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Bio-efficacy of plant derivatives and natural oils against two spotted spider mite, *Tetranychus urticae* Koch

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Abstract
An *in-vitro* study was conducted to screen certain plant derivatives and natural oils against *Tetranychus urticae* Koch based on percent mortality of mites and percent reduction in egg laying by mites. The study revealed that propargite 57 EC @ 2.00ml/l (standard check) was significantly superior than the botanical extracts and natural oils tested in terms of mortality of adults (84.63 percent) and reduction of eggs (81.82 percent) respectively. However among the different plant derivatives and natural oils evaluated, tulsi leaf extract @10 percent, neem oil @ 5 percent, neem oil @ 3 percent, nochi leaf extract @10 percent and nochi leaf extract @ 5 percent recorded the maximum mortality of mites (81.15, 80.72, 80.58, 80.29 and 79.98 percent) and the maximum reduction of eggs as well (74.93, 74.41, 73.99, 73.52 and 73.10 percent) respectively, which were statistically on par in their efficacy. From the present investigation it is evident that tulsi leaf extract @10 percent, neem oil @ 3 percent and nochi leaf extract @ 5 percent were found to be the best candidates which can be recommended as an alternative to synthetic chemical acaricides for the management of *T. urticae* Koch.

Keywords: Bio-efficacy, Plant derivatives and Natural oils, two spotted spider mites, *Tetranychus urticae* Koch

1. Introduction
Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan and polyphagous pest with great economic importance. Owing to the changing farming scenario as well as climatic conditions, *T. urticae* has become a serious problem on many crops in protected and field conditions [5, 23, 15]. *T. urticae* affects the crops by direct feeding; thereby reducing the area of photosynthetic activity and causing leaf abscission in severe infestations [9]. Piercing of cells by mite stylets leads to the mechanical damage of cells and injection of saliva by mites into plant cells causes changes in cell physiology, cytology and biochemical processes of punctured as well as nonpunctured adjacent cells. *T. urticae* feeding can also damage stomata and the palisade layer in the leaves, which ultimately results in typical "stippling" damage, with white or grayish coloured speckles on the leaves due to the punctures made by feeding [17]. Chlorosis, bronzing of leaves, defoliation and even plant death may also occur in case of severe infestation [14].
Conventional management of *T. urticae* includes synthetic chemical acaricide treatments that could lead to undesirable side effects, such as death of non-target organisms, development of pesticide-resistant races and residue concerns [6], outbreak of secondary pests [4], pest resurgence [16], dermal toxicity to the labours exposed in the field [13], environmental pollution through accumulation of pesticides in soil, water and air [2].
Hence to overcome the adverse effects of synthetic acaricides, nowadays certain plant derivatives and natural oils have been proved to be effective as suitable alternatives [11, 19]. The plant derivatives and natural oils have been traditionally regarded as a rich source of biochemical substances that may perhaps play a considerable role in the management of phytophagous insect and mite pests. The plant derivatives and natural oils in general are deemed as more eco-friendly in comparison to synthetic chemical acaricides. They are by and large characterized by reduced impact on non-targeted organisms, lower mammalian toxicity, and short persistence in the environment [8]. Therefore keeping the potentiality of plant derivatives and natural oils in view, an attempt was made to test certain plant derivatives and natural oils against *T. urticae* Koch in *in-vitro* condition.

2. Materials and Methods

A laboratory experiment was conducted to screen certain plant derivatives and natural oils based on percent mortality of mites and percent reduction in egg laying capacity by the mites. Certain plant derivatives and natural oils *viz.*, soapnut extract (*Sapindus marginatus* L.), garlic bulb extract (*Allium sativum* L.), pongamia oil (*Pongamia pinnata* (L.) Panigrahi), neem oil (*Azadirachta indica* A. Juss.,), nochi leaf extract (*Vitex negundo* L.), tulsi leaf extract (*Ocimum sanctum* L.), fish oil rosin soap (FORS), cashew nut shell liquid (*Anacardium occidentale* L.) (CNSL), propolis and horticultural mineral oil (HMO) were tested against *T. urticae* with different concentrations and the treatments were imposed by the leaf dip method. The popular acaricide (propargite 57 EC @ 2.00ml/l) was used as standard check for comparison besides keeping an untreated check.

