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Toxicity of clove (*Syzygium aromaticum*) plant extract and essential oil to the two-spotted spider mite *Tetranychus urticae* (Acari: *Tetranychidae*) and predatory mite *Phytoseilius persimilis* (Acarina: Tetranychidae and Phytoseiidae)

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Abstract:

In the present study plant extracted clove (*Syzygium aromaticum*) was extracted either by hexane or acetone. Also, commercial essential oils for the same plant and vertimec acaricid, were tested against *Tetranychus urticae* Koch (Acari: *Tetranychidae*) eggs and adults and phytoseiid predatory mite *Phytoseiulis persimilis* Athias-Henriot (Acari: Phytoseiidae) by using spray technique. The clove hexane extracts high toxic against eggs and adult of *T. urticae* than acetone extract. The mortality percentage for *P. persimilis* using hexane extract, recorded that clove caused high toxicity against *P. persimilis* followed by the acetone extracts. The clove essential oil exhibited a high degree of efficiency against eggs and adult female of *T. urticae*. The LC_{50} value of the biocide (Vertemic) against *T. urticae* adult were 0.0005 ml/L**.** The Identification was carried out using GC/MS analysis, as mentioned before in material and methods. Eleven compounds were identified by comparing with instrument database library. Thin Layer Chromatography, (TLC) was used to separate and isolate of various compound present in experimental clove hexane extracts of *S. aromaticum*, three compounds eugenal, caryophyllene and eugenyl acetate were identified by comparing with instrument data base library.

Introduction

Two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), is widely distributed globally and a common pest of many plants. In this context, the use of some plant extracts can present a realistic alternative to synthetic acaricides because of their efficiency against pests. Plant extracts can affect pest behavior, including repelling the pest or prohibiting feeding activity, and pest physiology, including molting and respiratory inhibition, growth and fecundity reduction, and cuticle disruption (Enan, 2001 and Gokce *et*

al., 2011). Plant extracts are also an environmentally interesting tool because of their biodegradability and minimal side effects on non-target organisms as well as on the environment (Attia *et al*., 2012).

Many predaceous are now used as biological control agents in various agricultural ecosystems and are important as predators of phytophagous mite in IPM programs. *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is one of the most important generalist indigenous predators of tetranychid mite (Ebadollahi *et al*., 2014). Many studies proved that *Syzygium aromaticum* has considered as important aromatic spices which contains a necessary essential and responsible compound of antimicrobial activity (Kumar *et al*., 2012 and Pandey and Singh, 2011). The oil of *S. aromaticum* has inhibition activity for the germs, fungi and Insect repellent (Liu , 1987).

The aim of this study used the Clove hexane extract against of *T. urticae* followed by evaluation of their chemical constituents by Gas chromatography-Mass spectrometry (GC-MS).

Material and methods

1. Rearing of mites *Tetranychus urticae* :

Initial colony of the two spotted spider mite, *T. urticae* was taken from the Laboratory of Acarology in Plant Protection Research Institute Giza Egypt. Bean (*Phaseolus vulgaris*) seeds were planted in plastic pots (15 cm. diameter) at a rate of 6-7 seeds per pot and incubated under muslin cage to prevent any infestation. Pots containing lima bean seedlings (15cm long) were taken to the laboratory, then infested leaves of *T. urticae* were transferred to these plants and left to reproduce under laboratory condition (25± 2℃ and 60%±5 RH.). The initial colony was supplied with fresh bean plants from time to time.

2. Preparation of plant materials and extraction of crude bioactive compound:

Plant materials were obtained from Cairo University Faculty of Agriculture Ornamental Horticulture Department. Flower buds of clove (*S. aromaticum*.). This part was chosen to be extracted either by hexane or acetone.

3. Extraction procedure:

A weight of 250 g of each plant was ground in an electric grinder into fine powder then soaked in solvents, extracted using two different solvents; either hexane or acetone. The fine powder was weighted then soaked in 350 mL hexane or 500 mL acetone and left for 2hrs then filtered to dryness under vacuum using a rotary evaporator in water bath at 60℃ the crude extract was then weighed and adjusted to 10 mL volume with acetone, and kept in a refrigerator until testing (Su and Horvat, 1981).

4. Bioassay tests:

Laboratory evaluation of the crude plant extracts and a biocide were conducted against *T. urticae* using either; hexane or acetone. Four concentrations of each extract were used.

