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Evaluation of Insecticidal activity of selected medicinal plants against *Tetranychus urticae* (Mites)

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Research Article

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ABSTRACT

Aqueous extract of some selected medicinal plants *Allium sativum*, *Zingiber officinale*, *Capsicum annuum* and *Azadirachta indica* were evaluated for their insecticidal activity against on two spotted mites *Tetranychus urticae*, which is a pest that causes serious damage to several plants. Propargite was used as a standard for insecticidal activity and percentage mortality was found to be 100%. All individual extracts exhibited concentration and time dependent insecticidal activity. The formulation consists of equal ratio of extracts of *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica* showed a high toxicological effect, producing 90% mortality after 72 hours. Results clearly indicates that the formulation of *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica* can be used as an effective agent for the control of two spotted mites *Tetranychus urticae* and it could be tested against other pest affecting crops throughout the world there by reducing use of synthetic pesticides.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* (Acari: *Tetranychidae*), is a generalist herbivore that feeds on many crop and ornamental plants. Hot, dry weather is conducive to spider mite outbreaks. Damage can be seen as chlorosis of the leaves where the mites have been feeding [1]. Its short life cycle and high reproductive potential predispose this mite to evolving resistance to many chemical control methods.

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Their colour is generally pale yellow or orange with no spots. Eggs are round, almost clear, about 0.1 mm in diameter, and these may become pale brown just before hatching. Female two spotted mites can lay more than 100 eggs during their lives of approximately 30 days [2]. Eggs hatch into first instar larvae, which are white, oval, and about 0.1 mm long and have six legs. Most spider mites prefer warm and dry conditions, and suitable conditions could speed up the mite's life cycles. The two-spotted mite *T. urticae* takes 8-12 days at 30°C-32°C to complete its life cycle [3].

Some spider mites can act as vectors in transmitting plant diseases. The potato virus y, which affects potato, tomato, tobacco, and other *Solanaceae*, can be transmitted by the two-spotted mite. The mite does not actually inject the virus into the plant, instead excretes the virus into the leaf surface and permits entry of the virus into the plant through feeding damage. The two-spotted mite can also transmit tobacco ring spot virus, tobacco mosaic virus, southern bean mosaic virus, and cotton curliness [4,5].

The neem tree*, Azadirachta indica A. Juss* is a member of the Mahogany family, *Meliaceae*. It is a fast growing tree of multipurpose use. One of the important uses of neem is its use as a source of botanical pesticides. As far as its use as a source of insecticides is concerned the main interest of the scientists has focussed on the products derived from neem seed kernels and the pure compound azadirachtin [6-10].

Garlic, *Allium sativum* Linnaeus (*Amaryllidaceae*), is a native of temperate western Asia and has been used throughout the world as a food spice and medicine. Antimicrobial, cardiovascular, anticancer, hypo- and hyper-glycaemic, and other beneficial properties of garlic have been reported. In different studies, garlic essential oil was demonstrated to possess insecticidal activity against *Blattella germanica Linnaeus* (Blattodea: Blatellidae), *Lycoriella ingénue Dufour* (Diptera: Sciaridae), *Reticulitermes speratus Kolbe* (Isoptera: Rhinotermitidae), and several grain storage insects as *Ephestia kuehniella Zeller* (Lepidoptera: Pyralidae), *Sitophilus oryzae Linnaeus, Sitophilus zeamais Motschulsky* (Coleoptera: Curculionidae), and *Tribolium castaneum Herbst* (Coleoptera: Curculionidae).

Ginger, *Zingiber officinale* Roscoe (Family: *Zingiberaceae*) used worldwide as a traditional medicine, has recently been found possessing an insecticidal effect on many insects. *Capsicum annum* (Family: *Capsaicidae*), contains capsaicin compound and has many uses as spice and public medicine, as well as its ability to kill certain insects [11].