2.1 Preparation of ethanolic extracts of plant derivatives and ethanol based natural oil formulation

The botanical extracts were prepared as per the methodology suggested by Premalatha [20]. The botanicals which are indigenous and locally available were collected from field / medicinal plant garden. Plant parts like leaves / rhizome / fruits / bulb were shade dried, before preparing the ethanolic extracts, (Soxhlet's apparatus method) for foliar application and comparative efficacy studies in laboratory condition. The plant parts were washed with water, then shade dried and ground separately from which 50g of the well powdered material was soaked in 100ml of solvent (Ethanol) for 48 hrs at room temperature. The content was often stirred, after complete soaking; the extract was decanted and filtered through Whatman No.1filter paper. The filtrate was then made up to 100 ml by adding 5ml of Triton X 100 (emulsifier) and the required quantity of solvent. The natural oils used in the study were purchased from commercial venders and were diluted in ethanol + water (70 + 30 by volume) mixtures then the solutions were made upto 100 ml by adding 5ml of Triton X 100 (emulsifier) and the required quantity of solvent. The final material was equivalent to 50 EC of the respective natural oil formulations.

2.2 Screening for the efficacy of plant derivatives and natural oils

The screening was performed using leaf discs placed on a moist cotton pad on a Petridish, surrounded with vaseline to prevent the escape of mites. The test solutions were diluted to prepare different concentrations and the assays were carried out. The experiment was carried out in a Completely Randomized Design with three replications which were compared with standard check (propargite 57 EC @ 2.00ml/l) besides keeping an untreated check. The toxicity of the test compounds were evaluated by leaf disc dip technique as suggested by Seigler [22].

The formulated compounds were diluted to required concentrations by dilution method. Leaf discs of mulberry were dipped in each test concentration for 60 seconds and shade dried. Then 30 adult females of *T. urticae* were released to each disc. The discs were then placed on a moist cotton pad contained in Petri dishes and kept under controlled conditions of 25 $\pm$ 1 ^{0}C & 75 $\pm$ 5% RH. The response of phytophagous mites in terms of number of eggs laid and mortality was recorded at 24, 48, 72, 96, 120 and 144 hours after treatment. At the end of the experiment, the mean population of eggs and mites were worked out after square root transformation as suggested by Goulden [10], for calculating percent mortality of mites over untreated check and percent reduction in egg laying capacity over untreated check.

3. Results and Discussion

The cumulative mean data revealed that (Table 1 & 2 and Fig. 1 & 2) among the plant derivatives and natural oils tested for their acaricidal property; tulsi leaf extract @10 percent, neem oil @ 5 percent, neem oil @ 3 percent, nochi leaf extract @10 percent and nochi leaf extract @ 5 percent recorded the maximum percent mortality of mites (81.15, 80.72, 80.58, 80.29 and 79.98 percent) and the maximum percent reduction of eggs (74.93, 74.41, 73.99, 73.52 and 73.10 percent) respectively, which were statistically on par in their efficacy, followed by pongamia oil @ 5 percent, pongamia oil @ 3 percent, soap nut extract @ 10 percent and garlic bulb extract @ 10 percent recorded 69.94, 69.72, 69.28 and 68.70 percent mortality of mites and 61.87, 61.44, 61.11and 60.85 percent reduction of eggs respectively, which were statistically on par in their efficacy.

However propargite 57 EC @ 2.00ml/l (standard check) was significantly superior than the botanical extracts and natural oils tested with the highest mortality of adults (84.63 percent) and the highest reduction of eggs (81.82 percent) respectively. Among all the botanical extracts and natural oils evaluated against *T. urticae*, tulsi leaf extract @10 percent, neem oil @ 3 percent, nochi leaf extract @ 5 percent was found to be the best promising candidates; which can be recommended as an alternative to synthetic acaricides. In case of neem oil @ 3 and 5 percent and nochi leaf extract @ 5 and 10 percent, the lower concentrations of these candidates are recommended since both the higher and lower concentrations of these candidates were statistically significant in their effectiveness.

