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Evaluation of indigenous biopesticides against Red Spider Mite, *Oligonychus coffeae* (Nietner) in tea

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Abstract
Fish extract at 0.25, 0.5 and 1%, *Polygonum hydropiper* at 2.5, 5 and 7.5% and Azadirachtin-5% (Neemazal-F) were evaluated in the laboratory against red spider mite, *Oligonychus coffeae* (Nietner) to determine its effect on viability of eggs and mortality of adult and nymphs mites. The result showed that highest reduction in hatchability of eggs of red spider mite was achieved by Fish extract 1% followed by *Polygonum hydropiper* extract 7.5% in both the periods of study. Both fish extract 1% and *Polygonum hydropiper* extract 7.5% caused significant mortality on nymphs of red spider mite. The potency of both fish extract 1% and *Polygonum hydropiper* extract 7.5% were observed on adult mortality of red spider mite upto the extent of 81-85%. Highest reduction in hatchability of eggs, mortality on nymphs and adults was achieved by fish extract followed by *P. hydropiper* extract.

Keywords: Red Spider Mite, *Oligonychus coffeae,* biopesticides

1. Introduction
The red spider mite, *Oligonychus coffeae* (Nietner) (Acarina: Tetranychidae) causes considerable crop loss in South India[7] and also causing havoc at Terai, Dooars and Assam regions[12]. The damage to the tea plant is caused by larvae, nymphs and adult mites which feed on the sap of the leaves and occasionally on petioles [1]. Their attack is mainly confined to the upper surface of the mature foliage and feeds along the mid rib and veins and gradually spreads to the entire surface of leaf thereby changes the colour of the leaf to ruddy bronze, resulting in crop loss from 17 to 46% [11]. In severe infestation, it damages the younger and older leaves and ultimately leads to defoliation and debilitation of the tea bush [9]. Red spider mite is active and breeds on tea throughout the year in North East India [6].
A wide range of acaricides and insecticides belonging to different chemical groups currently being used worldwide to control these pest have serious drawbacks such as toxicity to non-targeted organisms, pesticide induced resistance, health hazard and presence of residues in tea [13]. To overcome such crisis in the tea industry, it is essential to go for practices that are based on the use of non-chemical methods and are easily adoptable, cost effective and easily available. In this context, the re-evaluation and use of traditional botanical pest control agents (powder, water extracts, oil and wood ash) that farmers have been using over several decades provide a clue to indigenous use of pest control strategies. Traditional knowledge based practices including indigenous plant extracts and plant products would be a better option. In the present study an attempt was made to explore the potential and utilization of commonly available fish waste, *Polygonum hydropiper* and Azadirachtin against red spider mite in relation to ovicidal and acaricidal action under laboratory condition.

2. Materials and methods
To study the efficacy, laboratory works were carried out in the Department of Tea Husbandry and Technology, Assam Agricultural University, Jorhat, Assam, India during the period 2015-2016.

2.1 Methods of preparation
2.1.1 Fish extract
The fish extraction was prepared by mixing 80 kilograms of different parts of fish along with 50 litres cow urine, 15kg cow dung and 100 litres of water. The mixture was kept for 7 days in a plastic drum installed underground.

2.1.2 *Polygonum hydropiper* extract

The plants were collected from nearby places of Deha Tea Estate, Jorhat for preparation of aqueous extracts. 30 kilograms of *Polygonum hydropiper* were crushed and mixed with 10 litres cow urine and 100 litres of water and was kept in a cement tank for 10 days and then filtration was done before spraying. Treatment details of different extracts used in the present study are given in Table 1.

2.2.3 Azadirachtin- 5%

Azadirachtin-5% is a neem extract concentrate insecticide manufactured by Parry India Limited. It is used to control different pests of tea.

Table 1: Treatments details of different extracts used in the present study.

