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Effects of plant based formulations on management of red spider mite (*Oligonychus coffeae*)

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Abstract
In recent decades, the Indian tea industry has experienced a paradigm shift due to increased restrictions on pesticidal residues in exported tea. To address this crisis, non-chemical pest management methods are necessary. This study aimed to assess the effects of plant-based formulations of *Melia azedarach* and *Pongamia glabra*, with varying concentrations (2.50%, 5.00%, 7.50%), along with the inclusion of cow urine (5%), against one of the major pests in tea, the red spider mite. A commercial neem formulation (1:1500) was also used for comparison. The study was conducted at the Department of Tea Husbandry & Technology and Department of Entomology, Assam Agricultural University, Jorhat, from 2019 to 2021, following a complete randomized design (CRD) with 14 treatments and 6 replications. The application of these plant extracts significantly affected the red spider mites, including oviposition rate, hatchability reduction, nymph mortality, and adult mortality. Scientific validation and standardization of this work would greatly contribute to tea pest management.

Keywords: Red spider mite, *Melia azedarach*, *Pongamia glabra*, oviposition, hatchability, mortality, adult, nymph

Introduction
The commercially grown, multi-billion dollar industry oriented evergreen crop 'Tea', *Camellia sinensis* (L.) O. Kuntze, belongs to the *Theaceae* family and *Gordonaceae* tribe. (Wight, 1962). This woody perennial plantation crop is grown as monoculture on big and small land holdings from which 'made tea' is prepared from their tender leaves and buds. The big players of tea includes countries like China, India, Kenya and Sri Lanka. India ranks 2nd in production after China which produced 1257.53 million kg tea during 2020(Anonymous, 2020).Tea is widely consumed and cultivated in more than 13 states of India out of which Assam, West Bengal, Tamil Nadu and Kerala are the leading ones.

The tea crop ecosystem provides an ideal ecosystem for a number of insect-pests and disease causing microbes to thrive, (Agnihothrudu, 1964; Das, 1965) [1, 8] which includes about 1034 arthropod species, 82 nematode species, 1 algal disease and 350 fungal diseases, resulting in significant yield loss (Chen and Chen, 1989) [6].

Amongst the entomological pests, the red spider mite, *Oligonychus coffeae* Nietner (Acarina: Tetranychidae) is the most serious one. (Cranham, 1966) [7], discovered in Assam during 1868. (Hazarika *et al*., 2009) [12]. More than 12 species of mites have been reported from Bangladesh, China, India, Indonesia, Japan, Malaysia, Sri-Lanka, Taiwan and erstwhile USSR damaging tea crops. (Muraleedharan, 1992) [14]. India accounts for about 13 species of mites belonging to 8 families. (Gupta, 2001) [10]. The red spider mite enjoys a wide distribution in almost all the tea growing countries and has grabbed the attention of Entomologists and acarologists globally.

The current trend of overuse of synthetic pesticides in tea has caused imbalance to the ecosystem leading to pest resurgence, variation in susceptibility, residual problems in made tea, a burden for natural regulatory agents and varied effects on non-target organisms, including humans (Bora *et al*., 2007) [5]. But, during the past few decades, there has been a paradigm shift in the Indian tea industry as most of the countries we export to is imposing restrictions for acceptability due to higher than permissible limits of pesticidal residues in the made tea. Overcoming such a crisis in the tea industry necessitates the use of non-chemical pest management approaches, including integrated control strategies that are adoptable, cost

effective, easily available and eco-friendly.

The use of phyto extracts, having insecticidal properties is an alternative to these commonly used synthetic pesticides. Numerous plant extracts, including neem-based products have contributed greatly in tea pest control. Furthermore, spraying of cow urine too has been recommended for the pest management in different crops to reduce the toxic effects of synthetic pesticides (Ambwani *et al*., 2006) [2]. The present experiment has been attempted to determine the suitability of a few bio-intensive approach for sustainable management of one of the major tea insect pest, the red spider mite (*Oligonychus coffeae*) with the objective to study the efficacy of phyto extracts for management of red spider mite.

Materials and Methods

The research was conducted at the Department of Tea Husbandry and Technology and Department of Entomology, AAU, Jorhat during 2019-21 under room temperature and ambient relative humidity.

Matured leaves of *Pongamia glabra* and *Melia azedarach* was collected from their natural habitat inside the university premises, including the Experimental Garden for Plantation Crops (EGPC) Jorhat during October, 2020. The leaves were shade-dried, powdered with the help of an electric grinder and an aqueous extract was prepared using distilled water on a weight by volume (w/v) basis. The solution was then filtered & squeezed using a muslin cloth to extract all its content and kept in sealed air tight glass bottles in desired concentrations (*viz,* 2.50%, 5.00%, 7.50%) for use in the experiment.

