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Ovicidal, acaricidal and growth inhibitory activity of X*anthium strumarium* L., *Acorus calamus* L., and *Pongamia pinnata* L. (Pierre) against a major pest of tea, *Oligonychus coffeae* Nietner (Tetranychidae : Acarina)

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ABSTRACT

Ovicidal, post-embryonic development and adulticidal activity of petroleum ether, acetone and methanol fractions of X*anthium strumarium* (L.)*, Acorus calamus* (L.), and *Pongamia pinnata* (Pierre), prepared by following sequential extraction method, against the tea red spider mite, *Oligonychus coffeae* Nietner (Acarina: Tetranychidae) were determined by employing direct exposure method under laboratory conditions. Responses in terms of reduction in egg hatchability, larval-nymphal mortality, adult emergence and mortality of adults varied according to solvent fractions tested, their concentration and developmental stage of the insect. All the solvent fractions of *A. calamus* and methanolic fraction of *X. strumarium* possess the property of killing eggs but no ovicidal action was noticed in *P. pinnata*. All the plant extracts adversely affect the post-embryonic development as well as adult of red spider mite. The plant extracts described herein merit further study as potential miticides for *O.coffeae* control in tea ecosystem.

Key Words **:** *Oligonychus coffeae*, Solvent fraction, X*anthium strumarium, Acorus calamus*, *Pongamia pinnata*, ovicidal, growth inhibitory activity, Acaricide, Tea

INTRODUCTION

The tea, *Camellia sinensis* (L) O.Kuntze is one of the most important beverage and commercial crop covering an area of 4.35-lakh hectare in India producing annually 870 million kg made tea, out of which 78% is harvested from North-east India. At present the tea industry is facing various constraints as increased cost of production, pest outbreaks (especially tea mosquito bug and red spider mite) and more pesticide consumption (7.35-16.75 kg/l per ha. of insecticides and 1.0- 3.5 kg/l per hectare of acaricides) (Borbora and Biswas, 1996), stringent regulatory measures from different bodies (EU, HACCAP, FAO, CODEX, WHO) and countries (German, Japan, Poland, Russia, USA, Canada) regarding residues in made tea, consumers consciousness about ill effects of hard pesticides, environmental pollution and ecological imbalance. To overcome the above, safer and sustainable alternative strategies must be adapted to manage the pests of tea and reduce the pesticide load.

The tea red spider mite, *Oligonychus coffeae* (Nietner), wide distribution in most of the tea

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growing countries of Asia, Africa and South America, is a serious pest of tea in North-east India and causes considerable economic loss to the tune of 17 – 20 % (Das, 1959 ; Banerjee, 1966, 1971). The control of red spider mite populations primarily depends on application of acaricides as dicofol, ethion, sulfur, propargite, fenazaquin and neem products. Further, there was an outbreak of red spider mite in Assam and North Bengal during April – July 2004, 2005 and 2006 creating an alarming situation. In this juncture, the plant protection programme for controlling mites must be towards effective integrated pest management with reduced reliance on the hard chemicals. One of the approaches in IPM is the effective utilization of locally available and safe plant based products. The future use of safe plant based pesticides to produce residue-free tea will appeal to many consumers who wish to avoid the risks of adverse side effects associated with pesticides.

In view of these, the prime objective of this study was: (i) to determine the ovicidal and acaricidal action of *Xanthium strumarium* L., *Acorus calamus* L., and *Pongamia pinnata* (L.) Pierre, prepared in petroleum ether, acetone and methanol, following sequential extraction method and ii) to assess the post-embryonic development and adult emergence after exposing the eggs of *Oligonychus coffeae* to different solvent fractions of plant extracts.

MATERIALS AND METHODS

Collection, preparation and preservation of plants

Leaves (young and old) and succulent stems of *Xanthium strumarium* L., *Acorus calamus* L., and *Pongamia pinnata* (L.) Pierre were collected from tea gardens adjoining the Tocklai Experimental Station, Tea Research Association, Jorhat, Assam, during January – February 2005. All the collected plant materials were allowed to dry under shade for 20 –30 days, powdered in an electric grinder, passed through a 20-mesh sieve, and packed in a polypropylene bag of 1 kg capacity.

Preparation of Solvent Extracts – Sequential extraction method

100 grams of each plant powder material was soxhleted for 16 hours initially with 250 ml of petroleum ether and after that solvent was decanted, denoted as Fraction-I. The residue obtained from the first extraction was again soxhleted with Acetone (250 ml) for 16 hours. The acetone extract was poured out and designated as Fraction-II. The residue obtained from the second extraction was subjected to soxhlet extraction with Methanol (250 ml) and after 16 hours the methanol extract was collected as Fraction-III. Thus sequential extraction was done on the basis of solvents' polarity. The extracts were filtered through Whatman filter paper No.1 in a one litre capacity conical flask. The solvent from each extract was removed under reduced pressure in a rotary evaporator. The obtained residues after removing the respective solvents through rotarary evaporation were further dissolved in methanol to make 20% solution. This considered as a stock solution. Aqueous emulsions were made by adding distilled water to stock solution of three different fractions according to the concentration needed (0.5, 1.0, 2.0, and 5.0 % v/v) (Sarmah *et al*., 1999). Soap solution (Lifebuoy) (0.05% w/v) was added as an emulsifier.