5. Commercial essential oils:

Commercial essential oils were obtained from El Hawag factory. Essential oil was kept in a refrigerator until using. Series of aqueous concentrations of essential oil were prepared with Triton X-100 as a emulsifier agent at a rate of 0.1%.

6. Biocide (Vertimec):

Abamactin (Vertimec) 1.8% E.C. commercial formulation recommended as acaricid for controlling the two spotted spider mite *T. urticae*. Vertimec is produced by Sanganta Agro Egypt.

7. Treatment of adult stages:

Leaf discs of Acalypha (2 cm diameter) were placed separately upside –down on moist cotton Pads in Petri-dishes. Ten adults of *T. urticae* were placed on each disc then treated with one of the different treatments, sprayed by glass atomizer with different concentrations of the hexane extract which dissolved in acetone. The hexane extract concentrations of clove extract were used at 0.05, 0.0375, 0.018 and 0.009 mg/ml, and with acetone extract concentrations of Clove extract was used at 0.1, 0.05, 0.025 and 0.0125 mg/ml, Control discs were sprayed with acetone. While Clove oil was used as 2%, 1%, 0.05% and 0.025%; Control treatment was operated by water with Triton x-100 at rate of 0.1%. The concentrations with Biocide vertimec used as 0.0125, 0.0625, 0.003125 and 0.001 ml / L. Control was sprayed by water. Four replicates were made for each concentration. All treated discs were kept at 25±.2 ℃ and 55%±5 RH. Mortality was estimated for adult females after 24h of spraying. The percentage of mortality was determined and corrected by Abbott's formula (1925) as follows:

$$\text{Percentage of mortality} = \frac{\%\text{ tested mortality} - \%\text{ control mortality}}{\text{100-\% control mortality}} \times 100$$

LC_{50}, LC_{90} and slope values were calculated according to Finney (1971) and using (Ldp line) software by (Bakr, 2000).

8. Ovicidal action (Treatment of eggs):

To investigate the ovicidal activity of the clove extract, ten adult females were placed on Acalypha leaf discs (2 cm diameter) on wet cotton wool in a petri dish and allowed to put eggs. The petri dish was incubated for 24hrs at 25±.2 ℃ and 55%±5 RH. Then adult's females were removed from the leaf discs. There after eggs were counted and sprayed by a glass atomizer with a serial concentrations of clove plant hexane extracts 0.05, 0.025, 0.0125 and 0.00625 mg/ml; while with acetone extract the concentrations of clove extract were used at 0.1, 0.05, and 0.025. Control discs were sprayed with acetone. Essential oil as 5%, 2.5%, 1.25% and 0.05% for clove. Control treatment was operated by Triton x-100 at rate of 0.1%. The concentrations with biocide (Vertimec) used as 0.0125, 0.0625, 0.003125 and 0.001 ml / L. Control sprayed with water. Four replicates were used for each concentration. Treated eggs were incubated at 25±0.2 ℃ and 55%± 5 RH. for three days till hatching. The numbers of hatching and non-hatching eggs were recorded. Unhatchabilty was corrected by Abbott's formula (1925). LC_{50}, LC_{90} and slope values were computed according to Finney (1971) and using Ldp line software according to Bakr (2000).

9. Natural enemies and culture:

The phytoseiid predatory mite *Phytoseiulis persimilis* Athias-Henriot (Acari: Phytoseiidae) was used in this study. *P. persimilis* was obtained from Lab. of Acarology cultures of Plant Protection Department, National Research Centre, Dokki- Giza. Predatory mites were transferred to clean Acalypha, leaves put on wetted cotton in a large tray. Individuals of *T. urticae* were added as prey then kept in an incubator at 28 ± 1 ℃ and 70% R.H. Acalypha leaf discs (2 cm diameter) were placed separately upside – down on moist cotton wool in a Petri dish. Ten adults of *T. urticae* were placed on each disc with five adults of *P. persimilis* was sprayed with clove hexane extract 0.05, 0.025, 0.0125 and 0.00625 mg/ml. Acetone extract were used as 0.05, 0.025, 0.0125 and 0.00625 mg/ml for Clove. Control discs were sprayed with acetone. Biocide Vertimec concentrations used as 0.0125, 0.0625, 0.003125 and 0.001 ml / L. Control

sprayed with water. Essential oil concentration was used as 2%, 1%, 0.05% and 0.25% for Clove; Then kept in an incubator at 28±1℃.Control treatment was operated by Triton x-100 at rate of 0.1%. Four replicates were used for each concentration. Mortality percentages were calculated after 24h of treatment and corrected by Abott's formula, (1925). LC_{50}, LC_{90} and slope values were computed according to Finney (1971) and using Ldp line software according to Bakr (2000).