Treatment Symbol	Treatment	Dilution (H.V)
T_1	Control	----
T_2	Fish extract (0.25%)	1:400
T_3	Fish extract (0.5%)	1:200
T_4	Fish extract (1%)	1:100
T_5	*Polygonum hydropiper* extract (2.5%)	5:200
T_6	*Polygonum hydropiper* extract (5%)	10:200
T_7	*Polygonum hydropiper* extract (7.5%)	15: 200
T_8	Azadirachtin-5% (Neemazal-F)	1:1500

2.2 Rearing of mites

Rearing of mites in the laboratory was done on detached leaves in petridishes and was carried out by following a standard procedure adopted by Hazarika *et al.* (1995). The petiole was wrapped with wet cotton wool to keep the vigour of the leaf longer and was placed by keeping the upper surface upon a wad of wet cotton wool in a petridish (15cm diameter). The cotton wool was kept wet by adding tap water as and when the cotton wool dried up. The mite infested leaves from the field were collected in a polythene bag and brought to the laboratory. The mites were then released on the leaf culture with the help of a fine camel hair brush (size zero). When the leaves become crowded, the mites were transferred to fresh detached leaf cultures as shown in Figure 1.

2.3 Ovicidal test for red spider mite

For assessment of ovicidal properties of the extracts, healthy uninfested mature tea leaves of clone TV 1 were detached from the plant along with its petiole. All the leaves were thoroughly washed under tap water and placed in 15cm diameter petridishes. Ten pairs of mite were released in each leaf for a period of 30 minutes to oviposit. The eggs laid during this period were allowed to remain as such for 24 hours. After that the ovicidal test was carried out by direct spraying of treatment solutions with a glass atomizer. Twenty eggs were counted on each leaf and others were discarded of from the leaves along with the adult mites. Each treatment was replicated three times. The first observation was recorded as soon as the eggs in the control hatched. Those eggs which did not hatch beyond this period were regarded as non-viable.
The percentage reduction in hatchability was determined by the following formula:

$$\text{Per cent reduction in hatchability} = \frac{\text{Number of unhatched eggs before treatment - Number of hatched eggs after trea tment}}{\text{Number of unhatched eggs before treatment}} \times 100$$

2.4 Mortality of nymphs and adults of red spider mite

Healthy uninfested mature tea leaves of clone TV 1 were detached from the plant along with their petiole. All the leaves were thoroughly washed under tap water and placed in 15cm diameter petridishes. Twenty healthy adult mites were released in each leaf with the help of camel hair brush (size zero). Treatment solutions at different dilutions were used as foliar spray. The treatment solutions were sprayed with the help of a glass atomizer on the leaves. The experiment was replicated thrice and observations were recorded after 24, 48, 72 hours of treatment on the mite mortality. Same method was followed against the nymphal stage of red spider mite. The percentage mortality over pretreatment was determined by using the following formula:

$$\text{Percentage of mortality} = \frac{\text{Pre treatment population - Post treatment population}}{\text{Pretreatment population}} \times 100$$

2.5 Statistical analysis

All the data were analyzed statistically. Significance of variance due to treatment effect was determined by calculating the respective 'F' values [8].
The Standard Error (SE) of difference of mean was calculated by using the following formula:

$$\text{SEd}\pm = \sqrt{\frac{2 \times \text{Error mean square}}{\text{Number of replications}}}$$

To find out the significance of mean difference amongst the treatments critical difference (CD) was calculated by multiplying the standard error of difference of means with appropriate table value of 't' at 5 per cent and 1 per cent level of probability [8].
CD = SED ± x 't' (at 5% or 1%) for error degree of freedom.

3. Results

3.1 Effect of ITKs on hatchability of red spider mite (2015)

The data on per cent reduction in hatchability of red spider mite during both the period are presented in Table 2. In both the period of investigation, the highest reduction in hatchability was found during November (81.66%) with Fish extract at 1:100 dilution followed by *P. hydropiper* extract at 15:200 dilution (75.00%) and Azadirachtin 5% (70.00%).

3.2 Effect of ITKs on mortality of nymphs of red spider mite (2015)

3.2.1 24 hours after treatment

The mean data on per cent mortality after 24 hours of treatment are presented in Table 3. During August 2015, the highest per cent mortality of nymphs of red spider mite 80.00% was observed with Fish extract at 1:100 dilution. *P.*

hydropiper extract at 15:200 dilution recorded 75.00% mortality followed by Azadirachtin 5% (71.66%). During November 2015, Fish extract (1:100) exhibited the highest mortality of nymphs of red spider mite.

