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Vitex species, a potential store house for acaricidal principles

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Abstract
Biological activity of methanol extracts from leaves of four different species of *Vitex* namely, *V. negundo* (purple), *V. negundo* (white), *V. altissima*, *V. peduncularis* and *V. trifolia* was studied on red spider mite, *Tetranychus macfarlanei* infesting okra crop. Toxicity parameters such as repellence (walk- off), mortality were determined by releasing adult mites on okra leaves treated with extracts under laboratory conditions. Among the methanol extracts of different *Vitex* spp. Per cent walk-off of adult mites from treated okra discs differed significantly 6 and 12 hours interval. Repellent property showed marginal increase from 6 hours to 12 hours in most of the treatments except in *V. peduncularis* at 10% concentration, with which walk-off response remained lowest and same *i.e.*, 13% up to 12 hours after six hours per cent walk-off was maximum, 72 per cent with *V. negundo* (purple) extract at 20% concentration followed by *V. trifolia* at 15% with 59 per cent walk-off. *V. negundo* (purple) extract at 20% was superior with the highest walk-off of 73% upto 12 hours, so also the walk-off with *V. trifolia* extract at 15% concentration. Among the species of *Vitex*, repellent activity of *V. negundo* (purple) at 20% concentration and of *V. trifolia* at 15% was more apparent. Mortality of adult mites in various treatments differed significantly at all the intervals of 24, 48 and 72 hours. Extract of *V. negundo* (purple) at 20% recorded highest mortality of 89 per cent at 72 hours followed *V. peduncularis* at 14% (79% mortality) and *V. negundo* (white) at 12% (78% mortality) and were statistically on par with each other. It was evident that *V. negundo* (purple) methanol leaf extracts caused maximum mortality of mites *i.e.*, 89 per cent at 20% concentration. The lowest mortality record was less than 50 per cent *i.e.*, 48 per cent with the same species of *Vitex* at the lower concentration of 15%.

Keywords: *Vitex*, repellence and mortality

Introduction
Most of the insects are controlled using conventional chemical insecticides which are harmful to the environment, humans and beneficial organisms. In addition pest populations have developed resistance to synthetic insecticides. In response to this, several efforts have been made to identify alternative insecticides that are target specific, biodegradable, environmentally safe, and botanicals in origin for integrated pest management programmes, presently, several products of botanical origin, especially the secondary metabolites, have received significant renewed attention as potentially bioactive agents used in management of insect pests (Kiran and Devi, 2007) [10]. Plant derived insecticides comprise of an array of chemical compounds which act in concert on both behavioural and physiological process, Hence the chances of pests developing resistance to such substances are likely. Moreover, botanical insecticides are less likely to bioaccumulate as they are biodegradable (Saxena, 1987) [16].

Among the botanical insecticides, miticidal properties are present in the genus *Vitex* belonging to the family Verbenaceae. An extensive literature search on the genus *Vitex* revealed presence of miticidal/ acaricidal properties in a number of *Vitex* species found in tropical and subtropical regions. The genus *Vitex* has been reported to exhibit acaricidal activities against a number of mite pests. In the present study, biological activities of methanol extracts of *Vitex* spp. are presented.

Material and Methods
Laboratory investigation on the biological activities of methanol extracts of *Vitex* spp. leaves on red spider mite, *Tetranychus macfarlanei* Baker and Pritchard infesting okra was conducted at the Acarology section of Department of Agricultural Entomology, University of Agricultural Sciences, GKVK, Bengaluru.

Tetranychus macfarlanei
Okra leaves infested by red spider mites in the field were collected and reared on mulberry leaves kept on moist cotton wads in plastic trays in the laboratory.

Simultaneously these spider mites were also released and maintained on potted okra plants in glass house. After colonization, spider mites were used for bioassay studies with treated okra leaves.

Preparation of extracts

Organic solvent i.e., methanol having boiling point of 65°C with polarity index of 5.1 was selected for extraction from leaves of five different species of *Vitex viz*., *Vitex negundo* L. (purple), *Vitex negundo* L. (white) belonging to the botanical family Verbenaceae. Plant extracts were used to investigate their acaricidal activity against *T. macfarlanei* infesting okra.