10. Isolation and identification :

Chromatographic techniques thin - Layer Chromatography (TLC) was used to separate the constituents of one plant hexane extract. The method used is as described by Su and Horvat (1981). TLC is a useful technique to determine bioactive compound from plant extract.

11. Identification of hexane plant extracts (GC/ MS chromatogram):

The chemical constituent's crude of hexane clove extract which gave high toxicity to *T. urticae* was identified by GC/MS (gas chromatography- mass spectrometry). For complete and rapid characterization of the separated compounds we have used Gas chromatography Mass spectrometric (GC/MS) method. Total GC running time was 30 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

12. Purification of bioactive compound(s) using Silica Gel Chromatography:

The crude hexane flower buds extract of clove (*Syzygium aromaticum*) (5g) was subjected to silica gel column chromatography (After adding 5ml of acetone to crude), to separate the extract into its component fractions.

Chromatography was performed on 20X20 cm were covered with a thin layer 0.5mm thick of silica gel Gf 254. The tested material was spotted 2.5 cm away from the edges and with 2.0 cm between each spot. Solvent system was used in attempt to fractionate investigated plant extract. Toluen: acetic acid (9:1v/v) was used in all plant extract. The plates were removed and allowed to dry under room condition ($20^oC\pm2$). Subsequently the sample components are identified by comparison of their retardation factor (R_f) Values with those of the separated standards.

Each plate was exposed to ultra violet light at wavelength UV 254 nm. The movement of each separating spot of the extract was expressed by its retardation factor (R_f).Values were calculated for each spot using the following formula:-

$$R_f = \frac{\text{distance traveled by solvte from the point of application to the center of spot}}{\text{distance traveled by the solvent front}}$$

This treatment may show some of spots the spots were recorded on the plates directly. The R_f value of each fraction was calculated. After developing of the plats, the silica gel layer at each fraction was abraded and collected on filter paper. The fraction was eluted with acetone. The acetone was evaporated and kept in freezing till used.

Leaf disc of Acalypha (2 cm diameter) were placed separately upside –down on moist cotton Pads in Petri-dishes. Fifteen adults of *T. urticae* were placed on each disc and sprayed with four bands then mortality percentages were calculated after 24h of treatment and corrected by Abott's formula, (1925).

Results and discussion

1. Toxicity of clove *Syzygium aromaticum* plant extract and essential oil to the two-spotted spider mite *Tetranychus urticae* and predatory mite *Phytoseilius persimilis*:

Results in Table (1) show that clove (*S. aromaticum*) hexane extract exhibited a high degree of efficiency against adult of *T. urticae* recording overall mean of mortality ranged from 72.5- 98.3%, while 0.009 mg/mL concentration caused 82.5% mortality after 3 day but 0.0375mg/mL concentration caused 90% mortality after 24 hours of application increased to 100% after 3 days from application where 0.05 mg/mL caused 95-100% after 1, 2 and 3 days of application. For acetone extract Results reveals that it is less toxic than hexane extract where 0.0125 mg/mL concentrate Caused 67.5% after 3 days the mortality increased by increasing the conc. from 67.5-100% with 0.0125-0.1 mg/mL conc. After 3 days. But Clove essential oil recording overall mean of mortality range of 15- 93% with 0.25-2% concentration, while 0.25 % concentration caused 10% mortality after 1 day, but 2%concentration caused 87.5% mortality after 1 day of application increased to 100% after 3days from application.

Slope values of clove, LC_{50} and LC_{90} shown in Table (2). The LC_{50} values were 0.0068, 0.01 and 0.83 for Clove with hexane, acetone extract and essential oil respectively. The LC_{90} values were 0.03, 0.07 and 2.1 with hexane, acetone extract and essential oil respectively, while slope value 1.76, 1.62 and 3.0 respectively.

Table (1): Toxicity of Clove *Syzygium aromaticum* plant extract (Hexane and acetone) and essential oil on *Teranychus urticae* adult stage.

