Insecticidal Activity of Commercial Oil and Petroleum Ether Extract of Clove Buds (*Syzygium aromaticum*) on Some Stored Grain Pests.

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ABSTRACT

This study aimed to evaluate the insecticidal activity of commercial clove oil and clove petroleum ether extract (*Syzygium aromaticum*) on the adults of rice weevil, *Sitophilus oryzae* (L.); cowpea beetle, *Callosobruchus maculatus* (F.); red flour beetle, *Triboleum casteneum* (H.) ; the mould mite, *Tyrophagus putrescentiae* (Shrank) *and Dermatophagoides farinae* (Shrank) at 30±2°C and 65±5% R.H. Besides, studying the efficacy of clove petroleum ether extract to protect grains during storage and if it had any effect on seeds germination plus analyzing the chemical constituents for both extract and commercial oil through GC/MS technique to study the difference in main constituents. Results showed complete mortality (%) at the highest concentration with *S.oryzae*, *C.maculatus* and *T.putrescentiae* while *T.casteneum* and *D. farinae* showed high resistance for both oil and extract. Accumulative mortality of pests increased gradually with the increase of concentrations and exposure period. The reproduction of females of all experimental pests was completely inhibited at the highest concentration. *T. castaneum* was less susceptible to tested oils compared with *S.oryzae* and *C.maculatus*. The extract was more powerful than commercial oil especially with *T.casteneum* and *D. farinae* which are known to have high resistance. Clove extract give complete protection for grains for 10 weeks with low significant on germination (%). The main constituents of clove commercial oil were eugenol (50.58%), cinnamaldehyde (15.72 %) and linoleic acid (9.52%) and those of the clove extract were eugenol (37.43%) eugenol acetate (11.47%), caryophellene (10.44 %), linoleic acid (9.42%) and caryophellene oxide (8.58%).

Key words: stored grain pests, clove oil, germination of seeds, Clove extract, residual toxicity and mites.

INTRODUCTION

There is increasing interest both in industry and scientific research for aromatic and medicinal plants because of their potential applications in medicine and plant disease control measures. The antimicrobial, antifungal and insecticidal properties of plant essential oils are well established against wide spectra of organisms such as fungi, bacteria and insects. These properties are mainly due to many active phytochemicals including vitamins, flavanoids, terpenoids, carotenoids, coumarins, curcumines, etc. and hence, they are of great importance in food industry and offer the possibility to substitute natural sources for synthetic preservatives and other products, (Gurdip and Sumitra, 2005).

Pests of stored grains include rice weevil, *Sitophilus oryzae* (L.); red floor beetle, *Tribolium castaneum*; cowpea beetle, *Callosobruchus maculatus* and the mould mite, *Tyrophagus putrescentiae*, (Park *et al.*, 2003; Demitry *et al.*, 2007 & Li, 2004).

The bioactivity of clove oil and extract as pest control agents against stored grain pests was studied by many investigators, (Lee *et al.*, 2001; Mahfuz and Khalequzzaman,2007 ; Sumadi *et al.*, 2010; Zeng, *et al.*,2010 & Khalequzzaman and Rumu, 2010).

The main chemical constituent of clove oil is eugenol which represents about 74.3% (Alma *et al.*, 2007; Ayoola1 *et al.*, 2008 & Nazrul *et al.*,2010) and known as powerful insecticide (Kwon Park and Sang-Chul Shin 2005 & Zeng *et al.*, 2010) .

Many researchers used commercial oils to prove its effectiveness against stored grain pests, therefore, this research aims to study the differences of toxicity between commercial oil and oil extract in the laboratory, also to study the effect of clove oil extracts in laboratory to protect seeds during storage.

MATERIALS AND METHODS

1) REARING:

1-A) INSECT REARING:

Tested insects were reared in glass jars (each of approximately 500 ml) containing about 250 gm of cowpea and wheat seeds. Each jar was covered with muslin cloths and fixed with rubber bands for egg laying and incubated at 30±2°C and 65±5 % R.H.

