African Journal of Biotechnology Vol. 8 (3), pp. 417-423, 4 February, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effect of aqueous plant extracts on tea red spider mite, *Oligonychus coffeae*, Nietner (Tetranychidae: Acarina) and *Stethorus gilvifrons* Mulsant

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Accepted 28 May, 2007

Four aqueous plant extracts (APEs) of *Acorus calamus* (L), *Xanthium strumarium* (L), *Polygonum hydropiper* (L) and *Clerodendron infortunatum* (Gaertn) were evaluated under both laboratory and field conditions at 2.5, 5.0 and 10.0% (w/v) concentrations against tea red spider mite, *Oligonychus coffeae* (Nietner). Also, the impact of APEs on survival and feeding of *Stethorus gilvifrons*, a potent coccinellid predator of red spider mite was studied. Parameters assessed were ovicidal activity and acaricidal activity in case of red spider mite, and feeding activity and adult mortality for the coccinellid. Strong ovicidal action was observed with *X. strumarium* (87.09%) and *A. calamus* (70.62%) whereas least action in *P. hydropiper* (30.86%) and *C. infortunatum* (20.58%). All the APEs showed > 50% mortality of red spider mite at higher concentrations (5 and 10%) under laboratory conditions. Field evaluation of APEs recorded 46.9 – 81.8% mite reduction at 5.0% and 64.7 – 100.0% at 10.0% concentration. More acaricidal activity was noticed in *C. infortunatum* and *X. strumarium* under field condition. The APEs, even at higher concentration (10%), caused no mortality to the adults of *S. gilvifrons* for 14 days, and no significant change in feeding after 24 h in comparison with untreated control. Crude plant extracts of *A. calamus*, *X. strumarium*, *P. hydropiper* and *C. infortunatum* can effectively be utilized as safer phytopesticidal products in both organic and inorganic tea estates as one of the potent tools in integrated mite management.

Key words: Tea, *Oligonychus coffeae*, aqueous plant extracts, *Stethorus gilvifrons*.

INTRODUCTION

Red spider mite, *Oligonychus coffeae* (Nietner), had been causing a considerable damage since 1960 but recently its havoc is more prominent at Terai, Dooars and Assam regions. Different classes of acaricides (dicofol, ethion, sulfur, propargite, fenazaquin) are being used by the tea planters to combat the menace from red spider mite. The latest surveillance report of the European Community (EC) in 2004 indicating the presence of residues in Assam teas is a cause of great concern. Authorization of 450 compounds have already been withdrawn by EC for use on agriculture products have adversely affected the tea exports of many countries including India since it withdraws the approval for ethion, which is extensively used for mite control in tea. Recently it has been reported that the incidence of ethion residue in Indian tea was higher than the prescribed maximum residue limit (MRL) to the tune of 14.2, 32.9 and 17.8% in the year 2002, 2003 and 2004 (Anonymous, 2002, 2003, 2004). Assam teas continue to record high number of positive values for organochlorine pesticide residues, very few of which exceeded the EU maximum residue level. Thus, incidence of DDT (13.8 and 47.1% in 2002 and 2004, respectively) and dicofol (70.4 and 82.4% in 2002 and 2004, respectively) remain comparatively high (Anonymous, 2002, 2004). The incidence of DDT in Assam teas is increasing and few samples contained more than the 0.2 mg/kg limit. Further, it is pointed out that impurity in dicofol, which contains DDT as contaminant might cause the adverse effect. It may be mentioned that DDT is a banned

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Table 1. Plants evaluated for ovicidal and acaricidal activities against *Oligonychus coffeae* (Nietner).