3.2.2 48 hours after treatment

Mortality of nymphs of red spider mite was found to be highest with Fish extract at 1:100 dilution (83.33%). *P. hydropiper* extract at 15:200 dilution recorded 78.33% mortality followed by Azadirachtin 5% (75.00%) during August. During November 2015, Fish extract (1:100) exhibited the highest mortality of nymphs of red spider mite.

3.2.3 72 hours after treatment

During August 2015, Fish extract at 1:100 dilution recorded the highest mortality 85.00% followed by *P. hydropiper* extract at 15:200 dilution (80.00%) and Azadirachtin 5% (76.66%). During November 2015, Fish extract (1:100) exhibited the highest mortality of nymphs of red spider mite (85.00%) followed by *P. hydropiper* extract at 15:200 (75.00%) and Azadirachtin 5% (73.33%).

3.3 Effect of ITKs on mortality of adult red spider mite (2015)

3.3.1 24 hours after treatment

The data presented in Table 4 on percent mortality of adult red spider mite after 24 hours of treatment. Among all the ITKs tested, the highest mortality of adult red spider mite was recorded during August with Fish extract at 1:100 dilution (78.33%) followed by *P. hydropiper* extract at 15:200 dilution (71.66%) and Azadirachtin 5% (70.00%). During November 2015, Fish extract (1:100) exhibited the highest mortality of nymphs of red spider mite (80.00%) followed by *P. hydropiper* extract at 15:200 (73.33%) and Azadirachtin 5% (71.66%).

3.3.2 48 hours of treatment

Among all the ITKs, Fish extract (1:100) recorded significantly highest percentage of mortality of red spider mite 81.66% followed by *P. hydropiper* extract at 15:200 dilution (75.00%) and Azadirachtin 5% (73.33%) during both the period of study.

3.3.3 72 hours of treatments

Significant variations in respect of mortality of adult red spider mite were observed during both the period of investigation. The highest mortality was found during August with Fish extract at 1:100 dilution (85.00%) followed by *P. hydropiper* extract at 15:200 dilution (81.67%) and Azadirachtin 5% (75.00%). During November 2015, Fish extract at 1:100 dilution (83.33%) followed by *P. hydropiper* extract at 15:200 dilution (75.00%) and Azadirachtin 5% (73.33%).

Plate 1: Eggs of *Oligonychus coffeae*

Plate 2: Nymph of *Oligonychus coffeae*

Plate 3: Adult Male of *Oligonychus coffeae*

Plate 4: Adult Female of *Oligonychus coffeae*

Plate 5: Cluster of mites

Table 2: Effect of ITKs on hatchability of red spider mite (2015)

Treatments	Dilution (HV)	Percentage reduction in hatchability (72 hours)	
		August	November
T_1 (Control)	Water spray	1.66	8.33
T_2 (Fish extract-0.25%)	1:400	38.33	40.00
T_3 (Fish extract-0.5%)	1:200	41.67	45.00
T_4 (Fish extract-1%)	1:100	75.00	81.66
T_5 (*P. hydropiper* extract-2.5%)	5:200	36.66	38.33
T_6 (*P. hydropiper* extract-5%)	10:200	40.00	41.67
T_7 (*P. hydropiper* extract-7.5%)	15:200	71.67	75.00
T_8 (Azadirachtin-5%)	1:1500	68.33	70.00
F test		-	-
S.Ed (±)		14.07	13.96
C.D. (P=0.05)		24.77	24.57
(P=0.01)		36.88	36.58

Table 3: Effect of ITKs on mortality of nymphs of red spider mite (2015)