Mature leaves of the tea clone TV 21 maintained at the Experimental Garden for Plantation Crops (EGPC) was utilized for mass rearing of mites (*Oligonychus coffeae*) and experimentation. Cow urine was acquired from the Dairy Farm, Assam Agricultural University, Jorhat.

Adults of *O. coffeae* were sourced from the Experimental Garden for Plantation Crops (EGPC), AAU, Jorhat-13 and were cultured following the detached leaf technique (Das *et al.*, 2012) [8] in the Physiology Laboratory of the Department of Entomology, AAU, Jorhat during 2019-2021.

From the stock culture of *Oligonychus coffeae* reared on detached leaves, 10 numbers of a-day-old adults were allowed to settle for 24 hours on a TV21 clone leaf disc of 2.5 cm^2 (Plate) and was sprayed with phyto-extracts at various desired concentrations using a hand atomizer (50 ml). The fourteen treatments, *Pongamia glabra* 2.5% (T_1), *Pongamia glabra* 2.5% + Cow urine 5% (T_2), *Pongamia glabra* 5% (T_3), *Pongamia glabra* 5% + Cow urine 5% (T_4), *Pongamia glabra* 7.5% (T_5), *Pongamia glabra* 7.5% + Cow urine 5% (T_6), *Melia azedarach* 2.5% (T_7), *Melia azedarach* 2.5% + Cow urine 5% (T_8), *Melia azedarach* 5% (T_9), *Melia azedarach* 5% + Cow urine 5% (T_{10}), *Melia azedarach* 7.5% (T_{11}), *Melia azedarach* 7.5% + Cow urine 5% (T_{12}), Commercial Neem Oil (1:1500) (T_{13}), Control (T_{14}) were replicated six times following complete randomized design (CRD) and adult mortality data was visually recorded at 24, 48 and 72 hours after treatment (HAT) using a stereoscopic microscope. A commercial neem based formulation (1:1500) and a control treatment containing water was also maintained to compare bio-efficacy against *Oligonychus coffeae.*

Again, to evaluate the ovicidal property of these treatments, ten red spider mite gravid females were allowed to lay eggs on leaf disc for 24 hours. Out of these, thirty eggs were counted and other eggs were discarded off from the leaves. These newly laid eggs were then sprayed with varied concentrations of plant extracts. Hatchability was recorded when 90% of the eggs in the control plate hatched.

The observations were recorded on Rate of oviposition, Percent reduction in egg hatchability**,** Nymph and adult mortality at various time intervals. The data obtained from various characters under study were analysed using analysis of variance (ANOVA) (Gomez and Gomez, 1984) [9].

Result and Discussion

Rate of oviposition: The data on rate of oviposition of red spider mite during both the experiment period are presented in Table 1. Two phyto-extracts, *viz. Melia azedarach* and *Pongamia glabra* were introduced to evaluate their efficacy against red spider mite, both of which were found to be effective for reducing the rate of oviposition. *Melia azedarach* was found to be more effective than *Pongamia glabra.* During October-November,2020, after 72 hours *Melia azedarach* (7.5%) + Cow urine (5%) showed more efficacy in reducing the rate of oviposition of red spider mite followed by *Melia azedarach* (7.5%), *Melia azedarach* (5%) + cow urine 5%, *Melia azedarach* (5%), *Melia azedarach* (2.5%) + 5% Cow urine. These treatments were at par with commercial neem formulation (1:1500).

Again, during March-April 2021, *Melia azedarach* (7.5%) + Cow urine (5%) found to be the most effective against rate of oviposition of red spider mite followed by *Melia azedarach* (7.5%), *Melia azedarach* (5%) + cow urine 5%, Melia *azedarach* (5%) These were at par with commercial neem formulation (1:1500) after 72 hours. This showed plant extracts to effective against rate of oviposition when leaves were covered by spray materials. The results are in agreement with the finding of Isman, (2006) [13] who reported botanical insecticide contain compounds that exhibit ovicidal, repellent, antifeedant and toxic effects on insects and have long been recommended as alternative to chemical pesticides for pest management. The present study was also comparable with the finding of Hazarika *et al*., (2008) [11] who reported that plant extracts contain certain alkaloids, tannins, quinines, phenolic compounds and phytoalexins which have potent pesticidal properties, including ovipositional deterrence, antifeedant, repellent and growth retarding properties.

