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## Exploration of acaricidal properties of certain seed oils against the Red Spider Mite (RSM), *Oligonychus coffeae* Nietner (Tetranychidae: Acarina) infesting tea

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### Abstract

*Simarouba glauca* and *Hydnocarpus pentandra* seed oils were evaluated against RSM under laboratory conditions. Among the tested treatments, *H. pentandra* and *S. glauca* @ 5ml/L were achieved significant mortality over adult RSM. Similarly, *H. pentandra* seed oil @ 4ml & 5ml/L have significantly achieved 100% egg mortality and other treatments such as Paraffinic oil @ 3ml/L and Neem oil @ 3ml/L and *S. glauca* seed oils were achieved at about 98%, 92% and 90% of egg mortality respectively. The oviposition deterrence study reveals that the least number of eggs were laid on the treatments of *H. pentandra* seed oil @ 5ml & 4ml/L and *S. glauca* seed oil @ 5ml/L which achieved the highest Discrimination Quotient (DQ) value of 0.65, 0.63 and 0.58 respectively. Both seed oils could be used as an effective alternative source to control RSM due to has admirable acaricidal, ovicidal and oviposition deterrence properties.

**Keywords:** biocontrol, fatty acids, ovicidal, oviposition deterrent

### Introduction

The Red Spider Mite (RSM), *Oligonychus coffeae* Nietner (Tetranychidae: Acarina) is considered as one of the major pest in tea industries (Radhakrishnan and Prabhakaran, 2014) [13]. It normally infests the upper surface of mature tea leaves and severe infestation could occur in both young and mature leaves and its leads to defoliation (Srikumar *et al.*, 2015) [22]. Severe infestation of RSM leads to significant crop loss of at about 17-46% (Babu *et al.*, 2008) [1] and could be attained at about 8–17 % increase in crop yield by controlling of RSM (Somnath Roy *et al.*, 2014) [21]. The control of RSM is mostly succeeded by various synthetic acaricides. Extensive use of these acaricides paves the way to environmental contaminations and residue issues in the made tea (Perumalsamy *et al.*, 2010) [10]. To overcome the issues, various alternating controlling measures are adopted for the management of RSM. Many previous investigations show that the plant extracts have a wide range of insecticidal and acaricidal properties and the same have been broadly evaluated against various polyphagous pests for the last two decades (Durdane *et al.*, 2011, Roy *et al.*, 2018; Rowyda *et al.*, 2018) [6, 17, 16]. As per the previous investigations, crude palm oil and cotton oils, orange peel oil (Hill and Schoonhoven, 1981) [8], eucalyptus oil (Rahman and Talukder, 2006) [14], *Delonix regia* seed oil (Obembe, 2017) [12] and seed oils of *Pongamia glabra*, *Azadirachta indica* and *Chrysanthemum cinerariifolium* (Pavela, 2009) [23] were identified as potential sources for pest control on various pests.

So, in the present study, two different seed oils such as *Simarouba glauca* and *Hydnocarpus pentandra* were evaluated for their acaricidal, ovicidal and oviposition deterrence against the RSM. *Simarouba glauca* belongs to the family Simaroubaceae and commonly known as a paradise tree. It originates from North America with the characteristics of a medium-sized tree (<20m), girth at about 50-80 cm and a life span of 70 years. It grows in various agro-climatic conditions and it produces oval elongated purple-coloured fleshy fruits from March to June (Mishra *et al.*, 2012) [11]. *Hydnocarpus pentandra* (Buch.-Ham.) Oken belongs to the family Flacourtiaceae and commonly known as Marotti. It is considered a medicinal herb and available in semi-evergreen forests of Western Ghats, India. Seeds yield chaulmoogra oil that has been using for various medicinal purposes due to the presence of its antifungal and antibacterial properties (Shyam Krishnan *et al.*, 2013) [20].

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In further both oils has a wide range of various biological properties. Apart from that, an attempt was made to explore the acaricidal property.

## Materials and Methods

### Seed collection and oil extraction

*S. glauca* seeds were collected from Kuruchukottai village (10°30'33.4"N 77°13'00.9"E), Udumalpet, Tamil Nadu, India and *H. pentandra* seeds were bought from Pollachi market, Pollachi, Tamil Nadu, India. Collected seeds were washed and shade dried under laboratory condition then stored in a tight plastic bag for further studies. 100g of seeds were weighted and grained into a fine powder using an electric blender and powdered samples were used for oil extraction in the Soxhlet apparatus by using n-Hexane and petroleum ether solvents for *S. glauca* and *H. pentandra* seeds respectively. Oil was collected at the end of the 10<sup>th</sup> cycle and concentrated by removing excess solvent using a vacuum evaporator. Concentrated oil was stored at -4°C for further analysis.