Indiscriminate use of synthetic pesticides for crop production and protection poses poisonous effects through contact, inhalation, and dietary exposure and has become a cause of carcinogenesis, fertility problems and mutagenesis in humans. These circumstances led towards searching for effective and eco-friendly pest control alternatives, especially from natural plant resources. Many insecticides derived from botanical sources are available and are easily affordable and accessible to the farming community; they are safer for human beings and for the environment with minimal residual effect, and they are target-specific and less toxic to vertebrates, pollinators and fish [12,13].

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So the current study was planned to investigate the Aqueous extract of some selected medicinal plants *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica* were evaluated for their insecticidal activity against on two spotted mites *Tetranychus urticae*, which is a pest that causes serious damage to several plants [14].

MATERIALS AND METHODS

Sample collection and processing

Fresh cloves of *Allium sativum*, rhizomes of *Zingiber officinale* and green peppers of *Capsicum annuum* and leaves of *Azadirachta indica* were collected from the local market of Coimbatore districts. The samples were rinsed with tap water and then were dried under shade for 2 weeks. The dried samples were powdered using an electrical grinder. 20 g of dry sample powder with 1 L distilled water and heated at 80^{o}C for 20 min on a hot plate. The solution was filtered by filter paper (Whatman No 42, Maidstone, England) and stored at 4^{o}C for further experiment.

Phytochemical analysis

The Phytochemical screening of *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica* aqueous extract were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures. The screening was carried out to discover the significant bioactive components such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The main phytocomponents of *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica* aqueous extract were identified by using GC-MS detection system. The samples were suspended with ethanol and subject to GC-MS analysis. Elucidation of phytocomponents was assayed by comparison of their retention times and mass with their regular authentic standard spectra using computer searches in NIST0. L and Wley 7n.l libraries.

Insecticidal activity

The insecticidal activity was evaluated. Twelve *Tetranychus urticae* were put into a 500 ml glass bottle and kept in the laboratory at 28 ± 1°C, 58 ± 5°% RH (12 h light cycle) and 25.5 ± 1°C, 45 ± 5% RH (12 h dark cycle) for 72 h. Different Ratio of Sample Extraction diluted in Distilled water were tested on *T. urticae*. The extract was applied to filter paper (Whatman number 1, cut into 7 cm diameter) and immediately introduced into a glass bottle and then sealed. For the control group, the insects were placed in the glass bottles under the same condition but without addition of extraction. Each ratio and control was replicated three times. Insect Mortality was determined by observing the recovery of immobilized insects in 24 h intervals up to 72 h. When no antennal or leg movements were observed, insects were considered dead. One synthetic pesticide PROPARGITE is used as control weighed to achieve the appropriate concentrations and was applied according to manufacturer's directions. Percentage of mortality of *T. urticae* was observed and overall mortality rate was calculated after 48 hrs using the Abbott's formula.

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RESULTS

Phytochemical screening

Phytochemical screening of sample extract of *Allium sativum*, *Zingiber officinale*, *Capsicum annuum* and *Azadirachta indica* confirmed the presence and absence of alkaloids, flavonoids, saponin, phenol, steroids, and glycosides, Phlobactin, Coumarins, Anthraquinone, Leucoanthocyanin, Triterpenoids and Carbohydrate in individual sample. The negative sign depicted the absence of secondary metabolites (Table 1).

Table 1. Phytochemical screening of aqueous extracts of *Allium sativum*, *Zingiber officinale*, *Capsicum annuum* and *Azadirachta indica*.

