The study successfully isolated and characterized bioactive phyto-compounds from natural sources that demonstrate effective pesticidal properties against groundnut beetles, a significant pest in stored groundnuts. Using GC-MS, the compounds were identified and assessed for their bioactivity. The following conclusions can be drawn:

- **Effective Phyto-Compounds:** Several plant-derived compounds were identified with potent insecticidal and repellent properties. Among these, compounds such as **limonene, neral, citronellal, and other monoterpenes** exhibited strong pesticidal activity against groundnut beetles.

- **Mechanism of Action:** The efficacy of these compounds was attributed to their ability to interfere with the beetles' sensory perception, causing deterrence or mortality. Their aromatic nature also plays a role in creating an unfavorable environment for pest infestation.
- **Eco-Friendly Alternative:** These natural phyto-compounds provide an environmentally sustainable alternative to synthetic chemical pesticides, reducing harmful residues in food storage systems and contributing to safer agricultural practices.
- **Economic Viability:** The study highlights the cost-effectiveness of using plant-derived pesticides, especially in rural and agrarian settings where groundnuts are a staple crop.
- **Future Scope:** Further research and formulation studies are required to develop these phyto-compounds into commercial pesticidal products with enhanced stability and prolonged efficacy. Exploring synergistic combinations of these compounds may yield even better results in pest management.

In summary, the study underscores the potential of natural phyto-compounds as a sustainable and effective solution for managing groundnut beetles, ensuring the safety and quality of stored groundnuts while promoting eco-friendly pest control practices.

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