1-B) MITE REARING:

Strains of mould mites, *Tyrophagus putrescentiae* and *Dermatophagoides farinae* were collected from infested wheat samples for obtaining a pure culture, adults were placed in rearing plastic rings containing yeast for feeding at 30±2°C and 65±5 % R.H.

2) BIOASSAY TESTS:

2-a) PLANT EXTRACTS:

To obtain the plant extracts, 500 gm of clove buds (*Syzygium aromaticum*) was ground in an electric mill into fine powder then soaked in petroleum ether solvent in a large flask for 5 days. The flask was shacked for one hour in a shaker and its content was filtered. The solvent was evaporated at 50°c under reduced pressure using a rotary evaporator as

described by (Su, 1985). The extract in the form of a crude gum was weighted and dissolved by the same solvent to get 10% (w/v) stock solution. Concentrations of 3, 2, 1, 0.5, 0.25 and 0.125 (w/v) were prepared by diluting the stock solution.

To investigate the effect of clove extract on three insects, *S. oryzae, T. casteneum, C. maculatus* and two mite species, *Tyrophagus putrescentiae and Dermatophagoides farinae*, about 10 grams of wheat and cowpea seeds were put separately into glass jars of 50 ml, mixed with clove extract and left for dryness (for 24hrs). Twenty adult insects (1-2 week old *S. oryzae* and *T. casteneum*) and (1-24 hrs old mites, *T. putrescentiae* and *D. farinae*) were confined with treated wheat seeds and (1-24 hrs old *C.maculatus*) was confined with the treated cowpea seeds. Jars were covered with muslin fixed with rubber bands and kept at 30±2°C and 65±5 % R.H. Every treatment was replicated three times. A set of jars contained untreated seeds were used as control.

To evaluate the efficacy of clove extract on tested pests, the mortality percentages were estimated after 2, 3, 5, 7 and 10 days of exposure for S. *oryzae* and *T. casteneum*; while for *C. maculatus* after 1, 2, 3, 4, 5 days and after 1, 3, 5, 7 days for mites. Mortality percentages were corrected using Abbot's formula (1925). The number of offspring (progeny) was also determined after 35 days from treatment for *C.maculatus* and after 65 days for *S.oryzae* and *T. casteneum*.

Reduction percentages in progeny of offspring were calculated by the following equation (El-Lakwah *et al.*, 1996).

% reduction = {(Offspring emerged in control – offspring emerged in treatment)/ Offspring emerged in control}x 100

2-b) Commercial oil:

Samples of 10 grams of each of cowpea and wheat seeds were mixed with four different concentrations; 2, 4, 6 and 8 mg/kg of oil. Three replicates were used for each concentration. The jars were covered with muslin and sealed with rubber bands, and kept for 48hrs at room temperature for oil adsorption. Three replicates of non treated seeds were made as control. Twenty adult insects (1- 2 weeks old *S. oryzae* and *T. casteneum*) and (1-24 hrs old mites, *T. putrescentiae* and *D. farinae*) were confined with the treated wheat seeds and (1-24 hrs old *C. maculatus*) confined with the treated cowpea seeds. Jars were covered with muslin fixed with rubber bands and kept at 30±2°Cand 65±5 % R.H. Mortality (%) counts were calculated as mentioned before. Reduction percentage in progeny of offspring was calculated.

3) DATA ANALYSIS

The mortality of the tested pest adults was probit analyzed using a computer program named ldp-line according to Finney (1971), from which the toxicity values (Lc_{50} and Lc_{95}) and Slope values of the tested compounds were estimated.