	A. Sativum	*Z. Officinale*	*C. Annuum*	*A. Indica*
Alkaloids	+	+	+	+
Flavonoids	-	+	+	-
Saponin	+	+	-	-
Glycosides	+	+	+	+
Carbohydrate	+	+	+	-
Phenols	+	+	+	+
Steroids	+	+	+	+
Phlobactin	-	+	+	+
Coumarins	-	+	+	+
Anthraquinone	-	-	+	-
Leucoanthocyanin	-	-	+	-
Triterpenoid	-	-	-	+

GC-MS Analysis

The presence of biologically active compounds from the ethanol extract of *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica* were evaluated by conducting GC-MS analysis. The principal active compounds, molecular weight (g/mol, M.W), Molecular Formula (M.F.), Retention time (R.T.) and Peak area (%) are presented in Table 2. The result of GC-MS analysis of the extracts led to the determination of several biological compounds (Table 2).

Table 2. GCMS analysis of aqueous extracts of *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica*

	IUPAC name	RT (min)	Area %	Molecular formula	MW
1	Borneol	8.109	0.6	$C_{10}H_{18}O$	154.2 g/mol
2	Decanal	8.487	1.79	$C_{10}H_{22}$	156.2 g/mol
3	2,6-octadienal,3,7-dimethyl-	9.264	0.62	$C_{10}H_{16}O$	152.23 g/mol

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4	Cyclohexane,1-ethenyl-1-methyl-	11.209	0.65	$C_{15}H_{24}$	204.35 g/mol
5	Ethyl cyclopropane carboxylate	11.386	0.63	$C_6H_{10}O_2$	114.14 g/mol
6	Pentanal	11.608	1.7	$C_5H_{10}O$	86.134 g/mol
7	Benzene,1-(1,5-dimethyl-4-hexen	11.742	3.67	$C_{15}H_{22}$	202.3352 g/mol
8	Pyridine,2-propyl	11.886	11.38	$C_8H_{11}N$	121.18 g/mol
9	Alpha-Farnesene	11.942	3.3	$C_{15}H_{24}$	204.36 g/mol
10	Naphthalene,1,2,3,4,4a,5,6, 8a-0.....	12.031	4	$C_{10}H_{16}$	136.23 g/mol
11	Cyclohexene,3-(1,5-dimethyl-4-h....	12.208	4.63	$C_{15}H_{24}$	204.35 g/mol
12	Cyclohexanemethanol,4-ethenyl-....	12.486	0.82	$C_{15}H_{26}O$	222.3663 g/mol
13	2-Deoxy-D-glucose	13.286	1.11	$C_6H_{12}O_5$	164.16 g/mol
14	Butan-2-one,4-(3-hydroxy-2-meth	13.397	10.25	$C_{11}H_{14}O_3$	194.2271g/mol
15	Sucrose	13.486	0.99	$C_{12}H_{22}O_{11}$	342.3 g/mol
16	Galactitol	13.575	3.24	$C_6H_{14}O_6$	182.172 g/mol
17	2-Tridecen-1-ol,(E)	13.664	0.63	$C_{13}H_{26}O$	198.34 g/mol
18	m-Toluicacid,hexadecyl ester	13.808	0.74	$C_{24}H_{40}O_2$	360.5732
19	Tridecanoic acid	16.141	5.52	$CH_3(CH_2)_{11}COOH$	214.348 g/mol
20	Sorbitol	16.463	3.05	$C_6H_{14}O_6$	182.17 g/mol
21	Phytol	17.341	4.93	$C_{20}H_{40}O$	128.1705 g/mol
22	2-Chloroethyl linoleate	17.519	2.27	$C_{20}H_{35}ClO_2$	342.9 g/mol
23	9,12-Octadecadienoic acid (Z,Z)	17.563	3.67	$C_{18}H_{32}O_2$	280.4472 g/mol
24	Linoleic acid ethyl ester	17.73	1.36	$C_{20}H_{34}D_2O_2$	310.5148 g/mol
25	Cyclododecyne	17.774	0.81	$C_{12}H_2O$	164.29 g/mol
26	2-Butanone,4-(4-hydroxy-3-metho...	17.93	0.58	$C_{11}H_{14}O_3$	194.2271
27	4-Amino-2,3-xylenol	18.23	0.75	$C_8H_{11}NO$	137.18 g/mol
28	Benzenesulfonamide.N-(2-(dimeth....	18.308	0.66	$C_{10}H_{20}O_2$	172.26 g/mol
29	Benzeneamine,3-ethyl-4-hydroxy	18.763	6.48	$C_{10}H_{20}O_2$	172.26 g/mol
30	Phenol,2-methoxy-4-propyl-	19.03	2.2	$C_{10}H_{14}O_2$	166.217
31	Butan-2-one,4-(3-hydroxy-2-meth...	19.241	1.06	$C_{11}H_{14}O_3$	194.23 g/mol
32	2-Butanone,4-(4-hydroxy-3-metho...	19.441	3.77	$C_{11}H_{14}O_3$	194.2271
33	2H-Pyran-2-one,5,6-dihydro-6-[2...	20.041	1.27	$C_{10}H_{16}O_2$	168.23 g/mol
34	Pyrazine,2-methoxy-3-(1-methyle...	20.196	2.49	$C_8H_{12}N_2O$	152.19 g/mol
35	Capsaicin	20.541	1.37	$C_{18}H_{27}NO_3$	305.41 g/mol