Clove extract with	Con.	% mortality after			Overall mean
		1 day	2 day	3 day	
Hexane	0.009 mg/ml	60	75	82.5	72.5
	0.018 mg/ml	77.5	90	97	88.1
	0.0375 mg/ml	90	96	100	95.0
	0.05 mg/ml	95	100	100	98.3
Acetone	0.0125 mg/ml	52.5	60	67.5	60.0
	0.025 mg/ml	75	80	85	80.0
	0.05 mg/ml	87.5	90	97	91.5
	0.1 mg/ml	92.5	95	100	95.0
Essential oil	0.25%	10	15	20	15.0
	0.5%	35	45	52.5	44.0
	1%	62.5	75	80	72.5
	2%	87.5	92.5	100	93.0

Table (2): LC_{50}, LC_{90} and Slope values of Clove *Syzygium aromaticum* plant extract (Hexane and acetone) and Essential oil on *Teranychus urticae* adult stage.

Tested materials	LC_{50}	LC_{90}	Slope
Clove hexane extract mg/ml	0.0068	0.03	1.76
Clove acetone extract mg/ml	0.01	0.07	1.62
Clove essential oil %	0.83	2.1	3.0

Table (3) showed that clove hexane extract caused a high degree of efficiency against *T. urticae* eggs recording overall mean of hatchability ranged from 59-33% with 0.00625-0.05 mg/mL conc. while 0.00625 mg/mL caused 66% hatchability after 3 days, and 0.025 mg/mL conc. Caused 33and 50% hatchability after 1 and 3 days. By increasing the conc. Increased the hatchability as used 0.05 mg/mL conc. caused 37% hatchability after 3 days. While clove acetone extract recording overall mean of hatchability ranged from 72-25% with 0.0125- 0.1 mg/mL, while 0.0125 mg/mL caused 67 and 78% hatchability after 1and 3 days, but 0.1 mg/mL concentration caused 20 and 30% hatchability after 1and 3 days of application. Clove essential oil recording overall mean of hatchability ranged from 78 and 23.6% with 0.625

and 5% concentrations, while 0.625% concentration caused 77% hatchability after 1 day, but 5% concentration caused 22% hatchability after 1 day of application increased to 25% after 3 days. Slope values, LC_{50} and LC_{90} shown in Table (4). The LC_{50} values were 0.01, 0.02 and 2.1 for *S. aromaticum* with hexane, acetone extract and essential oil. The LC_{90} values were 0.4, 0.2 and 10 with hexane, acetone extract and essential oil, while slope value 0.79, 1.57 and 1.92 respectively

.Table (3): Toxicity of clove *Syzygium aromaticum* plant extract (Hexane and acetone) and essential oil on *Teranychus urticae* eggs.

Extract	Con.	% hatchability after			Overall mean
		1 day	2 day	3 day	
Hexane	0.00625 mg/ml	53.1	59	66	59
	0.0125 mg/ml	46	52	57	51
	0.025 mg/ml	33	44	50	42
	0.05 mg/ml	29	34	37	33
Acetone	0.0125 mg/ml	67	72	78	72
	0.025 mg/ml	55	67	74	65
	0.05 mg/ml	30.5	36.9	41	36
	0.1 mg/ml	20	25	30	25
Essential oil	0.625%	77	79	80	78.0
	1.25%	64	69	70	67.6
	2.5%	40	43	45	42.6
	5%	22	23.9	25	23.6

Table (4): LC_{50}, LC_{90} and slope values of clove *Syzygium aromaticum* plant extract (Hexane and acetone) and Essential oil on *Teranychus urticae* eggs.

Tested materials	LC_{50}	LC_{90}	Slope
Clove hexane extract mg/ml	0.01	0.4	0.79
Clove acetone extract mg/ml	0.02	0.2	1.57
Clove essential oil %	2.1	10	1.92

Table (5) showed that *S. aromaticum* hexane exhibited a high degree of efficiency against *P. persimilis* recording overall mean of mortality ranged from 38-86% when used 0.00625- 0.05 mg/mL concentrations, while 0.00625mg/mL caused 35% mortality after1 day but 0.05 mg/mL caused 85%mortality after 1day of application, while with acetone extract results recorded that *S. aromaticum* caused high toxicity against *P. persimilis*, the overall mean for *S. aromaticum* caused 30-81% mortality with 0.006- 0.05mg/mL concentration, while after 1 day the mortality was 30, 50, 60 and 80% for 0.006, 0.0125, 0.025 and 0.05mg/mL. Results with essential oil recorded that the overall mean for *S. aromaticum* caused 26-81% mortality with 0.25- 2 % concentration, while after 1-day mortality were 25, 40, 60 and 80% with 0.25, 0.05, 1 and 2% concentrations. Slope values, LC_{50} and LC_{90} shown in Table (6). The LC_{50} values were 0.009, 0.017 and 1.01 for *S. aromaticum* with hexane, acetone extract and essential oil. The LC_{90} values were 0.05, 0.11 and 3.4 with hexane, acetone extract and essential oil, while slope value 1.65, 1.57 and 2.3 respectively.