Culture and maintenance of *Oligonychus coffeae* Nietner

Red Spider mite infested tea branches were collected from field and kept in conical flask containing water. This was placed in an open cylindrical glass jar (60cm Height x 45cm Diameter). This was the stock culture. From where adults were shifted to fresh mature leaves of TV 1 [*Camellia assamica* (Masters)] and allowed them to lay eggs for 24 hours. After 24 h the adults were removed from the

leaves by using fine brush. Those eggs were maintained as culture for obtaining adults of equal age groups by following the modified method of Helle and Sabelis (1985). This set up was kept under laboratory conditions at the temperature of 25± 2° C, 70-80% RH and a 16:8 LD photoperiod for a period of two months.

Ovicidal Activity

For the assessment of ovicidal properties of the extracts, fifteen gravid females of red spider mite were introduced on TV 1 clone (mature fourth leaf from the top of the shoot) for oviposition and kept it for over night in the petri dish. The mature leaves padded with water soaked cotton. After 18 hours 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 up to 30 eggs and 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 plant solvent fraction at 0.5, 1.0, 2.0, and 5.0 % v/v concentration by using glass atomizer. The exposure of eggs to different plant extracts was scheduled separately. The control eggs totaling 150 were also segregated as above manner and treated with water solvent. 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 :

$$\text{Per cent reduction in hatchability} = \frac{\text{No. unhatched eggs/treatment}}{\text{Total No. of eggs/treatment}} \times 100$$

Post-embryonic development and survival of *Oligonychus coffeae* after exposure during egg stage

Observations were taken periodically after treating the eggs of *O.coffeae* to different plant extracts for their hatching. From the larvae that emerged from these eggs, 10 individuals were randomly picked, placed carefully on fresh mature TV 1 tea leaves with the help fine brush and reared. The tea leaves were padded with water soaked cotton pads and kept in the petri

Table 1. Per cent reduction in egg hatchability in following exposure of eggs of *Oligonychus coffeae* to different solvent fractions of *Xanthium strumarium, Acorus calamus* and *Pongamia pinnata*

PE	Petroleum ether fraction Concentration (%)					Acetone fraction Concentration (%)					Methanol fraction Concentration (%)				
	C	0.5	1.0	2.0	5.0	C	0.5	1.0	2.0	5.0	C	0.5	1.0	2.0	5.0
XS	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	1.6 ± 0.85 (7.27)	4.5 ± 1.88 (12.25)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	37.3 ± 2.97 (37.64)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	9.4 ± 1.41 (17.85)	22.6 ± 1.62 (28.38)	91.2 ± 1.77 (72.84)
AC	0.0 ± 0.0 (0.57)	84.2 ± 2.14 (66.58)	91.2 ± 0.70 (72.74)	100.0 ± 0.0 (84.20)	100.0 ± 0.0 (84.20)	0.0 ± 0.0 (0.57)	59.1 ± 2.22 (50.24)	74.2 ± 1.26 (59.47)	81.4 ± 2.74 (64.45)	95.2 ± 1.72 (78.17)	0.0 ± 0.0 (0.57)	0.8 ± 0.68 (5.13)	9.1 ± 1.66 (17.56)	54.6 ± 2.28 (47.64)	97.7 ± 1.38 (81.28)
PP	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)	0.0 ± 0.0 (0.57)

Mean (± SE) of 5 observations; Data in parentheses are arc sine transformed values; PE – plant extract; XS – *Xanthium* strumarium; AC – *Acorus calamus*; PP – *Pongamia pinnata*; C- Control.

dish. Mortality of the larvae and nymphs, larval and nymphal development duration, the total post-embryonic development period of surviving individuals and the number of adults that eventually emerged were recorded (Gurusubramanian and Krishna, 1996). Observations were made for three times.

Acaricidal Activity - Direct Spray Method

Thirty numbers of two-day-old adult stages of *O.coffeae* were segregated by using fine brush and shifted to fresh and cleaned uninfested TV1 (mature fourth leaf from the top of the shoot) tea leaves padded with wet cotton swabs placed in petri dish (20 cm diameter) and kept the leaves for 4 hours for settlement of mites. After settlement, pretreatment count was taken under microscope for checking the mite count. Different chosen concentrations (0.5, 1.0, 2.0, and 5.0 % v/v) of different solvent extracts of *X. strumarium*, *A. calamus*, and *P. pinnata*, prepared in methanol, acetone and petroleum ether, were sprayed separately by using atomizer over both sides of tea leaves (0.75 ml) and kept in a petri plate. Mortality counts were taken after 24 and 48 h. Each treatment was observed for five times. In all the cases water was sprayed as control (Sarmah *et al*., 1999).