4) RESIDUAL TOXICITY STUDIES:

4-a) PERSISTANCE TESTS:

To study the residual efficacy of clove extract against *C. maculatus* and *S. oryzae*; LC_{95} conc. was mixed separately with 100 gm of cowpea and wheat seeds and stored for 10 weeks under laboratory conditions. One hundred gm of untreated seeds were used for control. Three replicates were used for each group. Five gm of each treated and untreated seeds were placed into a glass pot and infested with 5 pairs of insects. Three replicates were used for each group. The mortality percentages of insects after 5 days of treatment and reduction in F_1-progeny after 35 (for *C. maculatus*) and 65 days (for *S. oryzae*) were recorded. The toxicity was tested every week.

4-b) SEED GERMINATION TEST:

The germination of treated seeds with LC_{95} conc. and control seeds was tested after 24 hours and 10 weeks from storage. For this assay, 20 seeds from each treated and untreated replicate were placed separately in Petri dish, under laboratory conditions but without insects. The germination of seeds was evaluated for each treatment. Each group of seeds was placed on moist filter paper in Petri dishes and incubated at 25° C.

5) GC/MS CHROMATOGRAM:

The chemical constituents of both clove extract and commercial oil were identified by GC/MS (Gas chromatography-mass spectrometry) gas chromatograph equipped with an agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column pas-5 ms (30mm x 0.25 um film thickness). Samples were injected under the following conditions:

Helium was used as carrier gas at approximately 1ml/min., pulsed splitless 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 ionizing energy 70 e.v. scanning from m/z 50 to 500. The ion source temperature was 230°C and the quadrupole temperature was 150°C. The electron multiplier voltage (EM voltage) was maintained 1250V above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60°C then elevated to 280°C at rate of 8c /min, and 10 min. hold at 280°C the detector and injector temperature were set at 280°C and 250°C, respectively.

Identification of the compounds:

Compound identification was made by comparing the NIST and WILEY libraries and with the authentic spectra (Adams, 1995). Data of the peaks with those reported in literature, mass spectra of the peaks with literature data. Percentage composition was computed from GC peak areas on BP-I column without applying correction factors.

RESULTS AND DISCUSSION

Results in tables 1, 2 and 3 indicated that the mortality rate increased with the increasing of concentration and exposure period with both the commercial oil and the extract. Both extract and oil have high insecticidal activity against *C. maculatus* and *S. oryzae* causing 100% mortality with the highest conc. at the end of the experiment; while with *T. casteneum* the extract caused 70% at the highest conc. and show high resistance to commercial oil.

Both oil and extract gave 100% reduction in F_1- progeny at most tested concentrations with *C. maculatus* and *S. oryzae*. Although the oil and extract were not powerful against *T.casteneum* they caused 100 % reduction in F_1- progeny with the highest concentration.

These results were in harmony with those obtained by Abd El-Salam (2010), who tested the fumigant toxicity of clove oil on both *C.maculatus* and *S. oryzae* and found that the oil gave high mortality values; *C.maculatus* was more susceptible to the oil than S. oryzae. Fouad (2013a), who found that, clove oil, repelled all *C.maculatus* adults at 1% conc. Mondal and Khalequzzaman (2006), found that the clove oil didn't give high mortality values at LC50 concentration with adults and larvae of *T.casteneum* in both contact and fumigant bioassay. Zeng *et al.*, (2010), found that clove oil and its two main chemical constituents 2-methoxy-4-(2-propenyl)-phenol and trans-caryophyllene gave high toxicity and repellency grade with *R. dominica*, *S.oryzae* and *T. castaneum*. Mahfuz and Khalequzzaman (2007) tested 5 essential oils against *Callosobruchus maculatus* and found that the toxicity of the oils followed in the order: eucalyptus > clove > cinnamon > cardamom > neem. In the fumigation bioassay after 24 and 48 h of treatments. Mishra *et al.*, 2014 screened the chronic activity of clove oil against rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) in laboratory assay and showed that fumigation with sub-lethal concentration of essential oil significantly ($p<0.01$) reduced oviposition and exhibited ovicidal activity. Fouad (2013b) found that clove oil had high repellency rate at 4% concentration against the faba bean beetle *Bruchidius incarnates*. Mahdi and Khaladur Rahman (2008) recorded that clove oil was effective as protectant of black gram seeds against the pulse beetle, *Callosobruchus maculatus* (F.). Sabbour and Abd-El-Aziz (2009) tested clove oil against *Bruchidius incarnates* and recorded it as a strong repellent after 7 days from treatment.