### Treatment details

A total of eight treatments were evaluated for its acaricidal, ovicidal and oviposition deterrence against RSM under laboratory conditions. Both *S. glauca* and *H. pentandra* seed oils were evaluated at five different concentrations viz., T<sub>1</sub>@1ml/L, T<sub>2</sub>@2ml/L, T<sub>3</sub>@3ml/L, T<sub>4</sub>@4ml/L and T<sub>5</sub>@5ml/L. Neam oil @ 3ml/L (T<sub>6</sub>) and Paraffinic oil @ 3ml/L (T<sub>7</sub>) were considered as a positive control. Distilled water spray (contains 0.05% of Tween 80) was considered as a negative control. The surfactant Tween 80 @ 0.05% were added for the preparation of *S. glauca*, *H. pentandra* and neem oil formulations. Paraffinic oil used as such in the treatments.

### Laboratory mass rearing and Bio-efficacy studies

Red spider mites were collected from the field and reared on the potted tea plant as a stock culture. Laboratory mass rearing was done by the "Leaf flotation" technique. The "Leaf disc method" (Selvasundaram *et al.*, 1999)<sup>[19]</sup> was adopted to the bio-efficacy studies against adult RSM. A 2cm diameter leaf discs were prepared and kept over the wet cotton in the Petri plate. 10 adult mites were introduced on the disc using a single bristle camel hair brush and spraying was done with a fine atomizer. Each treatment was replicated five times. Adult mortality was observed every 24 hours until 96 hours.

### Ovicidal activity

For the ovicidal activity, washed small whole leaves (between 2.5-3.5cm diameter and 3cm length) were kept on the wet cotton layer and a small cotton piece was kept on the petiole of each leaf to ensuring the maximization of freshness in the leaf throughout the study period. Five days old 10 adult female RSMs were introduced into the leaf by using a single bristle camel hair brush and allowed to lay eggs for 24 hours. Adults were removed after 24 hours and eggs were counted on each leaf using a light microscope before application. The same eight treatments were evaluated and replicated thrice. The ovicidal activity was recorded up to the 15<sup>th</sup> day after the application.

### Ovipositional deterrence

For the ovipositional deterrence study also small flatted whole mother leaf method was adopted. The treated leaves were left in the room for evaporating surface water than five days old

female RSMs were introduced into each leaf by using a single bristle camel hairbrush. The number of eggs was counted every 24 hours until its oviposition period. The ovipositional degree of deterrence was calculated by comparing the egg laid in both treated and untreated leaves. The ovipositional deterrence was indexed with the Discrimination Quotient (DQ) value from 0 to 1. DQ value was calculated using the following formula. Each treatment was replicated thrice.

$$\text{Discrimination Quotient (DQ)} = \frac{\text{No. of eggs on control leaves} - \text{No. of eggs on treated leaves}}{\text{No. of eggs on control leaves} + \text{No. of eggs on treated leaves}}$$

### Statistical analysis

All collected data were pooled together for statistical analysis. Bio-efficacy data were subjected to Duncan Multiple Range Test (DMRT) to determining the significant results at each time interval. Probit analysis also performed to determine the LD<sub>50</sub> and LD<sub>90</sub> values. DMRT also performed for determining the significant ovicidal activity of all tested treatments. All the statistical analysis was done by SPSS v16.0 software and results with  $p < 0.05$  was considered statistically significant.