Scientific name	Family	Parts used	Common name
Acorus calamus	Araceae	Rhizome	Sweet flag
Clerodendron infortunatum	Verbenaceae	Leaves and succulent stem	Clerodendron
Xanthium strumarium	Compositae	Whole plant	Bur weed
Polygonum hydropiper	Polygonaceae	Whole plant	Smart weed

substance for application in tea in India. Currently, the Central Insecticide Board (CIB) and Prevention of Food Adulteration (PFA) regulation committee have reviewed the Maximum Residue Limit (MRL) position for tea and have recommended the use of only sixteen insecticides and acricides, eight herbicides and eight fungicides for use in tea (Gurusubramanian et al., 2005).

To overcome the current crisis being faced by the tea industry, there is need to switch over to alternatives such as the use of biorational products (plant insecticides, hydrocarbon oils, microbials, etc.), which are abundant in source and not being tapped properly. From the academic point of view, plants represent a vast storehouse of potentially useful natural products, and indeed, many laboratories worldwide have screened thousands of species of higher plants not only in search of pharmaceuticals, but also for pest control products (Arnason et al., 1989; VanBeek and Breteler, 1993; Sarmah et al., 1999; Rahman et al., 2005; Sarmah et al., 2006). These studies have pointed to numerous plant species possessing potential pest-controlling properties under laboratory conditions, but the step from the laboratory to the field eliminates many contenders, even when judged only on their efficacy against pests under realistic field conditions. Hence in this study an attempt was made to explore the potential and utilization of commonly available weed and medicinal plants in and around tea ecosystem (*Acorus calamus*, *Xanthium strumarium*, *Polygonum hydropiper* and *Clerodendron infortunatum*) against red spider mite in relation to ovicidal and acaricidal action under laboratory and field conditions. The effect of APEs on feeding potential and toxicity against *Stethorus gilvifrons*, an effective predatory coccinellid of red spider mite was also tested.

MATERIALS AND METHODS

Preparation of plant extracts

Leaves and succulent stems of *P. hydropiper, X. strumarium* and *C. infortunatum* and rhizome of *A. calamus* were collected locally from nearby areas of Tocklai experimental station, Tea Research Association, Jorhat (Table 1). Each plant material was dried under shade and powdered by using electric grinder and pass through a 20 mesh sieve and kept in a 1 kg capacity polypropylene bag. 300 g of each powdered plant material were taken into a 2 litre capacity conical flask and 1000 ml of distilled water was added to it and shaken for 8 h in a mechanical shaker and then kept it for 24 h. The extract was separated using fine muslin cloth and then filtered. The filtrate was collected in a 2 litre capacity conical flask and volume was made up to 1000 ml. This was considered as stock solution. Required concentrations (2.5, 5.0 and 10.0%) were prepared from the stock solution.

Rearing of red spider mite

A culture of red spider mite was maintained in the laboratory at 25 ± 2°C and 70 - 80% relative humidity on a susceptible tea clone, TV1, by following detached leaf culture method of Helle and Sabelis (1985) with slight modifications.

Ovicidal activity

For the assessment of ovicidal properties of the extracts, 15 gravid females of red spider mite were introduced on TV1 mature fourth leaf from the top of the shoot for oviposition and kept overnight in the petri dish. The mature leaves were padded with water soaked cotton. After 18 h the introduced mites were removed with the help of fine brush. The eggs laid on tea leaves were counted under microscope as pre-treatment count. Tea leaves containing more than 30 eggs were removed cautiously by using fine needle. 150 eggs were considered for each ovicidal treatment of the plant extract and observed for five times (30 eggs/observation). After counting, the eggs are subjected to spraying of different APEs at 2.5, 5.0, and 10.0% (w/v) by using glass atomizer. The control eggs were also segregated as above manner and treated with water. Hatchability was determined for both experimental and control batches of eggs for a period of 12 days after oviposition. Those eggs that did not hatch after this period were regarded as non-viable (Sarmah et al., 1999). Per cent reduction in hatchability was calculated by using the following formula:

$$\text{Egg mortality (\%)} = 100 - \left(\frac{\text{No. unhatched eggs/treatment}}{\text{Total No. of eggs/treatment}} \times 100\right)$$

Laboratory acaricidal test

For laboratory evaluation of plant extracts, 30 healthy adult female of red spider mite (24 h old) were released on a healthy detached tea leaf of TV1 from the culture maintained in the laboratory. Final count of mite population was taken for confirmation on number of mite after 4 h (after proper settlement of mite). Each concentration was sprayed on both the surfaces of leaf using glass atomizer. The number of live red spider mite was counted 24, 48 and 72 h after treatment. Each treatment was replicated five times. Data were subjected to ANOVA analysis.