As shown in tables 4 and 5, mortality rate increased with increasing of concentration and exposure period. Both oil and extract were powerful against the mould mite, *T. putrescentiae*; while with *D. farinae* the extract showed high mortality compared with commercial oil.

This result agreed with the results of kim *et al.*, 2003b who tested the acaricidal activity of clove bud oil compounds (acetyleugenol, v-caryophyllene, eugenol, a-humulene), and congeners of eugenol (isoeugenol, methyleugenol) against adult *Tyrophagus putrescentiae* and found that in fumigation tests with adult *T. putrescentiae*, all four phenylpropenes were more effective against the mite in closed containers than in open ones, indicating that the mode of delivery of these compounds was largely due to action in the vapor phase. Pumnuan and Insung (2011) who investigated the acaricidal activity of essential oils obtained from 28 selected medicinal plants against stored product mite, *Suidasia pontifica* Oudemans and found that at the dose of 1.0%, essential oils of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum bejolghota*), myrtle grass (*Acorus calamus*), betel vine (*Piper betle*), and turmeric (*Curcuma longa*) were highly toxic to *S. pontifica* with more than 70% mite mortality. Kim *et al.*, 2003a examined the acaricidal activity of clove (*Syzygium aromaticum*) bud oil-derived eugenol and its congeners (acetyleugenol, isoeugenol, and methyleugenol) against adults of *Dermatophagoides farinae* using direct contact application and fumigation methods. They found that on the basis of LD(50) values, the compound

Table (1): Toxicity of both clove oil and extract on *S.oryzae* adults infesting wheat seeds

Tested material	Conc. (ml/kg)%	(%)Adult mortality after indicated days					No. of F1 progeny after 60 days	% reduction in F1 progeny
		2	3	5	7	10		
Clove oil	8	91.6±3.3	100±0	100±0	100±0	100±0	0	100%
	6	86.6±3.3	100±0	100±0	100±0	100±0	0	100%
	4	85±5.78	100±0	100±0	100±0	100±0	0	100%
	2	21.6±4.4	100±0	100±0	100±0	100±0	2	97.5%
Control		0	0	0	0	0	83	
Clove petroleum ether extract	3	100±0	100±0	100±0	100±0	100±0	0	100%
	2	100±0	100±0	100±0	100±0	100±0	0	100%
	1	91.6±4.4	98.3±1.6	98.3±1.6	100±0	100±0	0	100%
	0.5	75±10	85±7.6	91.6±4.4	91.6±4.4	91.6±4.4	0	100%
	0.25	61.6±9.2	80±8.6	85±5.7	88.3±4.4	88.3±4.4	3	96%
Control		0	0	0	0	0	77	

Table (2): Toxicity of both clove oil and extract *on T. casteneum* adults infesting wheat seeds

Tested material	Conc. (ml/kg)%	(%)Adult mortality after indicated days					F1 progeny after 60 days	% reduction in F1 progeny
		2	3	5	7	10		
Clove oil	8	0±0	0±0	1.3±3.3	8.3±4.4	18.3±4.4	0	100%
	6	0±0	0±0	1.6±1.6	1.6±1.6	10±2.9	8	89%
	4	0±0	0±0	0±0	0±0	3.3±1.6	13	82.4%
	2	0±0	0±0	0±0	0±0	1.6±1.6	21	71.6%
Control		0	0	0	0	0	74	
Clove petroleum ether extract	3	31.6±6	41.6±6	55±7.6	55±7.6	70±10.4	0	100%
	2	6.6±3.3	13.3±4.4	15±5	16.6±6	21.6±4.4	0	100%
	1	3.3±3.3	5±2.8	5±2.8	6.6±1.6	13.3±4.4	0	100%
	0.5	0±0	0±0	1.6±1.6	3.3±3.3	8.3±3.3	14	78.5%
Control		0	0	0	0	0	65	

Table (3): Toxicity of both clove oil and extract *on C.maculatus* adults infesting cowpea seeds.