The results obtained from the present study is in conformity with the reports of Kanniammal [12] who reported that the *O. sanctum* leaf extract was found very promising with 77.30 percent adult mortality on *T. urticae*. Bussaman [1] also reported similar findings to vouch that leaf extracts of *O. sanctum* recorded the maximum of cent percent mortality of Mushroom mite, *Luciaphorus* sp. Roy [21] reported that 3 percent methanolic extract of *Ocimum tenuiflorum* Linn. exhibited acaricidal activity against *T. neocaledonicus* Andre with 97 percent mortality. So also Patel [18] reported that neem oil 1 percent caused upto 64.40 percent mortality on *T. cinnabarinus*. Premalatha [20] have also proved that neem oil @ 3 percent recorded 74.00 percent reduction in population of *T. urticae* over the untreated check which is in close conformity with the present findings. Gajalakshmi [7] reported that the leaf extract of *V. negundo* @ 10 percent recorded 65.83 percent mortality of *T. urticae* at 72 hours after treatment. Chiasson [3] also proved that aqueous leaf extract of *V. negundo* (@ 5 percent) registered 70-91 percent mortality of mite, at 72 hours after treatment.

Table 1: *In- vitro* bio-assay of plant derivatives and natural oils against TSSM (*T. urticae* Koch) (At $25^0 \pm 1^0$C and 75 ± 5% RH)