### Results and Discussion

All the treatments shows significant mortality between and within the tested treatments at after 24 hours (df = 64, F=21.169,  $p < 0.01$ ), after 48 hours (df = 64, F=28.703,  $p < 0.01$ ), after 72 hours (df = 64, F=46.838,  $p < 0.01$ ) and after 96 hours (df = 64, F=64.427,  $p < 0.01$ ) of the application. The results were tabulated in Table 1. Among the tested treatments on adult mortality, a maximum of 87% and 86% of the adult mortality was observed in *H. pentandra* @ 5ml/L and *S. glauca* @ 5ml/L respectively, whereas positive standards such as neem oil @ 3ml/L and paraffinic oil @ 3ml/L were achieved only 64% and 24% respectively at after 96 hours of application. Paraffinic oil @ 3ml/L was initially showed significant highest mortality after 24 hours however its mortality was on par with the treatments such as *S. glauca* @ 3ml/L, 4ml/L after 48 hours and also shows equivalence mortality with *S. glauca* @ 3ml/L, 4ml/L, 5ml/L at after 72 hours. *H. pentandra* seed oil @ 3ml/L and Paraffinic oil @ 3ml/L were gives nil mortality after 96 hours of the observation. *S. glauca* seed oil @ 4ml/L, 5ml/L and *H. pentandra* seed oil @ 5ml/L were achieved significant highest mortality of 82%, 86% and 87% respectively at after 96 hours of the application. Probit analysis results were tabulated in Table 4 and it shows that *S. glauca* and *H. pentandra* seed oils required 1.23ml and 2.66ml/L respectively to achieve 50% of the adult mortality. Likewise, same it requires 5.03ml and 7.18ml/L to achieve 90% mortality after 96 hours of the application. Santhana Bharathi *et al.*, (2020)<sup>[18]</sup> reported that application of 1.5 and 2% of *S. glauca* seed oil produced 100% mortality on *Eligma narcissus* after 96 hours of application due to the presence of fatty acids such as oleic acid, palmitic acid, stearic acid and linoleic acid as key compounds. Also, he reported that *S. glauca* seed oil required 0.399ml and 0.72ml/L to achieve 50% and 90% mortality respectively on *E. narcissus*. So, *S. glauca* acts much better in lepidopteran pests when compared to mites. Sivaraman *et al.*, (2017)<sup>[24]</sup> found that crude seed oil of *H. pentandra* exhibits 41.6% of pupal mortality and adult malformation against *Helicoverpa armigera* at 2% concentration. Similarly, Sivaraman *et al.*, 2014<sup>[25]</sup> also documented that the application of hexane, chloroform, ethyl acetate and methanol extracts of *H. pentandra* seed oils shows that the chloroform

extract was the most effective treatment against the *Aedes aegypti* and *Culex quinquefasciatus*. Equally, Prashith kekuda *et al.*, (2017) [26] evaluated *H. pentandra* seed oils against II and IV instar larvae of *A. aegypti*. The results show that 96% and 70% of larval mortality was observed in II and IV instars respectively. But in the present study, a maximum of 87% of mortality was observed in *H. pentandra* seed oils @ 5ml/L after 96 hours of the application. Chowdhury *et al.*, (2003) [4] found that the application of *S. delagoense* and *Lippia javanica* oils @ 2% (v/v) concentration against the RSM have achieved 55% and 66.5% mortality respectively. Similarly, the application of *Annona Squamosa* seed oil extract was found efficient against the adults of *Aphis gossypii* and *Tetranychus kanzawai* (Chin *et al.*, 2009) [3]. A similar observation was made by Dheeraj *et al.* (2013) [5] that potassium salts of fatty acid mixed with synthetic pyrethroids acts as an effective insecticide.

In the ovicidal activity study, increasing oil concentration resulted in high ovicidal activity and low hatchability. Likewise, hatchability was high and ovicidal activity was very low in low oil concentrations. The results showed (Table 2) that *H. pentandra* seed oil @ 4ml/L & 5ml/L were achieved 100% ovicidal action on RSM eggs. Paraffinic oil @ 3ml/L and Neem oil @ 3ml/L were achieved at about 98% and 92% of egg mortality respectively. Similarly, *S. glauca* seed oil @ 5ml/L was achieved 90% of egg mortality. However, Among the tested treatments, *H. pentandra* seed oil @ 4ml/L & 5ml/L were given better control over the other treatments. Normally volatile oils can reduce egg hatchability by affecting vital

processes which are associated with their embryonic development (Hanem Fathy Khate, 2013) [7]. Roobakkumar *et al.*, (2010) [10] observed that application of Neem Kernel Aqueous Extract (NKAE), pongam oil and garlic extracts on RSM's eggs caused 90%, 70% and 50% of egg mortality respectively. When the application of *Jatropha curcas* seed oil showed that eggs were more susceptible as compared to the pre-adult stages of *Callosobruchus maculatus* and *Dinarmus basalis* insects (Boateng and Kusi, 2008) [2].