Tested material	Conc. (ml/kg)%	(%)Adult mortality after indicated days					F1 progeny after 27 days	% reduction in F1 progeny
		1	2	3	4	5		
Clove oil	8	100±0	100±0	100±0	100±0	100±0	0	100%
	6	100±0	100±0	100±0	100±0	100±0	0	100%
	4	100±0	100±0	100±0	100±0	100±0	0	100%
	2	83.3±4.4	91.6±3.3	93.3±1.7	98.3±1.7	98.3±1.7	0	100%
Control		0	0	0	0	0	88	
Clove petroleum ether extract	3	100±0	100±0	100±0	100±0	100±0	0	100%
	2	100±0	100±0	100±0	100±0	100±0	0	100%
	1	86.6±6	91.6±4.4	95±2.8	95±2.8	96.6±1.6	0	100%
	0.5	70±7.6	81.6±8.3	85±7.6	86.6±6	90±7.6	0	100%
	0.25	51.6±7	61.6±6	73.3±6	75±5.7	76.6±7	0	100%
	0.125	21.6±7	43.3±6	58.3±13	63.3±10	71.6±8.3	0	100%
Control		0	0	0	0	0	138	

Table (4): Toxicity of both clove oil and extract *on T. putrescentiae* adults infesting wheat seeds.

Tested material	Conc. (ml/kg)%	(%)Adult mortality after indicated days			
		1	3	5	7
Clove oil	2	76.6±3.3	100±0	100±0	100±0
	1	66.6±6.6	96.6±3.3	100±0	100±0
	0.5	53.3±8.7	76.6±12	80±10	90±5.7
	0.25	46.6±6.6	50±5.7	60±5.7	60±5.7
Control		0	0	0	0
Clove petroleum ether extract	3%	75±0.8	90±0.57	100±0	100±0
	2%	35±8.82	70±5.7	80±0	90±5.7
	1%	10±8.8	30±5.7	60±5.7	70±5.7
	0.5%	0±0	23.3±3.3	50±5.7	60±0
control		0	0	0	0

Table (5): Toxicity of both clove oil and extract *on D. farinae* adults infesting wheat seeds

Tested material	Conc. (ml/kg)%	(%)Adult mortality after indicated days			
		1	3	5	7
Clove oil	2	10±5.7	26.6±3.3	33.3±6.6	46.6±3.3
	1	6.6±6.6	23.3±3.3	23.3±3.3	33.3±8.8
	0.5	3.3±3.3	13.3±6.6	13.3±6.6	20±10
	0.25	3.3±3.3	10±1	10±1	10±1
Control		0	0	0	0
Clove petroleum ether extract	3%	30±1.5	93.3±3.3	96.6±3.3	96.6±3.3
	2%	20±5.7	55±8.8	70±8.82	77±3.33
	1%	15±8.8	40±03.3	60±5.7	60±5.7
	0.5%	10±0	20±3.3	20±0	30±5.7
control		0	0	0	0

Table (6): Relative potency values of clove commercial oil on tested pest adults.

Tested pests	Lc50	Lc95	Slope	Toxicity index (%)
S.oryzae	0.0286	0.0791	3.7162±0.4223	11.72
T.casteneum	0.2089	1.0839	2.3004± 0.7511	1.62
T. putrescentiae	0.0034	0.2112	0.9195±0.3555	100
D. farinae	0.0223	0.3968	1.3158± 0.3930	15.45

Table (7): Relative potency values of clove petroleum ether extract on tested pest adults.