Leaves of *V. negundo* (purple), *V. negundo* (white) were collected during May – June 2015 from the Medicinal and Aromatic plants section of the Division of Horticulture and from the Botanical Garden of GKVK campus. Leaves collected from the plants were shade dried and powdered separately using a Waring blender. Two fifty grams of each of these powdered material was used for hot extraction with petroleum ether using Soxhlet apparatus for 8-10 hours. The extracts were then concentrated to remove the solvent using Vacuum Flash Evaporator. Thick semi-solid extract thus obtained was stored at 4°C in a refrigerator for further used to investigate on their acaricidal activity on *T. macfarlanei* infesting okra.

For preliminary assay of getting mortality record of 10-20 to 80-90% and determination of relative toxicity of different solvent extracts from leaves of *Vitex* spp., concentrations of methanol extracts used were as below.

Organic Solvent	*Vitex* spp.	Concentration (%)
Methanol	*Vitex negundo* (purple)	2, 4, 8, 10, 15 & 20
	Vitex negundo (white)	2, 4, 6, 8, 10 & 12
	Vitex altissima	2, 4, 8, 10, 12 &15
	Vitex peduncularis	2, 4, 6, 8, 10 &14
	Vitex trifolia	2, 4, 6, 10, 12 &15

Leaf spraying method and evaluation of extracts for biological activities

Fresh okra leaves were sprayed uniformly with the desired concentration of the extract and were air dried under a ceiling fan. Leaf bits measuring 2.5cm X 2.5cm were prepared from the treated leaves and kept on moist cotton wad in Petri plates. Thirty adult female mites released on each of these leaf bits served as one replication and three such replications were maintained for each concentration of the extract. Leaf bits treated with water (+ teepol @ 0.1%) served as control (teepol was used as emulsifier when solvent extracts were not miscible with water).

i. To ascertain the walk-off response or repellency, the number of individuals which were drowned in water found drowned or entangled to moist cotton wad was recorded at 6 and 12 hours after release and per cent walk-off was computed (Penman *et al*., 1986) [14]. At each observation, adult mites which were found drowned/entangled, but were live, were again released back onto the respective leaf bit carefully using a fine camel hair brush and the observations were continued.
ii. To determine the mortality, the number of female adults found dead was recorded after 72 hours and per cent mortality was computed treatment-wise. Individuals, which were unable to move (moribund) were considered as dead or killed.

Statistical analysis

The data in percentages were subjected to angular transformation, while the numerical data were subjected to √X+0.5 transformations and analyzed statistically following the Analysis of Variance technique for Completely Randomized Design and the results were interpreted at five per cent level of significance.

Results and Discussion

The data on the biological activity of methanol extracts of *Vitex* spp. treated against red spider mite are provided in table 1 to 5.

Table 1 provides the data on the efficacy of methanol extract of *Vitex negundo* (purple) against red spider mite. Walk-off of *T. macfarlanei* adults from okra leaves treated with different concentrations of methanol extract of *V. negundo* (purple) leaves recorded at different intervals differed significantly. Progressive increase in the walk-off of adult mites was observed from 6 hours to 12 hours at all the concentrations. Maximum repellence of adult mites recorded was 73.33 per cent in treatment with 20% methanol extract from *V. negundo* (purple) leaves. With the increase in concentration of extracts from 2 to 20% there was increase in the mortality of adult female mites recorded at 72 hours after treatment. Maximum mortality was 88.89 per cent at 20 % concentration of methanol extract.

Data with respect to walk-off response in *T. macfarlanei* adult mites induced by different concentrations of methanol extract (2 to 12%) from *V. negundo* (white) leaves at 3 hours interval up to 12 hours are presented in Table 2. Maximum walk-off response with adult mites exposed to *V. negundo* (white) methanol extract was 47.78 per cent. Maximum mortality recorded was 76.67 per cent at 72 hours with 12% methanol extract treatment.

Similarly data with respect to walk-off response/repellence, mortality of red spider mites exposed to various concentrations of methanol extract of *V. altissima* leaves are presented in Table 3. Walk-off of adult mites across different concentrations at 6 hours ranged from 20 to 41.44 per cent and it ranged from 20 to 48.89 per cent at 12 hours. The maximum repellence of adults was 48.89 per cent at 4% methanol extract from *V. altissima*.

Mortality of mites was highest, 68.89 per cent on okra leaves treated with 10% methanol extract and the mortality records did not show any definite trend with the incremental increase in the concentration of extracts.