The Discrimination Quotient (DQ) values of *S. glauca* and *H. pentandra* are given in Table 3. The results show that all treated leaves were harboured a minimum number of eggs when compared with untreated control and the DQ values were increased positively towards the increasing oil concentrations on the tested oils. In all the treatments, adult mortality was started after the 5<sup>th</sup> day of the application. So, eggs were counted up to 96 hours for the study. The DQ values were started from 0.10 to 0.65. The least number of eggs were laid on the treatments of *H. pentandra* seed oil @ 5ml & 4ml/L and *S. glauca* seed oil @ 5ml/L were achieved the highest DQ value of 0.65, 0.63 and 0.58 respectively. Meanwhile, the application of positive standards such as neem oil @ 3ml/L and paraffinic oil @ 3ml/L have achieved the DQ value of 0.31 and 0.44 respectively. Roobakkumar *et al.* (2010) [10] has reported that aqueous extract of the garlic bulb and neem kernel confirms the ovipositional deterrence on RSM. Similarly, leaf and seed extracts of *Datura stramonium* and acetone extract of garlic bulb shows reduced fecundity of *Tetranychus urticae* (Kalaivani *et al.*, 2013) [9].

**Table 1:** Laboratory evaluation of *S. glauca* and *H. pentandra* seed oils against RSM's adults

Treatments	Dosage	*Adult Mortality %			
		After 24 h	After 48 h	After 72 h	After 96 h
<i>S. glauca</i> seed oil	1ml/L	14±2.44 <sup>bc</sup>	24±2.44 <sup>bc</sup>	30±3.16 <sup>b</sup>	38±3.74 <sup>c</sup>
	2ml/L	14±2.44 <sup>bc</sup>	34±2.44 <sup>c</sup>	54±5.09 <sup>c</sup>	68±3.74 <sup>d</sup>
	3ml/L	32±3.74 <sup>de</sup>	56±2.44 <sup>de</sup>	66±2.44 <sup>d</sup>	78±2.00 <sup>ef</sup>
	4ml/L	36±2.44 <sup>ef</sup>	60±3.16 <sup>de</sup>	70±3.16 <sup>d</sup>	82±2.00 <sup>f</sup>
	5ml/L	40±3.16 <sup>ef</sup>	64±2.44 <sup>e</sup>	72±3.74 <sup>d</sup>	86±2.44 <sup>f</sup>
<i>H. pentandra</i> seed oil	1ml/L	6.0±2.44 <sup>ab</sup>	16±2.44 <sup>b</sup>	22±2.00 <sup>b</sup>	28±2.00 <sup>b</sup>
	2ml/L	24±2.44 <sup>ab</sup>	34±2.44 <sup>b</sup>	50±3.16 <sup>b</sup>	62±2.00 <sup>c</sup>
	3ml/L	38±4.89 <sup>cd</sup>	50±4.47 <sup>c</sup>	70±3.16 <sup>c</sup>	70±3.16 <sup>d</sup>
	4ml/L	40±4.47 <sup>ef</sup>	52±3.74 <sup>d</sup>	72±2.00 <sup>d</sup>	78±3.74 <sup>d</sup>
	5ml/L	44±4.00 <sup>ef</sup>	56±2.44 <sup>d</sup>	74±2.44 <sup>d</sup>	87±2.00 <sup>f</sup>
Neem oil	3ml/L	10±3.16 <sup>ab</sup>	18±4.89 <sup>b</sup>	24±4.00 <sup>b</sup>	24±4.00 <sup>b</sup>
Paraffinic oil	3ml/L	44±6.00 <sup>e</sup>	56±7.48 <sup>de</sup>	64±4.00 <sup>d</sup>	64±4.00 <sup>d</sup>
Control	-	0 ± 0 <sup>a</sup>	2.0±2.00 <sup>a</sup>	4.0±2.44 <sup>a</sup>	4±2.44 <sup>a</sup>

\*Mean±SE of five replications

(The values followed by the same alphabet in the column indicates that not significant at 5% level in DMRT)