Treatments	No. of mites/ leaf disc of 50mm size (hours after treatment)*								% mortality over untreated check
	No. of mites released / leaf disc	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	Mean	
T_1- Soapnut extract @ 5%	30	25.30 (5.03)^h	23.31 (4.83)^i	22.00 (4.69)^i	20.31 (4.51)^l	19.64 (4.43)^k	18.63 (4.32)^i	21.53 (4.64)^i	38.59
T_2- Soapnut extract @ 10%	30	15.53 (3.94)^c	12.51 (3.54)^c	10.66 (3.26)^c	9.51 (3.08)^{ef}	8.63 (2.94)^{de}	7.79 (2.79)^c	10.77 (3.28)^c	69.28
T_3- Garlic bulb extract @ 5%	30	26.67 (5.16)^i	25.00 (5.00)^j	23.33 (4.83)^j	21.67 (4.66)^m	21.00 (4.58)^l	19.64 (4.43)^j	22.89 (4.78)^j	34.73
T_4- Garlic bulb extract @ 10%	30	15.79 (3.97)^c	12.83 (3.58)^c	10.80 (3.29)^c	9.79 (3.13)^f	8.81 (2.97)^e	7.83 (2.80)^c	10.98 (3.31)^c	68.70
T_5- Pongamia oil @ 3%	30	15.34 (3.92)^c	12.39 (3.52)^c	10.58 (3.25)^c	9.36 (3.06)^{ef}	8.35 (2.89)^d	7.67 (2.77)^c	10.62 (3.26)^c	69.72
T_6- Pongamia oil @ 5%	30	15.31 (3.91)^c	12.33 (3.51)^c	10.43 (3.23)^c	9.21 (3.03)^e	8.33 (2.89)^d	7.63 (2.76)^c	10.54 (3.25)^c	69.94
T_7- Neem oil @ 3%	30	11.83 (3.44)^b	8.56 (2.93)^b	6.83 (2.61)^b	5.30 (2.30)^{bc}	4.53 (2.13)^{bc}	3.80 (1.95)^b	6.81 (2.61)^b	80.58
T_8- Neem oil @ 5%	30	11.76 (3.43)^b	8.51 (2.92)^b	6.80 (2.61)^b	5.26 (2.29)^{bc}	4.46 (2.11)^b	3.76 (1.94)^b	6.76 (2.60)^b	80.72
T_9- Nochi leaf extract @ 5%	30	11.96 (3.46)^b	8.79 (2.96)^b	6.93 (2.63)^b	5.71 (2.39)^d	4.79 (2.19)^c	3.93 (1.98)^b	7.02 (2.65)^b	79.98
T_{10}- Nochi leaf extract @ 10%	30	11.89 (3.45)^b	8.65 (2.94)^b	6.89 (2.62)^b	5.53 (2.35)^{cd}	4.61 (2.15)^{bc}	3.90 (1.97)^b	6.91 (2.63)^b	80.29
T_{11}- Tulsi leaf extract @ 5%	30	22.30 (4.72)^g	20.33 (4.51)^h	18.67 (4.32)^h	17.00 (4.12)^k	16.33 (4.04)^j	15.67 (3.96)^h	18.38 (4.29)^h	47.57
T_{12}- Tulsi leaf extract @ 10%	30	11.66 (3.41)^b	8.33 (2.89)^b	6.67 (2.58)^b	5.00 (2.24)^b	4.33 (2.08)^b	3.67 (1.92)^b	6.61 (2.57)^b	81.15
T_{13}- CNSL @ 3%	30	21.36 (4.62)^g	19.03 (4.36)^g	17.38 (4.17)^g	16.02 (4.00)^j	15.37 (3.92)^i	14.69 (3.83)^g	17.31 (4.16)^g	50.63
T_{14}- CNSL @ 5%	30	21.33 (4.62)^g	19.00 (4.36)^g	17.33 (4.16)^g	16.00 (4.00)^j	15.33 (3.92)^i	14.67 (3.83)^g	17.28 (4.16)^g	50.72
T_{15}- FORS @ 15%	30	27.63 (5.26)^i	26.00 (5.10)^k	24.33 (4.93)^k	22.67 (4.76)^n	22.00 (4.69)^m	20.67 (4.55)^k	23.88 (4.89)^k	31.88
T_{16}- FORS @ 20%	30	18.33 (4.28)^d	15.33 (3.92)^d	13.67 (3.70)^d	12.00 (3.46)^g	11.33 (3.37)^f	10.67 (3.27)^d	13.56 (3.68)^d	61.34
T_{17}- Propolis @ 0.75%	30	20.33 (4.51)^f	18.04 (4.25)^f	16.00 (4.00)^f	14.67 (3.83)^i	14.04 (3.75)^h	13.33 (3.65)^f	16.07 (4.01)^f	54.17
T_{18}- Propolis @ 1%	30	20.31 (4.51)^f	18.00 (4.24)^f	15.97 (4.00)^f	14.63 (3.82)^i	14.00 (3.74)^h	13.31 (3.65)^f	16.04 (4.00)^f	54.26
T_{19}- HMO @ 2%	30	19.33 (4.40)^e	16.33 (4.04)^e	15.00 (3.87)^e	13.33 (3.65)^h	12.67 (3.56)^g	12.03 (3.47)^e	14.78 (3.84)^e	57.84
T_{20}- HMO @ 3%	30	19.00 (4.36)^{de}	16.31 (4.04)^e	14.98 (3.87)^e	13.31 (3.65)^h	12.63 (3.55)^g	12.00 (3.46)^e	14.71 (3.83)^e	58.06
T_{21}- Propargite 57 EC @ 2ml/l	30	10.66 (3.26)^a	7.33 (2.71)^a	5.67 (2.38)^a	4.00 (2.00)^a	2.67 (1.63)^a	2.00 (1.41)^a	5.39 (2.32)^a	84.63
T_{22}- Untreated check	30	31.06 (5.57)^j	32.63 (5.71)^l	34.36 (5.86)^l	35.96 (6.00)^o	37.66 (6.14)^n	38.69 (6.22)^l	35.06 (5.92)^l	-
SEd CD (p=0.05) CV%	NS*	0.0529 0.1067 1.53	0.0447 0.0900 1.40	0.0448 0.0902 1.49	0.0357 0.0719 1.26	0.0368 0.0743 1.35	0.0398 0.0802 1.51	0.0504 0.1016 1.68	-

* NS – Non significant

* Each value is the mean of three replications

Figures in parentheses are square root transformed values

In a column, means followed by common letter(s) is /are not significantly different by LSD at P=0.05%.