Table 4 represents the data on walk-off responses of *T. macfarlanei* adults on okra leaves treated with various concentrations of methanol extracts of *V. peduncularis.* Walk-off was highest 36.67 per cent on okra leaves with 8% methanol extract treatment. 72 hours after treatment the highest mortality of mites, 78.89 per cent was caused by 14% extract, on par with 10 % extract.

The data from Table 5 revealed that walk-off of *T. macfarlanei* adults on okra leaves treated with methanol extract of *V. trifolia* leaves was apparent up to 12 hours and with 15% concentration of the extract, the walk-off was highest *i.e.*, 73.33 and the highest mortality was 75.56 per cent at 15% methanol extract.

From Table 1-5, it is evident that methanol extract of *Vitex* spp. caused maximum repellence of 73.33 per cent at 20 % concentration of *V. negundo* (purple) and 15% of *V. trifolia* extract. Highest mortality recorded was 88.89% with 20% *V. negundo* (purple) leaf extract supported by Chowdhury *et al*. (2009a & b) [3, 4], who assayed sequential extraction with

different organic solvents like (petroleum ether, ethyl acetate, acetone and methanol) from *Vitex* leaves. Methanol extract was most toxic to *Tribolium castaneum* adults with low LD_{50} values followed by petroleum ether, acetone and ethyl acetate extracts. Similarly, Deepthy *et al*. (2010) [5] recorded higher activity of methanol leaf extracts from *V. negundo* at 1-6% concentration on III instar larvae of *Spodoptera litura*, which also significantly reduced pupation (>90%) and pupal weight (45-73% reduction) compared to extracts from hexane, acetone and water (aqueous extract). Higher concentration of biologically active principles like alkaloids, terpenoids and phenolic compounds in the methanol leaf extract of *V. negundo* resulted in more than 50 per cent nymphal mortality of red cotton bug *Dysdercus cingulatus* according to Ranilalitha *et al*. (2015) [15]. Mitra *et al*. (2015) [12] suggested the use of green pesticides for the control of tetranychid mite, *Petrobia harti* infesting medicinal herb *Oxalis corniculata*, which contain phenols, saponins, xanthoproteins, triterpenoids, tannins and flavonoids, being responsible for their acaricidal activity.

Srinivasa and Sugeetha (1999) [17] recorded comparatively lower walk-off of *T. macfarlanei* adults (56%) from okra leaves up to 12 hours, when treated with *V. negundo* methanol extract at 5-6% concentrations. Gopal (2000) [8] reported 50 per cent repellence of two spotted spider mite *T. urticae* adults from rose leaves treated with 6-10% concentrations of *V. negundo* methanol leaf extract and walk-off of *Oligonychus indicus* adults on treated sorghum leaves was 50-69 per cent. Repellent activity of *V. negundo* extracts against certain species of insects and Acari (ticks) have also been documented. Methanol leaf extracts of *V. negundo* caused 38 per cent repellence of Tea mosquito bug at 5 % concentration (Deka *et al*., 2016) [6], 89 per cent repellence of pink mealy bug *Maconellicocus hirsutus* at 10% concentration and 87 per cent repellence of *Tribolium castaneum* adults at 2.5-10% concentrations (Ramamurthy *et al*., 2001). Mehlhorn *et al.* (2005) [11] recommended the application of crude leaf extracts from *V. agnus-castus* (monk's pepper) on animals and human beings to protect at least for 6 hours from blood sucking ticks, *Ixodes ricinus* and *Riphicephalus sanguineus.*

Table 1: Effect of methanol extract of *Vitex negundo* (purple) leaves on red spider mite infesting okra

Concentration	Walk -off response / repellence (%) after		Per cent mortality after
	6 h	12 h	72 h
2%	3.33 (10.52)	5.56 (13.48)	5.56 (13.48)
4%	18.89 (25.35)	20.00 (26.24)	32.22 (33.06)
8%	22.22 (27.96)	27.78 (31.77)	33.33 (35.11)
10%	25.56 (29.50)	26.67 (30.14)	53.33 (46.91)
15%	40.00 (38.97)	44.44 (41.65)	47.78 (43.71)
20%	72.22 (58.36)	73.33 (59.18)	88.89 (73.94)
Control (water treated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
F-test	*	*	*
SEM±	(3.45)	(4.05)	(4.50)
CD at P=0.05	(10.64)	(12.51)	(13.89)