Tested pests	Lc50	Lc95	Slope	Toxicity index (%)
S.oryzae	0.0617	0.6186	1.6427± 0.6303	100
C.maculatus	0.0927	1.2118	1.4700± 0.2829	66.6
T.casteneum	2.6266	12.8426	2.3865± 0.3698	2.36
T. putrescentiae	1.198	5.2277	2.5708±0.4543	5.17
D. farinae	0.8357	3.944	2.4410± 0.4582	7.42

Toxicity index = LC50 of the most effective pesticide/ LC50 of the tested pesticide x 10

most toxic to *D. farinae* adults was methyleugenol (0.94 microg/cm(2)) followed by isoeugenol (5.17 microg/cm(2)), eugenol (5.47 microg/cm(2)), benzyl benzoate (9.22 microg/cm(2)), and acetyleugenol (14.16 microg/cm(2)). Eugenol and its congeners merit further study as potential house dust mite control agents or as lead compounds.

The advantages of using essential oils as grain protectants are: (1) they can be easily extracted by steam distillation or chemical solvents; (2) they have very low toxicity to mammals since they are popular spices consumed by people in various parts of the world; and (3) the essential oils are volatile and this can be potentially used as fumigants.

In conclusion: clove extract was powerful than commercial oil in controlling stored grain pests especially those known to have high tolerance.

2) DATA ANALYSIS

The probit statistics estimate of LC_{50}, LC_{95} and the slope of regression lines of clove commercial oil and extract are presented in Tables (6 & 7) and Figs. (1 & 2).

From probit analysis, it was found that the highest toxicity of commercial oil was with *T. putrescentiae* and the lowest with *T. casteneum*; while the highest toxicity of clove extract was with *S.oryzae* and the lowest was with *T.casteneum.*

When probit regression lines of both clove oil and extract against all tested pests were calculated, they showed a linear relationship between mortality percentage and concentration.

3) RESIDUAL TOXICITY STUDIES:

3-a) PERSISTANCE TESTS:

The residual effect of clove petroleum ether extract was experimented to evaluate its efficacy during storage for 10 weeks. The toxicity was tested every week. The used concentrations were the lowest values of LC_{95}. i.e., 0.77 and 0.62 (w/w) with *C. maculatus* and *S. oryzae*, respectively.

The obtained results revealed that LC_{95} concentration of clove buds extract was very