Table 2: *In- vitro* bio-assay of plant derivatives and natural oils for their impact on oviposition of TSSM (*T. urticae* Koch) (At $25^0 \pm 1^0$C and 75 ± 5% RH)

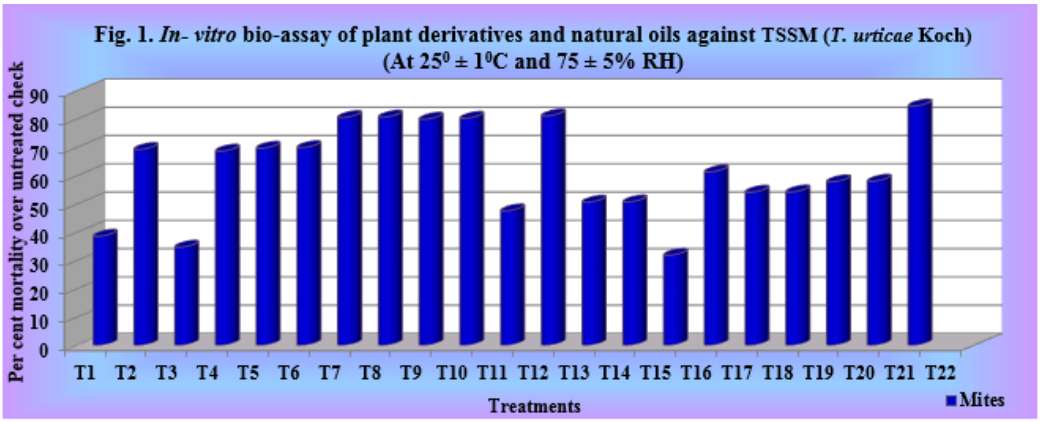
Treatments	No. of eggs layed / leaf disc of 50mm size (hours after treatment)*								% reduction over untreated check
	No. of mites released/ leaf disc	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	Mean	
T_1- Soapnut extract @ 5%	30	7.66 (2.77)^{k}	14.96 (3.87)^{l}	18.96 (4.35)^{k}	20.36 (4.51)^{k}	23.76 (4.87)^{i}	26.63 (5.16)^{j}	18.72 (4.33)^{j}	21.32
T_2- Soapnut extract @ 10%	30	4.43 (2.10)^{e}	8.03 (2.83)^{f}	8.83 (2.97)^{de}	10.03 (3.17)^{e}	11.03 (3.32)^{c}	11.53 (3.40)^{cd}	8.98 (3.00)^{d}	62.26
T_3- Garlic bulb extract @ 5%	30	8.00 (2.83)^{l}	15.53 (3.94)^{m}	19.93 (4.46)^{l}	21.86 (4.68)^{l}	24.93 (4.99)^{j}	27.89 (5.28)^{k}	19.69 (4.44)^{k}	17.25
T_4- Garlic bulb extract @ 10%	30	4.51 (2.12)^{e}	8.13 (2.85)^{f}	9.01 (3.00)^{e}	10.26 (3.20)^{e}	11.13 (3.34)^{c}	11.69 (3.42)^{d}	9.12 (3.02)^{d}	61.67
T_5- Pongamia oil @ 3%	30	4.36 (2.09)^{e}	7.86 (2.80)^{ef}	8.69 (2.95)^{de}	9.96 (3.16)^{e}	10.83 (3.29)^{c}	11.16 (3.34)^{cd}	8.81 (2.97)^{d}	62.98
T_6- Pongamia oil @ 5%	30	4.33 (2.08)^{e}	7.63 (2.76)^{e}	8.43 (2.90)^{d}	9.89 (3.14)^{e}	10.76 (3.28)^{c}	11.03 (3.32)^{c}	8.68 (2.95)^{d}	63.53
T_7- Neem oil @ 3%	30	3.67 (1.92)^{cd}	6.00 (2.45)^{cd}	6.93 (2.63)^{c}	8.43 (2.90)^{bcd}	9.01 (3.00)^{b}	9.58 (3.10)^{b}	7.27 (2.70)^{bc}	69.45
T_8- Neem oil @ 5%	30	3.49 (1.87)^{bc}	5.93 (2.44)^{c}	6.86 (2.62)^{bc}	8.26 (2.87)^{bc}	8.93 (2.99)^{b}	9.51 (3.08)^{b}	7.16 (2.68)^{bc}	69.90
T_9- Nochi leaf extract @ 5%	30	3.83 (1.96)^{d}	6.26 (2.50)^{d}	7.13 (2.67)^{c}	8.83 (2.97)^{d}	9.16 (3.03)^{b}	9.73 (3.12)^{b}	7.49 (2.74)^{c}	68.52
T_{10}- Nochi leaf extract @ 10%	30	3.76 (1.94)^{d}	6.13 (2.48)^{cd}	7.03 (2.65)^{c}	8.76 (2.96)^{cd}	9.09 (3.01)^{b}	9.65 (3.11)^{b}	7.40 (2.72)^{c}	68.89