**Table 2:** Ovicidal activity of *S. glauca* and *H. pentandra* seed oils against eggs of Red Spider Mites

S. No.	Treatments	Dosage (ml or g/L)	Mean No. of eggs observed	*Hatching %	*Nymphal mortality % after hatching	*Adult emerging %	*Ovicial %
1	<i>Simarouba glauca</i> seed oil	1 ml	64	46.08±5.79	14.08±7.38	40.42±8.41	53.91±5.79 <sup>b</sup>
2		2 ml	57	24.80±7.94	0	21.38±6.93	75.19±7.94 <sup>c</sup>
3		3 ml	37	16.67±4.73	6.66±2.72	13.64±2.97	83.32±4.73 <sup>d</sup>
4		4 ml	32	14.89±1.24	0	14.89±1.24	85.11±1.21 <sup>de</sup>
5		5 ml	29	9.75±2.07	0	9.75±2.07	90.25±2.07 <sup>def</sup>
6	<i>Hydnocarpus pentandra</i> seed oil	1 ml	26	43.24±5.20	10.66±1.20	30.73±8.22	56.75±5.20 <sup>b</sup>
7		2 ml	27	23.83±7.43	6.66±2.72	21.68±6.98	76.16±7.43 <sup>c</sup>
8		3 ml	35	4.34±1.22	0	4.34±1.22	95.65±1.22 <sup>gh</sup>
9		4 ml	31	0	0	0	100 <sup>h</sup>
10		5 ml	35	0	0	0	100 <sup>h</sup>
11	Neem oil	3 ml	49	7.95±1.81	0	7.95±1.81	92.04±1.81 <sup>efg</sup>
12	Paraffinic oil	3 ml	57	1.87±1.87	0	0	98.12±1.87 <sup>gh</sup>
13	Untreated control	-	41	91.27±2.97	2±1.02	89.67±2.46	8.62±2.97 <sup>a</sup>

\* Mean ± SE of three replications

(The values followed by the same alphabet in the column indicates that not significant at 5% level in DMRT)

**Table 3:** Ovipositional deterrence of *S. glauca* and *H. pentandra* seed oils on RSM

S. No.	Treatments	Dosage (ml or g /L)	No. of eggs at after 96 hours*	DQ Value
1	<i>Simarouba glauca</i> seed oil	1 ml	86.85±0.72 <sup>h</sup>	0.10
2		2 ml	81.71±0.95 <sup>g</sup>	0.13
3		3 ml	63.42±1.09 <sup>f</sup>	0.26
4		4 ml	41.14±1.21 <sup>d</sup>	0.44
5		5 ml	28.57±0.58 <sup>b</sup>	0.58
6	<i>Hydnocarpus pentandra</i> seed oil	1 ml	67.42±0.51 <sup>g</sup>	0.23
7		2 ml	54.85±1.03 <sup>e</sup>	0.32
8		3 ml	31.42±1.10 <sup>c</sup>	0.55
9		4 ml	24.00±1.18 <sup>a</sup>	0.63
10		5 ml	22.85±0.68 <sup>a</sup>	0.65
11	Neem oil	3 ml	56.00±0.96 <sup>e</sup>	0.31
12	Paraffinic oil	3 ml	41.71±0.75 <sup>d</sup>	0.44
13	Untreated control	3 ml	106.85±1.43 <sup>i</sup>	-

\*Values are represented as Mean±SE and the values followed by the same alphabet in the column indicates that not significant at 5% level in DMRT

**Table 4:** LD<sub>50</sub> and LD<sub>90</sub> values for *S. glauca* and *H. pentandra* at different time intervals

Seed oils	Time Interval (HRs)	LD <sub>50</sub>	95% Confidential limit		LD <sub>90</sub>	95% Confidential limit		Chi-Square value	Sig.
			LCL	UCL		LCL	UCL		
<i>S. glauca</i>	24	5.74	5.05	6.93	11.24	9.36	14.72	66.409	P<0.001
	48	3.24	2.94	3.55	7.85	6.96	9.18	54.01	P<0.001
	72	2.15	1.56	2.58	6.98	5.97	8.8	98.92	P<0.001
	96	1.23	0.622	1.66	5.03	4.49	5.87	89.65	P<0.001
<i>H. pentandra</i>	24	5.32	4.77	6.25	8.85	7.56	11.19	126.49	P<0.001
	48	4.52	4.07	5.17	8.99	7.71	11.2	86.722	P<0.001
	72	3.22	2.98	3.47	6.77	6.18	7.611	57.93	P<0.001
	96	2.66	2.31	2.98	7.18	6.34	8.48	65.33	P<0.001

## Conclusion

Based on the study, *S. glauca* and *H. pentandra* has admirable acaricidal, ovicidal and oviposition deterrence properties. The study paves the way to concentrate more on plant essential oils and seed oils for the management of RSM in tea plantations.

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