Table (5): Toxicity of clove *Syzygium aromaticum* plant extract (Hexane and acetone) and essential oil on *phytoseiulus persimilis* predator.

Extract	Con.	% mortality after			Overall mean
		1 day	2 day	3 day	
Hexane	0.00625 mg/ml	35	40	40	38
	0.0125 mg/ml	65	70	70	68
	0.025 mg/ml	80	80	85	81
	0.05mg/ml	85	85	90	86
Acetone	0.00625 mg/ml	30	30	30	30
	0.0125 mg/ml	50	55	60	55
	0.025 mg/ml	60	65	65	63
	0.05mg/ml	80	80	85	81
Essential oil	0.25%	25	25	30	26
	0.5%	40	45	45	43
	1%	60	65	65	63
	2%	80	80	85	81

Table (6): LC_{50}, LC_{90} and Slope values of clove *Syzygium aromaticum* plant extract (Hexane and Acetone) and Essential oil on on *phytoseiulus persimilis* predator.

Tested materials	LC_{50}	LC_{90}	Slope
Clove hexane extract mg/ml	0.009	0.05	1.65
Clove acetone extract mg/ml	0.017	0.11	1.57
Clove essential oil %	1.01	3.4	2.3

Data in Table (7) showed effect of the biocide (Vertemic) against *T. urticae* recorded 65- 92.5% mortality with 0.001-0.0125mL/l after 1 day, while after 3 day caused 87.5-100%. The overall mean recorded 76, 84, 94 and 96.8% with 0.001, 0.003, 0.006 and 0.0125 mL/l concentration. The hatchability recorded 80, 62.5, 38.7 and 27% with the same concentration after 1 day, while after 3day the hatchability increased to 87, 69, 44 and 31%, while toxicity against *P. persimilis* recoded 45, 55, 75 and 95% after 1 day and increased to 50, 65, 85 and 100% after 3 days. Slope, LC_{50} and LC_{90} shown in Table (8). The LC_{50}values of the biocide were 0.0005, 0.0029 and 0.0025 mL/L for *T.urticae* adult, *T. urticae* egg and *P. persimilis*, while LC_{90}were 0.009, 0.09 and 0.012 mL/Lfor *T.urticae* adult, *T. urticae* egg and *P. persimilis*.

Table (7): Toxicity of acaricide, vertimec on *Tetranychus urticae* adult, eggs and *Phytoseiulus persimilis* predator.

Trials	Con.	% mortality			mean
	ml/L	1 day	2 day	3 day	
***Tetranychus urticae* adult**	0.001	65	77.5	87.5	76.0
	0.003	77.5	85	90	84.0
	0.006	87.5	95	100	94.0
	0.0125	92.5	98	100	96.8
***Tetranychus urticae* eggs**	0.001	80	84	87	83.6
	0.003	62.5	66	69	65.8
	0.006	38.7	42	44	41.5
	0.0125	27	29	31	29.0
***Phytoseiulus persimilis* adult**	0.001	45	45	50	46.6
	0.003	55	60	65	60.0
	0.006	75	80	85	80.0
	0.0125	95	95	100	96.0

Table (8): LC_{50}, LC_{90} and Slope values of vertimec on *Tetranychus urticae* adult, eggs and *Phytoseiulus persimilis* predator.