T_{11}- Tulsi leaf extract @ 5%	30	6.67 (2.58)^{j}	14.33 (3.79)^{k}	17.13 (4.14)^{j}	19.03 (4.36)^{j}	22.22 (4.71)^{h}	24.46 (4.95)^{i}	17.31 (4.16)^{i}	27.27
T_{12}- Tulsi leaf extract @ 10%	30	3.33 (1.82)^{b}	5.63 (2.37)^{b}	6.53 (2.56)^{b}	8.10 (2.85)^{b}	8.86 (2.98)^{b}	9.42 (3.07)^{b}	6.98 (2.64)^{b}	70.67
T_{13}- CNSL @ 3%	30	6.34 (2.52)^{i}	13.46 (3.67)^{j}	16.22 (4.03)^{i}	18.73 (4.33)^{ij}	20.86 (4.57)^{g}	21.83 (4.67)^{h}	16.24 (4.03)^{h}	31.75
T_{14}- CNSL @ 5%	30	6.31 (2.51)^{i}	13.33 (3.65)^{j}	16.03 (4.00)^{i}	18.22 (4.27)^{i}	20.23 (4.50)^{g}	21.26 (4.61)^{h}	15.90 (3.99)^{h}	33.19
T_{15}- FORS @ 15%	30	8.33 (2.89)^{m}	16.22 (4.03)^{n}	21.23 (4.61)^{m}	23.66 (4.86)^{m}	25.73 (5.07)^{j}	28.96 (5.38)^{l}	20.69 (4.55)^{l}	13.06
T_{16}- FORS @ 20%	30	5.33 (2.31)^{f}	10.83 (3.29)^{g}	11.26 (3.36)^{f}	12.16 (3.49)^{f}	13.96 (3.74)^{d}	15.56 (3.94)^{e}	11.52 (3.39)^{e}	51.60
T_{17}- Propolis @ 0.75%	30	6.03 (2.46)^{h}	12.86 (3.59)^{i}	14.96 (3.87)^{h}	16.96 (4.12)^{h}	18.73 (4.33)^{f}	20.13 (4.49)^{g}	14.95 (3.87)^{g}	37.19
T_{18}- Propolis @ 1%	30	6.00 (2.45)^{h}	12.69 (3.56)^{i}	14.63 (3.82)^{h}	16.63 (4.08)^{h}	18.56 (4.31)^{f}	19.93 (4.46)^{g}	14.74 (3.84)^{g}	38.05
T_{19}- HMO @ 2%	30	5.69 (2.39)^{g}	11.89 (3.45)^{h}	13.43 (3.66)^{g}	13.93 (3.73)^{g}	15.86 (3.98)^{e}	18.26 (4.27)^{f}	13.18 (3.63)^{f}	44.62
T_{20}- HMO @ 3%	30	5.67 (2.38)^{g}	11.53 (3.40)^{h}	13.29 (3.65)^{g}	13.46 (3.67)^{g}	15.53 (3.94)^{e}	17.73 (4.21)^{f}	12.87 (3.59)^{f}	45.92
T_{21}- Propargite 57 EC @ 2ml/l	30	2.56 (1.60)^{a}	3.68 (1.92)^{a}	5.29 (2.30)^{a}	7.03 (2.65)^{a}	7.76 (2.79)^{a}	7.93 (2.82)^{a}	5.71 (2.39)^{a}	76.01
T_{22}- Untreated check	30	9.86 (3.14)^{n}	18.83 (4.34)^{o}	24.26 (4.93)^{n}	27.33 (5.23)^{n}	29.83 (5.46)^{k}	32.66 (5.71)^{m}	23.80 (4.92)^{m}	-
SEd CD (p=0.05) CV%	NS*	0.0260 0.0523 1.38	0.0296 0.0596 1.14	0.0366 0.0737 1.30	0.0457 0.0921 1.52	0.0422 0.0851 1.33	0.0467 0.0941 1.42	0.0371 0.0748 1.31	-