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36	3,6-Dimethyl-2,3,3a,4,5,7a-hexah..	20.696	0.68	$C_{10}H_{16}O$	152.23 g/mol
37	3-Buten-2-one, 4-(2,6,6-trimethy...	20.785	0.74	$C_{13}H_{20}O$	192.2973
38	9,12-Octadecadienoic acid (Z,Z)-...	21.352	2.15	$C_{18}H_{32}O_2$	280.4472 g/mol
39	3-Pyridineacetic acid	21.529	2.23	$C_7H_8ClNO_2$	173.6 g/mol
40	N-Formyl (4-hydroxy-2-methoxyphen...	21.785	1.22	$C_9H_{22}NO_2$	165.15 g/mol

Insecticidal activity

The insecticidal bioassay of *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica* showed a strong activity against *T. urticae* within 24 hours. At equal ration of all the 4 samples the extract produced 90% mortality after 72 h. The *Allium sativum* alone showed 58.3% mortality after 72 h. The Zingiber officinale showed 46.7% mortality at 72 h. The *Azadirachta indica* showed 83% mortality at 72 h. The *Capsicum annuum* showed 72% mortality at 72 h. The synthetic pesticide showed 100% mortality after 24 h and 48 h. Both Sample extract and pesticides reached 100% mortality at 72 h. The negative control showed no activity except at 72 h. The Insecticidal activity of sample extraction against *T. urticae* is shown below at (Table 3).

Table 3. Insecticidal activity of selected medicinal plants against *Tetranychus urticae* (Mites).

S.No	Extraction Ratio *A.sativum* *Z.officinale* *A.indica* *C.annuum*	Number of insect taken	Number of dead			Average number of deaths	Percentage of mortality
			Vial 1	Vial 2	Vial 3		
1	1:0:0:0	20	10	12	13	11.6	58.3 ± 1.52
2	0:1:0:0	20	9	9	10	9.33	46.7 ± 0.58
3	0:0:1:0	20	16	17	17	16.6	83.3 ± 0.57
4	0:0:0:1	20	13	14	15	14	70.0 ± 1.00
5	1:1:1:1	20	17	18	19	18	90.0 ± 1.00
6	Control	20	20	20	20	20	100

CONCLUSION

This study demonstrated that sample extracted from *Allium sativum, Zingiber officinale, Capsicum annuum* and *Azadirachta indica* was toxic to mites *Tetranychus urticae*. Therefore, it could be used as an alternative strategy for *Tetranychus urticae* control and as a substitute for synthetic pesticides. However further studies are necessary to

elucidate the mode of action and the environmental impact and development of formulations to improve the insecticidal efficacy. This would benefit agricultural sector of developing countries as these samples are readily available and biodegradable.

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