Tested materials	LC_{50} ml/L	LC_{90} ml/L	slope
***Tetranychus urticae* adult**	0.0005	0.009	0.97
***Tetranychus urticae* egg hatchability**	0.0029	0.09	0.85
Phytoseiulus persimilis	0.0025	0.012	1.88

2. Identification of flower buds of clove (*Syzygium aromaticum*) hexane extract by using GC/MS technique:

The chemical constituents of flower buds hexane extract. Was identified out using GC/MS analysis. Table (9) showed that the chemical constituents of flower buds of clove (*S. aromaticum*) hexane extract. Chemical names, percent area, R_t molecular weight M.W., structure and formula of the detected compound flower buds of clove Figure (1) Showed the chromatogram of the flower buds of clove (*S. aromaticum*) hexane extract. Eleven compounds were identified by comparing using instrument database library.Table (9) showed that the GC chromatogram show 10 peaks corresponding to 11 compounds. The compound number one was methylthio [2,3,7,8,12,13,17,18 octaethylporphyrinato] indium its area was 1.71% with R_t 13.67min. The compound number two was penitrem its area was 2.43% with R_t16.06 min. Compound number 3 was (3,6ditertbutyl1azulenyl) (3,5ditertbutyl4hydroxyphenyl) methane its area was 1.43% with R_t 18.35min. Compound number 4 was 2,5-Cyclohexadien-1-one,3-bromo-2 Methoxy-6-(methoxy methyl -4,4-dimethyl) its area was 7.05% with R_t 24.57 min. The compound number 5 was Caryophyllene its area was 11.13% with R_t 26.03min.The compound number 6 was 1carboxaldehyde5,5dimethy l2methylene 3cyclohexene its area was 2.52% with R_t 26.96min. The compound number 7 was Eugenol its area was 1.65% with R_t 28.58min. The compound number 8 was 2fluoropyridine its area was 5.16% with R_t 32.14min. The compound number 9 was Methyl 13 decarboxy methyl phaeophorpidea its area was 3.28% with R_t 37.13 min. The compound number 10 was Carotene its area was 6.81% with R_t 40.52 min. The compound number 11 was Lycoxanthin its area was 1.37% with R_t 43.82 min.

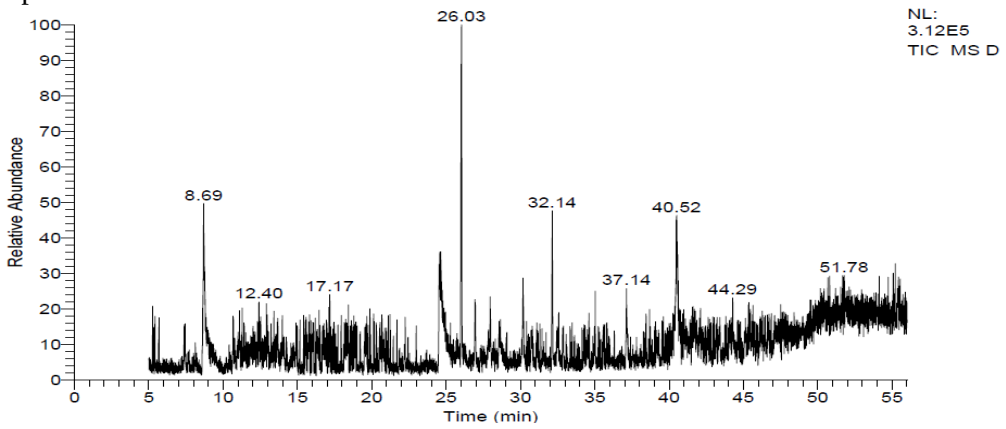
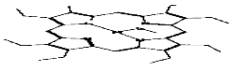
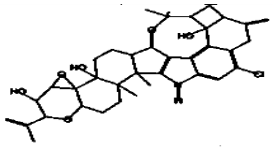
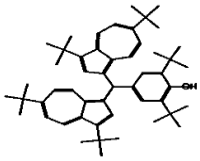
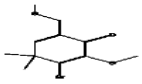
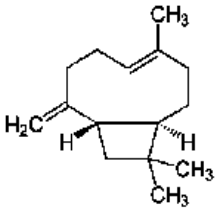
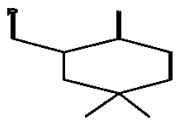
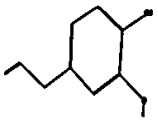
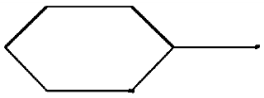
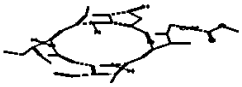
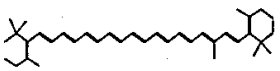


Figure (1): Gas chromatogram GC/MS of the of flower buds of Clove (*Syzygium aromaticum*.) hexane extract.

Table (9): Chemical constituents of flower buds of clove (*Syzygium aromaticum*) hexane extract.

