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Vitex species, a potential store house of acaricidal principles

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Abstract

Biological activity of methanol extracts from leaves of four species of *Vitex* namely, *V. negundo* (purple & white types), *V. altissima*, *V. peduncularis* and *V. trifolia* was studied on red spider mite, *Tetranychus macfarlanei* infesting okra crop. Toxicity effects such as repellency and mortality were determined by releasing adult female 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 leaf discs differed significantly upto 12 hours. With most of the extracts this repellent property showed only marginal increase from 6 hours to 12 hours. Among the species of *Vitex*, repellent activity of *V. negundo* (purple) at 20% concentration and of *V. trifolia* at 15% concentration was more apparent with 73 per cent walk-off of mites from treated leaf discs. Mortality of adult mites caused by extracts differed significantly between 24 & 72 h interval and also showed significant increase across different test concentrations. *V. negundo* (purple) extract at 20% recorded the highest mite mortality of 89 per cent followed by 79 per cent mortality caused by *V. peduncularis* extract treatment at 14% concentration.

Keywords: *Vitex*, okra spider mite, repellence and mortality

Introduction

Most of the insect and mite pests are controlled using conventional synthetic insecticides, which are harmful to environment, humans and non-target beneficial organisms. Consequently, many pest species or their populations have also developed resistance to many synthetic pesticides. In view of this, many efforts are being made to identify alternatives like, botanical insecticides that are target specific, biodegradable, environmentally safe *etc.*, to fit into integrated pest management modules. In the recent past, several products of botanical origin, especially secondary metabolites received significant attention as potential bioactive agents in the management of insect pests (Kiran and Devi, 2007) [5]. Plant derived insecticides comprise of an array of compounds which act on both behavioural and physiological processes of pest species and hence chances of pests developing resistance to such natural substances are more unlikely. Also botanical insecticides are less likely to accumulate in any biological system as they are biodegradable (Saxena, 1987) [10]. Acaricidal properties are known in the plant genus *Vitex* of botanical family Verbenaceae, widely distributed in tropical and subtropical regions of the world. Biological activities of methanol leaf extracts of four *Vitex* spp. are investigated in the present study.

Material and methods

Laboratory investigation on the biological activities of methanol leaf extracts of *Vitex* spp. on red spider mite, *Tetranychus macfarlanei* Baker and Pritchard infesting okra was carried out in the Acarology section of Department of Agricultural Entomology, University of Agricultural Sciences, Bangalore. Okra leaves infested by red spider mites in the field were collected and reared on mulberry leaves placed on moist cotton wads in plastic trays in the laboratory or on potted okra plants in glass house and used for laboratory bioassays with treated okra leaves. Organic solvent methanol (boiling point of 65°C & polarity index of 5.1) was used for extraction from leaves of four species of *Vitex* viz., *Vitex negundo* L. (purple & white types), *V. altissima*, *V. peduncularis* and *V. trifolia*. Leaves were collected during May – June period from the Medicinal and Aromatic plants garden and botanical garden in the GKVK campus of

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University of Agril. Sciences, Bangalore. Leaves were shade dried and powdered separately using a Waring blender. 250 grams of leaf powder was used for 8-10 hours hot extraction with methanol solvent using Soxhlet apparatus. The extracts were concentrated using Vacuum Flash Evaporator and viscous semi-solid extract obtained was stored at 4°C in a refrigerator for further use in laboratory assays. Preliminary assay with mite mortality record between 10 and 90 per cent was used to fix the test concentrations for studying biological activities of extracts on okra red spider mite.

Fresh okra leaves were treated with the desired test concentration of the extract using a handy laboratory sprayer (0.5 lit. capacity) and treated leaves were air dried under a ceiling fan. Leaf bits measuring 2.5cm X 2.5cm were prepared from the treated leaves and placed on moist cotton wad in Petri plates. Thirty adult female mites released on each leaf bit served as one replication and three replications were maintained for each treatment. Leaf bits treated with water (+ teepol @ 0.1%) served as control (teepol was used as emulsifier when methanol extract was not easily miscible with water).

The number of individuals which got drowned/stuck in the moist cotton wad in the petri plate was recorded at 6 and 12 hours and per cent walk-off was computed to ascertain the repellency of the extract (Penman *et al.*, 1986)^[8]. At each observation, mites which were found drowned/stuck, but alive, were gently released back onto the corresponding leaf bit using a fine camel hair brush and observations were continued. The number of female adults found dead was recorded at 24-hour interval up to 72 hours (3 days) and per cent mortality was computed treatment-wise to determine the killing effect of the extract. Mite individuals which were unable to move (moribund) were considered as dead or killed. The data in percentages were subjected to angular transformation and analyzed statistically following the

Analysis of Variance technique for Completely Randomized Design and results were interpreted at five per cent level of significance.