Feeding potential and mortality of *S. gilvifrons*

To assess the feeding potential and mortality of *S. gilvifrons*, matured healthy TV1 leaves (4^{th} leaf from the top) were collected from field, washed under tap water and allowed to dry under electric fan for 5 min. Dried leaves were treated with the chosen APEs by following dipping method as described by Rahman et al. (2005).

Table 2. Ovicidal action of aqueous plant extracts on the eggs of red spider mite under laboratory condition.

Treatment	Concentration (%)	Egg mortality (%)
Xanthium strumarium	10.0	87.09
	5.0	59.20
	2.5	44.90
Acorus calamus	10.0	70.62
	5.0	64.05
	2.5	33.33
Polygonum hydropiper	10.0	30.86
	5.0	26.84
	2.5	13.29
Clerodendron infortunatum	10.0	20.58
	5.0	10.81
	2.5	6.00
Control (water)	-	1.31
C.D. at (P=0.05)		7.89
C.V.%		8.64

Mean of five observations (30 eggs/observation).

100 numbers of adult red spider mites were introduced to the treated leaves of different APEs. After an hour, one adult of *S. gilvifrons* was introduced in each treatment and observations were made for 24 and 48 h regarding the consumption of mites and its mortality was observed for fourteen days. The experiment was replicated 5 times.

Field toxicity test

A field trial was conducted to evaluate the efficacy of different APEs (5 and 10%) against red spider mite. Mixed Assam tea clones TV 1, 18, 20, 22, 23, 24, 25 and 26 were chosen for the current study by following randomized block design with three replications. Each plot in the experiment was separated by two buffer rows of non-experimental tea. Thirty bushes per replication were considered for each treatment along with unsprayed control. Plots with heavy infestation of red spider mite were chosen for this study. After selection of the plots, pretreatment count was taken in the respective plots and two rounds of foliar spray were given at 15 days interval with hand operated Knapsack sprayer at 400 litres/ha. Post treatment observations were taken for four weeks after treatment. Observations on mite population were made on both adaxial and abaxial side of the thirty randomly collected mature leaves per replication for each treatment of different APEs along with unsprayed control by following the mangling method of Das (1960). Data were subjected to statistical analysis.

RESULTS

Ovicidal action of aqueous plant extracts (laboratory)

Highest egg mortality was registered at higher concentration (10.0%) of *X. strumarium* and *A. calamus* to the level of 87.09 and 70.62%, respectively, whereas in *P. hydropiper* (30.86%) and *C. infortunatum* (20.58%) lowest egg mortality was recorded. An egg mortality of 59.20 – 64.05% was obtained in *X. strumarium* and *A. calamus* at 5.0% concentration in contrast with *C. infortunatum* (10.81%) and *P. hydropiper* (26.84%). At lower concentration (2.5%), least ovicidal action was noticed in *C. infortunatum* (6.0%) and *P. hydropiper* (13.29%) but *A. calamus* and *X. strumarium* showed 33.33 and 44.90% egg kill, respectively (Table 2). Ovicidal action was maximum in *X. strumarium* and *A. calamus*, whereas minimum in *P. hydropiper* and *C. infortunatum* (Table 2). Petroleum ether and acetone extracts of *P. hydropiper* exhibited more than 80% egg mortality in red spider mite (Sarmah et al., 1999) unlike water extract as observed in the current study. Chemical substances present in the chosen plants may block the micropyle region of the egg thereby preventing the gaseous exchanges that will ultimately kill the embryo in the egg itself. Raja et al. (2003) screened 9 plants with various solvent extracts against *Spodoptera litura* in relation to ovicidal and ovipositional deterrent activity and varied responses were noticed irrespective of the concentrations and the solvents used for extraction. Slama (1974) reported that the incomplete blastokinesis and abnormal breakage of extra embryonic membranes in the embryo or unequal penetration of extracts through the egg chorion to different parts of egg at different times of the sensitive period could also be associated with observations on variability of morphological effects. These findings also corroborate with present work on ovicidal activity of the chosen plant extracts against *O. coffeae*.