No.	Compound	Area%	R_t	structure	M.W.	M.formula
1	Methylthio[2,3,7,8,12,13,17, 18octaethylporphyrinato] Indium	1.71	13.67		694	$C_{37}H_{47}InN_4S$
2	PENITREM	2.43	16.06		633	$C_{37}H_{44}ClNO_6$
3	(3,6 ditertbutyl 1 azulenyl) (3,5ditertbuty l4 hydroxypheny l) Methane	1.43	18.35		712	$C_{38}H_{40}N_4O_{10}$
4	2,5-Cyclohexadien-1-one,3-bromo-2 ethoxy-6- -(methoxy methyl -4,4-dimethyl)	7.05	24.57		274	$C_{11}H_{15}BrO_3$
5	Caryophyllene	11.13	26.03		204	$C_{15}H_{24}$
6	1carboxaldehyde5,5dimethy l2methylene 3cyclohexene	2.52	26.96		150	C10H14O
7	Eugenol	1.65	28.58		164	C10H12O2
8	2fluoropyridine	5.16	32.14		97	C5H4FN
9	Methyl 13 decarboxy methyl Phaeophorpidea	3.28	37.13		548	C34H36N4O3
10	Carotene	6.81	40.52		536	C40H56
11	Lycoxanthin	1.37	43.82		552	C40H56O

3. Isolation and Identification of the toxic components in plant extract:

Thin layer chromatogram indicated the different fraction obtained from hexane plant extracts. After the development with the solvent system, the band and region which have the same R_f values, were scraped from the glass plate and extracted with acetone. Such extracts were then used for bioactivity and identification studies. Four bands were evident in the TLC plate visualized under visible light (Figure 2). Compound with R_f values of 0.22, 0.32, 0.67 and 0.83 were visualized in TLC chromatograms.

Figure (2): Separation of clove (*Syzygium aromaticum*) compounds the toluene : acetic acid (9:1 v/v).

Tests were conducted to determine the bioactivity of different fractions against *T. urticae* compound having acaricide properties .The fraction with R_F 0.83 in hexane extract of *S. aromaticum* had a considerable acaricide activity.

4. Identification of best potent fraction component:

The best potent of flower buds of Clove (*S. aromaticum*.) was identified by GC/MS analysis. Analysis through GC-MS used to identify the volatile and semivalatile compounds present in the clove extract. To identify the chemical constituents of the fraction band number four which proved to be the highest potent fraction against mite adult. The identification was carried out using GC/MS analysis.

Table (10) represents the chemical composition of the fraction band from the bud of clove (*S. aromaticum*). As can be seen from this table, three compounds representing about clove, were characterized.Table (10) presents the compound names, percent area, R_t, molecular weight (M.W.) and chemical formula of the detected compound of fraction band four. Figure (3) showed the chromatogram of this fraction.
Three compounds eugenal, caryophyllene and eugenyl acetate were identified. The major components are shown in Table (10). The area of three compounds were 14.96, 2.16 and 1.77% with R_t 23.79, 27.87 and 29.46 min respectively.

Table (10): Chemical constituents of spot of flower buds of clove (*Syzygium aromaticum*) hexane extract.

R_t (min.)	Area%	Compound	M.W.	chemical formula
23.79	14.96	Eugenol	164	$C_{10}H_{12}O_2$
27.87	2.16	Caryophyllene	204	$C_{15}H_{24}$
29.46	1.77	Eugenyl acetate	206.10	$C_{12}H_{14}O_3$

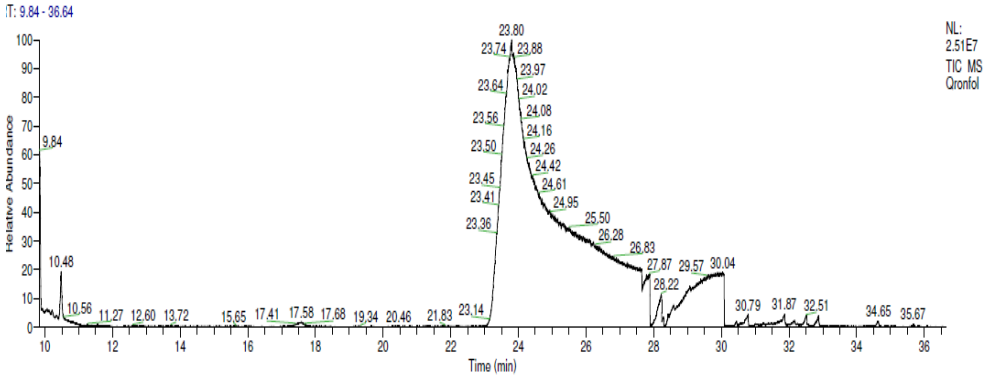


Figure (3): Gas chromatogram of the hexane extract isolated from clove buds fraction four.