Results and Discussion

Data indicating biological activities of methanol leaf extract of *Vitex* spp. against red spider mite are provided in Table 1 to 5.

Walk-off of *T. macfarlanei* adults from okra leaves treated with different concentrations of methanol leaf extract of *V. negundo* (purple) differed significantly. Progressive increase in the walk-off of adult mites was observed from 6 hours to 12 hours at all the test concentrations. Maximum repellence of adult mites recorded was 73.33 per cent in treatment with 20% methanol extract. Increase in treatment concentration from 2% to 20% also recorded increase in the mortality of mites when recorded at 72 hours after treatment. Maximum mortality was 88.89 per cent at 20 % concentration of the extract (Table 1). Data with respect to walk-off attributed to different concentrations of methanol extract (2% to 12%) of *V. negundo* (white) up to 12 hours shown in Table 2 revealed maximum repellence of 47.78 per cent with 10% treatment concentration, while maximum mortality was 76.67 per cent with the treatment concentration of 12%. According to walk-off and mortality data relevant to *V. altissima* indicated in Table 3, maximum repellence was 48.89 per cent, while maximum mortality was 71.77 per cent at treatment concentrations of 4% and 8%, respectively. With *V. peduncularis* leaf extract though walk-off was only 36.67 per cent at 8% extract treatment, higher mortality of 78.89 per cent was evident at 14% extract treatment (Table 4). Maximum walk-off of 73.33 per cent and maximum mortality of 75.56 per cent (Table 5) were apparent with *V. trifolia* leaf extract at 15% treatment concentration.

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

Concentration	Walk -off response / repellence (%) after		Per cent mortality after 72 hours
	6 hours	12 hours	
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 values; * - Significant

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

Concentration	Walk -off response / repellence (%) after		Per cent mortality after 72 hours
	6 hours	12 hours	
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 values; * - Significant

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

Concentration	Walk -off response / repellence (%) after		Per cent mortality after 72 hours
	6 hours	12 hours	
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 values; * - Significant

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

Concentration	Walk -off response / repellence (%) after		Per cent mortality after 72 hours
	6 hours	12 hours	
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 values; * - Significant

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

Concentration	Walk -off response / repellence (%) after		Per cent mortality after 72 h
	6 h	12 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 values; * - Significant

Among the methanol leaf extracts of *Vitex*, highest repellence of 73.33 per cent was evident with *V. negundo* (purple type) and *V. trifolia* at the test concentrations of 20% and 15%, respectively. Highest mortality of 88.89 per cent was recorded with *V. negundo* (purple type) methanol leaf extract at 20% concentration. Repellent activity of *V. negundo* extracts against certain species of insects and Acari (ticks) have been documented. As observed in the present study, Srinivasa and Sugeetha (1999) [11] recorded 56 per cent walk-off of *T. macfarlanei* adults from okra leaves up to 12 hours, when treated with *V. negundo* methanol extract at 5-6% concentrations. Gopal (2000) [4] 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. Mitra *et al.* (2015) [7] suggested the use of plant origin pesticides which contain phenols, saponins, xanthoproteins, triterpenoids, tannins and flavonoids for the control of tetranychid mite, *Petrobia harti* infesting medicinal herb *Oxalis corniculata*. Mehlhorn *et al.* (2005) [6] recommended the application of crude leaf extracts from *Vitex agnus-castus* (monk's pepper) on animals and human beings to protect them from blood sucking ticks, *Ixodes ricinus* and *Rhipicephalus sanguineus* for at least 6 hours. Similarly, successful and promising use of *Vitex* leaf extracts against chewing insect pest *Spodoptera litura* (Deepthy *et al.*, 2010) [2], sap sucking pests like red cotton bug, *Dysdercus cingulatus* (Ranilalitha *et al.*, 2015) [9] and tea mosquito bug *Helopeltis theivora* (Deka *et al.*, 2016) [3], stored product pest *Tribolium castaneum* (Chowdhury *et al.*, 2009) [1] have been reported.

Conclusion

Present study revealed the potential biological effects of methanol leaf extract of *Vitex* spp., particularly the repellent effect of *V. negundo* (purple type) & *V. trifolia* and killing effect of *V. negundo*, *V. peduncularis* & *V. trifolia* against okra red spider mite *T. macfarlanei*. Future work in this area should focus on separation and identification of active principles as well as the possibility of developing a suitable botanical formulation for use under field conditions.

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