Acaricidal activity of plant extracts on adult mites (laboratory)

Different concentrations (2.5, 5.0 and 10.0%) of aqueous extracts of *A. calamus*, *X. strumarium*, *P. hydropiper* and

Table 3. Acaricidal activity of aqueous plant extracts against adult red spider mite under laboratory condition.

Treatment	Concentration (%)	Per cent mortality		
		24 h	48 h	72 h
Xanthium strumarium	10.0	87.2	87.2	91.8
	5.0	56.7	56.7	61.5
	2.5	15.6	15.6	15.6
Acorus calamus	10.0	87.7	87.7	88.7
	5.0	54.2	54.2	57.1
	2.5	6.4	6.4	6.4
Polygonum hydropiper	10.0	77.7	77.7	84.2
	5.0	52.2	52.2	53.9
	2.5	12.8	12.8	12.8
Clerodendron infortunatum	10.0	100.0	100.0	100.0
	5.0	96.0	96.0	96.0
	2.5	23.0	23.0	23.0
Control (water)		3.4	3.4	3.4
CD (P=0.05)		6.24	6.24	6.78
C.V.%		10.73	10.73	11.13

Mean of five observations (30 adults/observation).

C. infortunatum were tested to evaluate their toxic effect at 24 48 and 72 h against adult red spider mites and the obtained results have been summarized in Table 3. A least acaricidal action (6.4 - 23.0%) was noticed after 24 h and up to 72 h at lower concentration (2.5%) of all the plant extracts. *A. calamus*, *X. strumarium*, and *P. hydropiper* aqueous extracts at 5.0% concentration exhibited pronounced 52.2 – 56.7% kill after 24 h whereas at the same concentration of *C. infortunatum* showed 96.0% mortality. Higher concentration (10.0%) of APEs manifested 77.7 – 100% (after 24 h) death of adult mites (Table 3). The mortality was in a linear trend i.e., increasing with increase in concentration. We have previously observed the same acaricidal activity by using different solvent extracts of *P. hydropiper* and *Lantana camara* (Sarmah et al., 1999). Maximum kill was attained in *C. infortunatum*, followed by *A. calamus* and *X. strumarium*, and finally by *P. hydropiper*. There were no marked changes in terms of toxicity between 24 and 72 h (Table 3).

Effect of plant extracts on *S. gilvifrons* in terms of feeding rate and toxicity – laboratory

S. gilvifrons was reduced significantly to 29.4 – 38.0 numbers of red spider mite (P = 0.05) in comparison with untreated control (43.8 red spider mite) when fed 5 and 10% of *X. strumarium, P. hydropiper* and *A. calamus* after 24 h exposure. However, after 48 h there was no significant difference in feeding rate. Leatemia and Isman (2004) experimented on the toxicity of crude aqueous seed extracts of *Annona squamosa* to natural enemies and showed that *Chrysoperla carnea* larvae were less susceptible to the extracts than *Orius insidiosus* adults and crude aqueous seed extracts of *A. squamosa* had moderate to high toxicity to *O. insidiosus*. In contrast, only consumption was affected slightly up to 24 h in our study and no mortality was observed up to 14 days in all the treatments (Table 4).