Percent reduction in egg hatchability: Data on Table 2 shows percent decrease in egg hatchability of red spider mite during both the seasons (Oct-Nov 2020 & Mar-Apr, 2021). After 72 hours of application *Melia azedarach* (7.5%) + (5%) showed the highest reduction in egg hatchability followed by *Melia azedarach* (7.5%) which were at par with commercial neem formulation (1:1500). Certain chemical substances present in plants were reported by Younoussa *et al.* (2016) [15] that blocks the micropyle region of the egg, thereby preventing the gaseous exchanges that ultimately eliminates the embryo in the egg itself. Bhuyan *et al*., 2017 [4] also observed another plant *Polygonum hydropiper* along with with cow urine and water significantly lead to a reduction in egg hatchability percentage during different periods of study which confirms the ovicidal effects of this combination.

Nymph mortality: Table 3 represents the mean data of percent nymph mortality post 24 hours of treatment. Here too, *Melia azedarach* (7.5%)+ Cow urine (5%) showed the highest mortality at 24, 48 and 72 hours post treatment during both

the study periods. During October- November, 2020 mortality rate was 95.66%, 96.33%, 97.89% at 24, 48 and 72 hours respectively. During March- April, 2021, significant mortality was recorded upto 95%, 96.33% and 97.67% at 24, 28 and 72 hours after application respectively. It was also found that *Melia azedarach* (5%), *Melia azedarach* (5%)+ Cow urine (5%), *Melia azedarach* (7.5%), *Melia azedarach* (7.5%)+ Cow urine (5%) showed significant mortality which were at par with commercial neem formulation (1:1500) after 72 hours post treatment. Among the two selected plant formulations *Melia azedarach* showed more efficacy on nymph mortality than *Pongamia glabra* at 24, 48, 72 hours after treatment for both the seasons *Melia azedarach* and *Pongamia glabra* alone or with the combination of cow urine showed effective results than control (water) for both the seasons.

Adult mortality: Table 4 represents the mean data on percent adult mortality post 24 hours after treatment. In this case too, results were similar to that of nymph mortality. The exposure of adults to *Melia azedarach* (7.5%)+ Cow urine (5%) showed the highest mortality at 24, 48 and 72 hours after treatment during both the study periods. Mortality was recorded up to 90.67%, 96.67% and 98.67% at 24, 48 and 72 hours after application respectively during the period of October- November (Table 4). During March-April, 2021 mortality rate was recorded upto 89.33%, 94.33% and 96.67% at 24, 48 and 72 hours respectively (Table 4.5). It was also observed that *Melia azedarach* (5%), *Melia azedarach* (5%)+ Cow urine (5%), *Melia azedarach* (7.5%), *Melia azedarach* (7.5%)+ Cow urine (5%) too showed significant mortality which were at per with commercial neem formulation (1:1500) at 72 hours post treatment. Among the two selected plant formulations, *Melia azedarach* again showed more efficacy on adult mortality than *Pongamia glabra*. The result of the experiment is at par with the findings of Babu *et al*., (2009) [12] who observed that 100% mortality in adults of *O. coffeae* sprayed with Derrimax (a commercial neem product) under laboratory conditions. Likewise, Kumar *et al*., (2010) [16] reported the application of Derrimax caused 100% mortality of adult red spider mite. The exposure of adult to the combination of *Polygonum hydropiper* with cow urine and water caused significant mortality in different period of study was also observed by Bhuyan *et al*. (2017) [4].

The findings of the investigation showed the application of plant extracts caused significant losses in red spider mite which were at par with commercial formulations for rate of oviposition, percent reduction in hatchability, nymph mortality and adult mortality. Among all the plant based formulations *Melia azedarach* (7.5%)+ Cow urine (5%) was the most effective formulation *in-vitro* for management of red spider mite in tea.