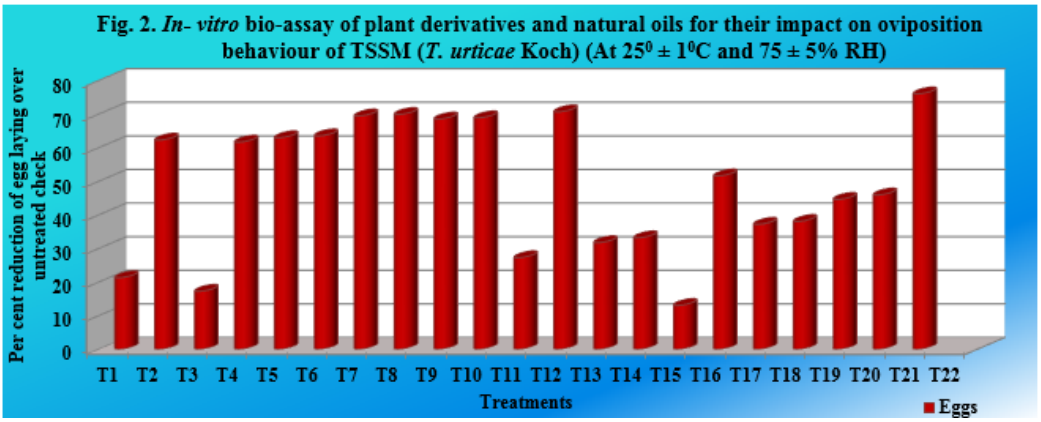
* NS – Non significant.

* Each value is the mean of three replications.

Figures in parentheses are square root transformed values.

In a column, means followed by common letter(s) is /are not significantly different by LSD at P=0.05%.





Treatments

T_1- Soapnut extract @ 5%
T_2- Soapnut extract @ 10%
T_3- Garlic bulb extract @ 5%
T_4- Garlic bulb extract @ 10%
T_5- Pongamia oil @ 3%
T_6- Pongamia oil @ 5%
T_7- Neem oil @ 3%
T_8- Neem oil @ 5%
T_9- Nochi leaf extract @ 5%
T_{10}- Nochi leaf extract @ 10%
T_{11}- Tulsi leaf extract @ 5%
T_{12}- Tulsi leaf extract @ 10%
T_{13}- CNSL @ 3%
T_{14}- CNSL @ 5%
T_{15}- FORS @ 15%
T_{16}- FORS @ 20%
T_{17}- Propolis @ 0.75%
T_{18}- Propolis @ 1%
T_{19}- HMO @ 2%
T_{20}- HMO @ 3%
T_{21}- Propargite 57 EC @ 2ml/l
T_{22}-Untreated check

4. Conclusion

From the present study it is evident that tulsi (*Ocimum sanctum* L.) leaf extract @10 percent, neem (*Azadirachta indica* A. Juss.,) oil @ 3 percent and nochi (*Vitex negundo* L.) leaf extract @ 5 percent were found to be the best candidates which can be recommended as an alternative to synthetic chemical acaricides for the management of *T. urticae* Koch.

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