Field evaluation of toxicity of plant extracts

At 5%, *P. hydropiper* and *A. calamus* aqueous extracts reduced the mite incidence to the tune of 45.4 – 46.9% and 50.1 - 57.7%, respectively, whereas at 10% concentration 67.3 – 64.7% and 77.4 – 86.4% reduction in prevalence of mites was attained. 56.3 – 70.9% and 79.9 – 90.2% reduction in mite population were observed at 5 and 10% concentrations of *X. strumarium*, respectively. *C. infortunatum* showed 62.2 – 81.8% and 94.5 – 100.0% reduction in incidence of mites at 5 and 10%, respecttively. Reduction in mite incidence was higher in *C. infortunatum* and *X. strumarium*. At higher concentration (10%) 86.4 – 100 per cent reduction was achieved during 4 week observation after second round of spray with *X. strumarium*, *C. infortunatum* and *A. calamus*. In case of *X. strumarium* and *C. infortunatum*, 70.9 – 81.8% reducetion was manifested even at lower concentration of 5% (Table 5). Similar observations were made by Rahman et al. (2005) when water extract of *C. infortunatum* (5 and 10%) was sprayed against *O. coffeae* (82.0 – 100.0% reduction in mite incidence) under field condition.

DISCUSSION

In spite of the wide recognition that many plants possess

Table 4. Effect of aqueous plant extracts on feeding potential and mortality of adult *Stethorus gilvifrons* Mulsant.

Treatment	Concentration (%)	Rate of consumption (mean+S.D.)		Mortality (%)
		0-24 h	24-48 h	
Xanthium strumarium	10.0	29.4 ± 1.35 **	37.6 ± 3.26	No adult Mortality Was observed till 14 days of observation in all the treatments
	5.0	37.0 ± 2.19 **	38.8 ± 3.18	
	2.5	39.6 ± 2.41	39.0 ± 3.46	
Acorus calamus	10.0	33.8 ± 2.48 **	39.0 ± 2.00	
	5.0	37.0 ± 0.63 **	38.0 ± 3.84	
	2.5	40.4 ± 0.80	39.2 ± 3.18	
Polygonum hydropiper	10.0	33.4 ± 4.40 **	38.2 ± 3.91	
	5.0	38.0 ± 3.03	39.0 ± 3.94	
	2.5	40.0 ± 3.40	40.6 ± 3.44	
Control(water)	-	43.8 ± 4.30	40.6 ± 1.62	

Mean of five observations.
**Significant at $P < 0.05$ (t-test).

Table 5. Field evaluation of toxicity of aqueous plant extracts against red spider mite.

Treatment	C (%)	PTP	Post treatment observations							
			1st spray				2nd spray			
			7 days		14 days		7 days		14 days	
			P	R	P	R	P	R	P	R
Polygonum hydropiper	5.0	330	180	45.4	185	43.9	160	51.5	175	46.9
	10.0	340	111	67.3	135	60.2	105	69.1	120	64.7
	5.0	331	165	50.1	170	48.6	140	57.7	140	57.7
Acorus calamus	10.0	333	75	77.4	90	72.9	60	81.9	45	86.4
	5.0	344	150	56.3	145	57.8	120	65.1	100	70.9
Xanthium strumarium	10.0	309	62	79.9	75	75.7	52	83.1	30	90.2
	5.0	381	72	62.2	80	79.0	65	82.9	69	81.8
Clerodendron infortunatum	10.5	333	18	94.5	20	93.9	0	100.0	0	100.0
Azadirachtin 0.03%	1:300	326	140	57.0	145	55.5	98	69.9	105	67.7
Control (untreated)		322	316	1.8	316	1.8	314	2.4	314	2.4
C.D at (P=0.05)			5.58		5.47		5.48		5.78	
C.V.%			5.33		5.41		4.82		5.04	