Treatments	Dilution (HV)	Mortality of nymphs (%)					
		24 hours		48hours		72hours	
		August	November	August	November	August	November
T_1 (Control)	Water spray	0.00	0.00	0.00	0.00	0.00	0.00
T_2 (Fish extract-0.25%)	1:400	36.66	40.00	40.00	41.66	43.33	43.33
T_3 (Fish extract-0.5%)	1:200	41.66	41.66	46.66	43.33	48.33	45.00
T_4 (Fish extract-1%)	1:100	80.00	83.33	83.33	85.00	85.00	85.00
T_5 (*P. hydropiper* extract-2.5%)	5:200	35.00	38.33	38.33	38.33	43.33	38.33
T_6 (*P. hydropiper* extract-5%)	10:200	38.33	40.00	43.33	41.66	45.00	43.33
T_7 (*P. hydropiper* extract-7.5%)	15:200	75.00	71.66	78.33	71.66	80.00	75.00
T_8 (Azadirachtin-5%)	1:1500	71.66	71.66	75.00	71.66	76.66	73.33
F test		-	-	-	-	-	-
S.Ed (±)		15.49	15.32	15.92	15.39	16.02	15.64
C.D. (P=0.05)		27.27	26.97	28.03	27.09	28.20	27.54
(P=0.01)		40.60	40.15	41.73	40.33	41.99	41.00

Table 4: Effect of ITKs on mortality of adult red spider mite (2015)

Treatments	Dilution (HV)	Mortality of adults (%)					
		24 hours		48hours		72hours	
		August	November	August	November	August	November
T_1 (Control)	Water spray	0.00	0.00	0.00	0.00	0.00	5.00
T_2 (Fish extract-0.25%)	1:400	40.00	41.66	43.33	43.33	46.66	45.00
T_3 (Fish extract-0.5%)	1:200	41.66	43.33	46.66	45.00	51.67	46.66
T_4 (Fish extract-1%)	1:100	78.33	80.00	81.66	81.66	85.00	83.33
T_5 (*P. hydropiper* extract-2.5%)	5:200	38.33	38.33	41.67	40.00	43.33	41.66
T_6 (*P. hydropiper* extract-5%)	10:200	40.00	41.66	45.00	43.33	48.33	45.00
T_7 (*P. hydropiper* extract-7.5%)	15:200	71.66	73.33	75.00	75.00	81.67	75.00
T_8 (Azadirachtin-5%)	1:1500	70.00	71.66	73.33	73.33	75.00	73.33
F test		-	-	-	-	-	-
S.Ed (±)		14.68	14.99	15.14	15.25	14.55	14.48
C.D. (P=0.05)		25.84	26.38	26.65	26.84	25.62	25.49
(P=0.01)		38.47	39.28	39.67	39.96	38.13	37.95

4. Discussion

Fish extract in combination with cow dung, cow urine and water at 1:100 dilution recorded 75.00-81.66% reduction in hatchability of red spider mite. In case of nymphs, significant reduction was recorded upto 80.00%, 83.33% and 85.00% in 24, 48 and 72 hours after application respectively during the month of August and 83.33%, 85.00% and 85.00% in 24, 48 and 72 hours after application during the month of November. However, mortality of adult red spider mite was found to be 78.33%, 81.66% and 85.00% in 24, 48 and 72 hours after application respectively during the month of August and 80.00%, 81.66% and 83.33% in 24, 48 and 72 hours after application respectively during the month of November. Effect of Azadirachtin 5% on hatchability, nymph and adult mortality of red spider mite was found to be at par with fish extract at 1:100 dilutions.

Polygonum hydropiper in combination with cow urine and water significantly reduce hatchability percentage after 72 hours of application during both the period of study which confirms the ovicidal effects of this combination [5]. The exposure of nymphs to this combination also caused mortality upto 71.66- 80.00% in both the period of studies, whereas in case of adults, mortality was found to be 71.66-81.67% [10]. Extracts of certain plants contains alkaloids, tannins, quinines, phenolic compounds and phytoalexins which are known for pesticidal activity [4]. Many plant extracts possess ovipositional detrence, antifeedant, repellent and growth retarding properties and have toxic effects on selected crop pests which may form the basis for future research to develop low cost formulations suitable for large scale application in tea plantations [2].

5. Conclusion

From the present investigation, it can be concluded that highest reduction in hatchability of eggs of *Oligonychus coffeae* was achieved by Fish extract at 1:100 followed by *P. hydropiper* extract at 15:200. Both Fish extract at 1:100 and *P. hydropiper* extract at 15:200 caused significant mortality on nymphs and adult of red spider mite whereas Azadirachtin 5% was at par with fish extract and *P. hydropiper* extract.

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