Clove (*S. aromaticum*) plant hexane, acetone extract and essential oil were the most effective compound on the susceptible strain of *Tetranychus urticae* where LC_{50} was 0.0068,0.01 and 0.83. Hanifah *et al*. (2012) indicated that generally, the plant extracts showed various degrees of repellency against the larval mites and the repellency increased with increasing concentrations of the extracts. At the lowest concentration (0.01%), onion extract gave the highest repellency (30%) followed by clove and cinnamon (23%). Syahputra and Endarto (2013). Found all the aqueous extracts assayed could not cause the death of the predator *H. axyridis*. Aqueous seed extracts of *J. curcas* and *M. elengi* at a concentration of 5% could not cause phytotoxicity symptoms on the citrus leaves of *Citrus sinensis*. The high acaricidal activity of the clove (*S. aromaticum*) essential oil was perhaps attributable to the high level of eugenol. Clove oil represented the most repellent property. This kind of activity may be due to the high content of eugenol compound. This agrees with Araújo *et al*. (2012) who reported that eugenol component had a strong repellency property on *T. urticae*.

Our results obtained that LC_{50} values of the biocide were 0.0005, 0.0029 and 0.0025 mL/L for *T.urticae* adult, *T. urticae* egg and *P. persimilis*, while LC_{90} were 0.009, 0.09 and 0.012 mL/Lfor *T.urticae* adult, *T. urticae* egg and *P. persimilis*. Trumble and Morese (1993) reported that the best economic returns were generated by abamectin against *T.urticae* in combination with *P. persimilis*. This indicates that the chemical application for two –spotted spider mite can be successfully integrated with biological control. Abou El-Ela (2014) studied that vertimec gave 76.34% and 77.31% in two seasons 2007 and 2008 against *T. urticae*. Also, Sayed *et al*. (2006) found that the vertimec is more effective than actellic and biofly against *T. urticae*.

GC/MS was performed to identify the semivolatile and volatile compounds present in the flower buds of clove (*S. aromaticum*) hexane extracts. Eleven compounds were identified by comparing with instrument data base library. The results of this study could lead to the compound Eugenol was 1.65% with R_t 28.58. The same result was obtained by Lee and Shibamoto (2001) aroma extract from dried clove buds (*S. aromaticum*) was obtained by using steam-distillation under mild conditions (55_C and 95 mm Hg). The major aroma constituents of clove buds were eugenol (24.371 mg/g) and eugenyl acetate (2.354 mg/g). The antioxidant activity of clove bud extract and its major aroma components were eugenol

and eugenyl acetate studied by Alma *et al*. (2007). Its chemical composition was analyzed by GC/MS. The result showed that the essential oil mainly contained about 87.00% eugenol, 8.01% eugenol acetat and 3.56% β-caryophyllene. Rana *et al*. (2011) used column chromatography to separate the eugenol rich fraction from clove oil. Thin Layer Chromatography (TLC) was used to separate and isolate of various compound present in experimental Clove hexane extracts of *S. aromaticum*, subsequent tests on their acaricidal activities were assessed. (TLC) was initially performed as a qualitative method to document the extract constituents, Valle Jr *et al*. (2016) This method has been widely used to separate secondary metabolites like polyphenols, flavonoids, saponins, alkaloids and steroids, including amino acides, proteins, peptides, hormones and pesticides, Bhawani *et al*. (2012).

In this study, chemical composition of clove (*S. aromaticum*) bud were analysis through GC/MS has identified the volatile and semivalatile compounds present in this extract. The compound eugenul, carophyllene and Eugenol acetate. The same result was obtained by Razafimamonjison *et al*. (2014) the oils were analyzed by GC and ten constituents were identified from the whole. The major constituent in bud, leaf and stem oils was eugenol, with increasing percentages from bud (72.08 - 82.36%) to leaf (75.04 - 83.58%) and stem (87.52 - 96.65%).

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