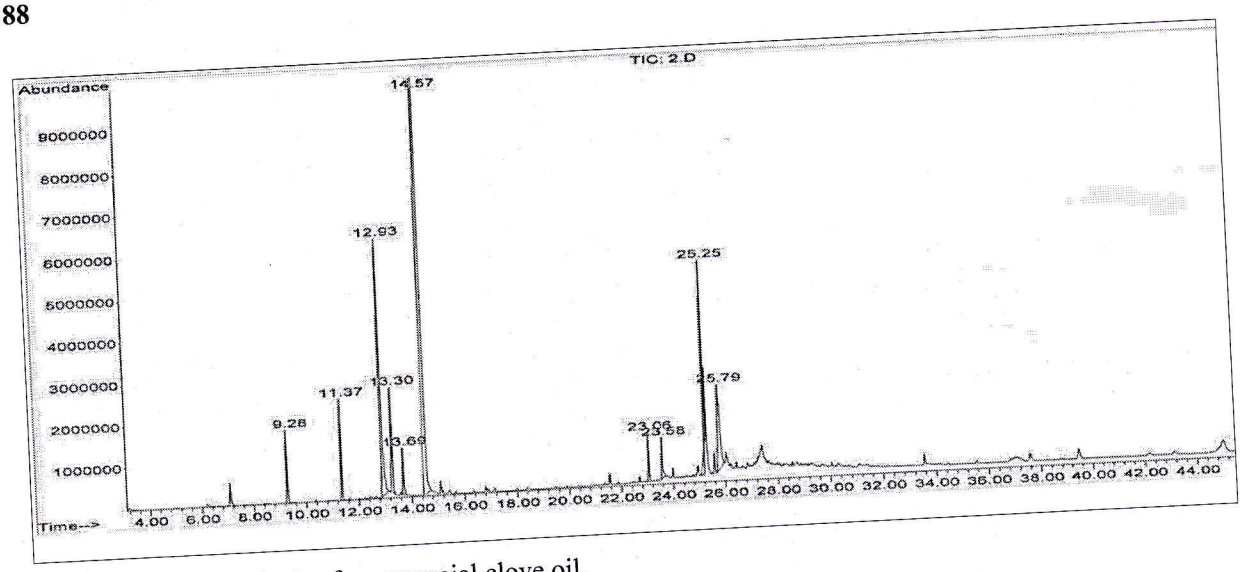

Fig. (1): GC/MS analysis of commercial clove oil.

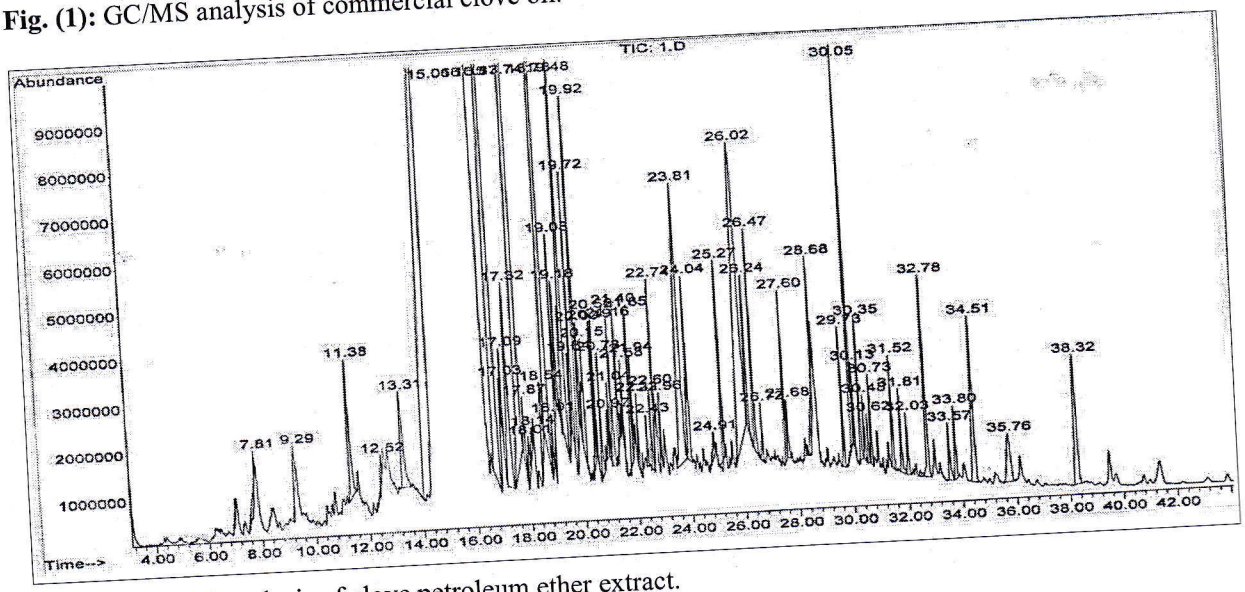

Fig. (2): GC/MS analysis of clove petroleum ether extract.

Table (8): Effect of clove extract at LC_{95} on germination of treated cowpea and wheat after 2 storage periods.