Figures in the parentheses are angular transformed for repellence and mortality data; are √ x+0.5 transformed for oviposition data.* Significant

Table 2: Effect of methanol extract of *Vitex negundo* (white) leaves on red spider mite infesting okra

Concentration	Walk -off response / repellence (%) after		Per cent mortality after
	6 h	12 h	72 h
2%	5.56 (13.48)	6.67 (14.64)	24.44 (29.62)
4%	14.44 (21.92)	15.56 (22.65)	21.11 (27.29)
6%	16.67 (23.91)	16.67 (23.91)	38.89 (38.55)
8%	36.67 (36.64)	37.78 (37.32)	57.78 (50.05)
10%	45.56 (42.34)	47.78 (43.70)	66.67 (54.87)
12%	38.89 (38.51)	38.89 (38.51)	76.67 (62.19)
Control (water treated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
F-test	*	*	*
SEM±	(4.40)	(4.58)	(3.48)
CD at P=0.05	(13.56)	(14.10)	(10.72)

Figures in the parentheses are angular transformed for repellence and mortality data; are√ x+0.5 transformed for oviposition data.* Significant

Table 3: Effects of methanol extract of *Vitex altissima* leaves on red spider mite infesting okra

Concentration	Walk -off response / repellence (%) after		Per cent mortality after
	6 h	12 h	72 h
2%	20.00 (26.03)	20.00 (26.03)	34.44 (35.75)
4%	46.67 (43.03)	48.89 (44.34)	62.22 (52.17)
8%	35.56 (36.57)	47.78 (43.74)	71.11 (57.64)
10%	38.89 (38.22)	40.00 (38.86)	68.89 (56.52)
12%	30.00 (32.86)	38.89 (38.55)	58.89 (50.14)
15%	41.11 (39.78)	45.56 (42.42)	53.33 (46.91)
Control (water treated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
F-test	*	*	*
SEM±	(4.03)	(4.11)	(2.76)
CD at P=0.05	(12.42)	(12.66)	(8.51)

Figures in the parentheses are angular transformed for repellence and mortality data; are√ x+0.5 transformed for oviposition data. * Significant

Table 4: Effect of methanol extract of *Vitex peduncularis* leaves on red spider mite infesting okra

Concentration	Walk -off response / repellence (%) after		Per cent mortality after
	6 h	12 h	72h
2%	0 (0.00)	0 (0.00)	10.00 (17.68)
4%	4.44 (9.65)	4.44 (9.65)	22.22 (27.96)
6%	6.67 (14.96)	6.67 (14.96)	25.56 (30.17)
8%	35.56 (35.84)	36.67 (36.73)	48.89 (44.33)
10%	13.33 (21.05)	13.33 (21.05)	66.67 (54.80)
14%	18.89 (25.53)	20.00 (26.36)	78.89 (63.90)
Control (water treated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
F-test	*	*	*
SEM±	(3.95)	(3.72)	(3.37)
CD at P=0.05	(12.17)	(11.45)	(10.37)

Figures in the parentheses are angular transformed for repellence and mortality data; are√ x+0.5 transformed for oviposition data. * Significant

Table 5: Effect of methanol extract of *Vitex trifolia* leaves on red spider mite infesting okra

Concentration	Walk -off response / repellence (%) after		Per cent mortality after
	6 h	12 h	72 h
2%	2.22 (4.99)	2.22 (4.99)	13.33 (21.14)
4%	1.11 (3.51)	3.33 (8.49)	16.67 (23.91)
6%	22.22 (28.11)	26.67 (31.01)	45.56 (42.45)
10%	28.89 (31.13)	33.33 (33.71)	37.78 (37.90)
12%	43.33 (41.07)	44.44 (41.75)	74.44 (59.63)
15%	58.89 (50.27)	73.33 (59.35)	75.56 (60.37)
Control (water treated)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
F-test	*	*	*
SEM±	(4.45)	(4.93)	(3.77)
CD at P=0.05	(13.72)	(15.18)	(7.62)

Figures in the parentheses are angular transformed for repellence and mortality data; are√ x+0.5 transformed for oviposition data. * Significant

Conclusion

The results of the present study indicate a potent effect of extracts from *Vitex* spp. The plant therefore holds great promise as a locally available miticidal/ acaricidal plant. Further work in this area should focus on the exploration of the efficacy of the isolated phytoextract under field conditions, with the aim of formulating a botanical acaricide.

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