C = Concentration; PTP = Pretreatment population; P = Population; R = Percent reduction.
Mean of three observations.

insecticidal properties, only a handful of pest control products directly obtained from plants, i.e., botanical insecticides, are in use in developed countries. At best, botanical insecticides presently constitute 1% of the world insecticide market, but annual sales growth in the range of 10 – 15% is entirely possible (Gentry, 1993; Wink, 1993). The impact of botanicals will perhaps be most noticeable in the home-and-garden sector, where they might conceivably achieve as much as a 25% market share within 5 years (Isman, 1997). The demonstrated efficacy of the botanical neem (based on seed kernel extracts of *Azadirachta indica*), and its recent approval for use in the United States, has stimulated research and development of other botanical insecticides. Unfortunately, efficacy against pests is only one of a number of important criteria that need be met for a plant extract or derivative to move successfully toward commercialization and use (Isman, 1995). Apart from efficacy and spectrum-of-action, biological criteria include favorable toxicology and minimal environmental impact (i.e., vertebrate selectivity; selectivity favoring natural enemies and pollinators; rapid environmental degradation). The chosen native plants meet these criteria admirably, yet the large scale utilization of these products for use in tea plantations has taken many years. Obviously, it is not enough to have an efficacious product that is relatively safe to the user and the environment – there are other considerations that must be satisfied. However, the large scale use of new botanical insecticides can be hindered by a number of issues. The principal barriers are (i) the relative scarcity or availability of the natural resource, (ii) standardization of extracts and quality control, based on active ingredients, and (iii) special problems in regulatory approval of botanicals (Isman, 1997).

Natural defenses of plants against herbivory consist almost always of mixtures of closely related compounds, rather than a single toxicant alone. This phenomenon is well exemplified among botanical insecticides. In other words, the overall efficacy is superior to that which could be obtained with the equivalent amount of the most active constituent alone, if isolated to purity or synthesized (Isman, 1997). This phenomenon was demonstrated over a decade ago (Berenbaum, 1985). Chen et al. (1995) observed that the growth inhibitory effect of refined bark extracts from *Melia toosendan*, containing 60 – 75% toosendanin, was significantly greater than that of pure toosendanin, indicating that lesser constituents were making a contribution to overall bioactivity greater than expected based on their mass. Complex mixtures of active constituents, as found in botanical insecticides, may also be advantageous in terms of pest resistance and behavioral desensitization in case of green peach aphid, *Myzus persicae* (Feng and Isman, 1995). Hence in this study only APEs were tried out which ward off the red spider mites and caused no effect on the survival of *S. gilvifrons*. The chosen native plants are abundant in and around fallow lands of tea plantations that could be effectively utilized in the current IPM programme. The procedure adopted in this study for the preparation of APE is one of the easiest methods and serve the purpose of the end user. This approach would help the tea industry in many ways (residue free tea, reduction in acaricide load, cost effectiveness, customer satisfaction) and remain important in red spider mite management for at least four reasons: (1) providing the most effective control, (2) short lived in the environment and posing relatively low risk to non-target organisms including the beneficial predators and parasites that help to regulate mites, the higher level predators in food chains and human consumers of treated crops, (3) naturally occurring or derived or manufactured with minimal technology, so they are accepted by organic certification programmes and by certain consumer groups and (4) more compatible with biological control efforts (Weinzierl, 2000; Siakh, 2004).

Furthermore, natural products are notoriously variable, and therefore consistency of the final product will be much harder to achieve. Also, it may be necessary to determine both the shelf stability of the active ingredient and its fate in the environment or in animals. Such studies can be complicated enough when only a single compound is being tracked, but the effort required to track several putative active principles in a single product should not be underestimated.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, Tocklai Experimental Station, Tea Research Association, Jorhat for providing necessary facilities and to the Statistics Department, TRA, Jorhat for statistical analysis. This work is part of a Tea Research Association in-house project (Ento/04/2004-2009), Kolkata.

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