% germination after	Seeds	
	Wheat	Cowpea
1 day	96.6%	98.3%
Control	98.3%	100%
significance	ns	ns
LSD 5%	1.3	0.93
8 weeks	83.3%	86.6%
Control	96.6%	100%
significance	*	*
LSD 5%	2.06	1.3

powerful in protecting grains during storage up to 10 weeks. Mortality (%) and % reduction F_1- progeny of the two tested insects were 100% during the experimental period. This could be very useful to decrease economic losses caused by pests attacking.

Sumadi *et al.*, 2010 studied the effect of clove oil dosage on controlling storage beetle *C. maculatus* F., and maintaining seed quality including seed viability and seed vigor of two soybean after three months storage duration and found that dosage of clove oil 5 mL/kg soybean seed showed better effect on suppression of storage beetle and maintaining seed viability and vigor.

3-b) SEEDS GERMINATION TESTS:

One of the important points when using plant extracts in controlling pests is if it has harmful effect on germination , this experiment was carried out to answer this question.

Germination (%) recorded in Table (8) was subject to analysis of variance and differences using anova test (a computer program costate). Mean values were adjusted by Duncan's Multiple Range test Duncan (1951) at 0.05% level of significance with Statistical software version 6.3.0.3. Data showed that

clove extract had low significant effect on germination (%) of both cowpea and wheat seeds after 10 weeks of storage comparing with untreated seeds.

In conclusion: clove extract is a powerful protectant for cereals and legumes seeds during storage against different pests with low significance on germination.

The previous results are incompatible with that of Mazzafera (2003) observed that clove ethanolic extract and pure eugenol, the major constituent of clove oil had an allelopathic effect on the germination of several seeds, as well as that some of these seedlings sprayed with the clove extract showed a lower dry mass accumulation. Kritzenger *et al.*, 2002 tested the antifungal activity of clove oil on storage fungi associated with cowpea seeds and found that the oil has no harmful effect on the germination and emergence of cowpea seeds.

4) GC/MS CHROMATOGRAM ANALYSIS:

The chemical constituents of clove commercial oil and petroleum ether extract89were analysed by GC/MS technique. The components were characterized by comparing their mass spectra with those of their analogous reported by the NIST and WILEY libraries and with the authentic spectra (Adams, 1995).

3-1) Identification of clove extract components by using GC/MS technique:

The GC chromatogram showed 10 peaks corresponding to 10 compounds. The main constituents were eugenol (50.58%), cinnamaldehyde (15.72 %) and linoleic acid (9.52%).

The GC chromatogram showed 24 peaks corresponding to 24 compounds. The main constituents were eugenol (37.43%) eugenol acetate (11.47%), caryophellene (10.44 %), linoleic acid (9.42%) and caryophellene oxide (8.58%).

Previous results showed that eugenol (%) in commercial oil was higher than that in clove extract and this is may be due to that commercial oil was artificially manufactured based on eugenol as it is the main constituent in clove.

These results are compatible with those of Abo-El-Saad *et al.*, 2011 who found that essential oil from clove buds (*Syzygium aromaticum*) was extracted using petroleum ether in Soxhlet apparatus. The resultant oil contained eugenol (48.92%), caryophyllene (18.55%), α-caryophyllene (3.25%), eugenol acetate (23%), *cis*-13-docosenamide (3.21%), presenting more than 96% of the oil. Nazrul *et al.*, 2010 analyzed essential oil obtained by hydrodistillation from dry buds of *Syzigium caryophyllatum* by Gas Chromatography Mass Spectrometry (GC-MS). Thirty one components were identified in bud oil with the main components being eugenol (49.7%), caryophyllene (18.9%), benzene,1-ethyl-3-nitro (11.1%) and benzoic acid,3-(1-methylethyl) (8.9%). Nassar *et al.*, 2007 recorded sixteen volatile compounds identified from the n-hexane extract of the buds of *Syzygium aromaticum* by using gas chromatography-mass spectroscopy (GC-MS). The major components were eugenol (71.56 %) and eugenol acetate (8.99 %).

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