Table 1: Effect of plant based formulation on rate of oviposition of red spider mite during October-November, 2020 and March-April, 2021

Treatments	Rate of oviposition (%) (72 HAT)	
	October-November, 2020	March-April, 2021
T_1	64.44	68.33
T_2	17.22	35.33
T_3	18.11	38.00
T_4	15.25	25.33
T_5	16.11	27.55
T_6	14.44	19.00
T_7	15.558	20.44
T_8	12.33	17.11
T_9	7.77	14.99
T_{10}	6.11	7.77
T_{11}	3.88	7.22
T_{12}	3.33	6.11
T_{13}	3.00	6.00
T_{14}	71.66	74.50
S.Ed	7.80	6.87
CDp= 0.05	11.86	10.85

HAT- Hours after treatment

Table 2: Effect of plant based formulations on percent reduction in egg hatchability of red spider mite during October-November, 2020 and March-April, 2021

Treatments	Percent reduction in egg hatchability (72HAT)	
	October-November, 2020	March-April, 2021
T_1	36.10	34.99
T_2	41.10	41.66
T_3	43.33	39.99
T_4	46.77	51.10
T_5	50.21	54.99
T_6	52.99	55.55
T_7	49.55	39.44
T_8	57.66	45.55
T_9	59.22	50.55
T_{10}	62.10	59.88
T_{11}	69.99	69.44
T_{12}	75.55	73.33
T_{13}	76.55	75.44
T_{14}	22.21	18.33
S.Ed	3.56	3.58
CDp= 0.05	7.71	7.73

HAT- Hours after treatment

Table 3: Effect of plant based formulations on red spider mite nymph mortality during October-November, 2020 &March-April, 2021

Treatments	Nymph mortality (%)					
	October-November, 2020		March-April, 2021			
	24 HAT	48 HAT	72 HAT	24 HAT	48 HAT	72 HAT
T_1	20.00	28.33	41.66	17.66	26.66	33.33
T_2	46.66	71.33	81.33	35.00	53.33	65.00
T_3	41.66	63.33	85.33	48.33	63.33	75.33
T_4	51.66	75.00	86.33	51.66	65.00	78.00
T_5	55.00	75.69	89.33	55.00	70.33	85.00
T_6	64.33	83.33	90.00	61.66	76.66	86.33
T_7	65.67	85.33	89.67	61.00	76.66	85.33
T_8	69.66	81.66	90.66	70.00	83.33	87.66
T_9	70.65	88.33	95.00	68.33	80.00	89.00
T_{10}	89.00	95.00	96.66	86.66	93.33	95.00
T_{11}	93.33	95.33	97.33	93.33	94.00	96.66
T_{12}	95.66	96.33	97.89	95.00	96.33	97.67
T_{13}	96.33	97.67	98.33	96	97	98
T_{14}	7.33	8.66	9	6.33	7.66	8.33
S. Ed	4.81	3.68	3.45	5.20	4.00	4.21
CD= 0.05	10.39	7.96	7.47	11.24	8.65	9.09

HAT- Hours after treatment

Table 4: Effect of plant based formulations on adult mortality of red spider mite during October-November, 2020 and March-April, 2021

Treatments	Adult mortality (%)					
	October-November, 2020			March-April, 2021		
	24 HAT	48 HAT	72 HAT	24 HAT	48 HAT	72 HAT
T_1	6.66	16.66	16.66	6.00	11.66	31.66
T_2	51.66	56.66	66.66	45.00	60.00	71.33
T_3	56.33	62.00	70.00	43.33	51.66	63.33
T_4	58.00	65.66	73.69	50.00	65.00	73.66
T_5	66.33	70.30	79.30	56.66	73.33	76.66
T_6	68.66	73.00	80.00	60.66	75.00	78.33
T_7	70.00	76.66	83.33	59.00	78.00	82.33
T_8	75.00	80.33	82.66	75.66	80.33	85.66
T_9	81.66	90.00	91.66	80.00	85.66	87.66
T_{10}	85.33	91.66	93.33	81.66	86.66	88.33
T_{11}	88.33	93.33	96.67	85.66	92.67	95.33
T_{12}	90.67	96.67	98.67	89.33	94.33	96.67
T_{13}	91.67	97.33	99	90.33	95	98.67
T_{14}	7.67	8.33	10	6.67	7.33	8.66
S. Ed	6.93	6.27	6.35	5.76	5.87	5.52
CD= 0.05	14.97	13.56	13.72	12.45	12.69	11.94

HAT- Hours after treatment

Conclusion

The continuous use of synthetic chemicals and improper pest control measures have resulted in various issues, such as secondary pest outbreaks, pesticide resistance development, pest resurgence, environmental contamination, adverse effects on non-target and beneficial organisms, and the presence of undesirable residues in tea production. Additionally it has contributed to increased input costs as well.

The present investigation explores the potential of botanicals, either alone or in combination with cow urine, for managing tea pests. It can be concluded that scientifically validated and standardized non-chemical methods commonly employed by growers can significantly aid in tea pest management. Considering the current global context, adopting organic and environmentally friendly practices can serve as a proven alternative to mitigate the negative impacts caused by harmful chemical pesticides.

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