All experiments were carried out at 25±2° C under 70-80% RH and a photoregime of 16:8 (LD) h. The data collected for per cent reduction in egg hatchability and acaricidal activity at 24 and 48 h were transformed into arc sine values and analysed statistically (Two way ANOVA) and for post-embryonic development, mortality and emergence, t-test was followed (Zar, 1974).

RESULTS

Per cent reduction in egg hatchability, larval/ nymphal mortality, duration from larval-nymphal stages to attaining adults, and adult emergence in *O.coffeae* following exposure of their eggs to different solvent extracts of *X. strumarium*, *A. calamus*, and *P. pinnata* are presented in tables 1 - 3. A severe per cent reduction in egg hatchability occurred in all the fractions of *A. calamus* to the tune of 84.2 – 100 % (petroleum ether) and 59.1 – 95.2 % (acetone) at 0.5 – 5.0 % concentrations and 54.6 – 97.7 % (Methanol) at 2.0 and 5.0 %. *X. strumarium* showed significant reduction in hatchability only at higher concentrations of acetone (37.3 per cent reduction at 5.0%) and Methanol (22.6 per cent reduction at 2.0 % and 91.2 per cent reduction at 5.0 %) fractions. *P. pinnata* , however, showed no ovicidal activity in all the three fractions compared to the control (Table 1). Through two way ANOVA, significant differences were observed in *A. calamus* and *X. strumarium* among the solvent fractions, among the concentration of the plant extracts and interaction between solvent fraction and concentration (Table 2).

Relatively significant higher larval and nymphal mortality ($p<0.05$) during pre-imaginal stages were obtained when larvae emerging from eggs treated with different solvent fractions of *X. strumarium* (43 – 85 % - petroleum ether; 58 – 85 % - acetone; 45 – 100 % - methanol) and *P. pinnata* (26 – 57 % - petroleum ether; 32 – 62 % - acetone; 43 – 63 % - methanol) in comparison with untreated control (7-15 %) and this in turn, led to the eventual production of <50 % adults ($p<0.05$) at higher concentrations (2.0 and 5.0 %) whereas 57 – 74 % and 41 – 55 % recorded at lower concentrations (0.5 and 1.0 %) of *X. strumarium* and *P. pinnata* respectively (Table 3). No adults of *O. coffeae* emerged following exposure of eggs to different fractions of *A. calamus* except 0.5 and 1.0 % methanol fractions and 5.0 % methanol fraction of *X. strumarium* as a result of the high mortality sustained during the larval and nymphal stages of development. There was, however, no significant variation ($p<0.05$) in the larval and nymphal duration and total post-embryonic development time among individuals subjected to treatment with different plant extracts at different concen-

Table 2. Two-way analysis of variance for per cent reduction in egg hatchability at different solvent fractions and concentrations of *Xanthium strumarium,* and *Acorus calamus* against *Oligonychus coffeae*

Plants	Source	SS	DF	MS	SEM ±	CD (0.05)	CV %
Xanthium strumarium	Total	30406.03	74	410.89	-	-	-
	Factor A	5961.17	2	2980.58	1.96	4.63	1.15
	Factor B	16333.79	4	4083.44	1.51	3.56	1.92
	Interaction A x B	7418.72	8	927.34	1.20	2.83	28.87
Acorus calamus	Total	79564.43	74	1075.19	-	-	-
	Factor A	13112.34	2	6556.17	1.99	4.70	0.27
	Factor B	55952.84	4	13988.21	1.48	3.49	0.46
	Interaction A x B	9839.34	8	1229.91	1.17	2.76	6.99

Factor A - Between different solvent fractions; Factor B – Between different concentrations;

AxB – Between different solvent fractions and concentrations.

trations during their embryonic growth (Table 3).

Regarding the acaricidal activity of different plant extracts (Table 4), petroleum ether and acetone solvent fractions of *A. calamus* observed 80.0 – 100.0 % mortality whereas 36.7 – 94.60 % kill was recorded in methanolic fraction within 24 h. *X. strumarium* registered a per cent kill of 54.5 – 100.0 %, 48.0 – 97.3 % and 13.2 – 98.0 % in methanol, acetone and petroleum ether fractions respectively. All the fractions of *P. pinnata* showed 42.6 – 85.4 % mortality. No significant changes in the per cent mortality of red spider mite were observed between 24 and 48 h recording (Table 4). Two way ANOVA analysis revealed the fact that significant variation was observed in solvent fraction, concentration and interaction between solvent fraction and concentration in terms of adulticidal activity (Table 5).

DISCUSSION AND CONCLUSIONS

The ovicidal (Table 1), larval and nymphal mortality (Table 3) and acaricidal (Table 4) action were more promising in the ascending trend whereas adult emergence of *O.coffeae* were in the descending order with the increase of concentrations of different fractions of *X. strumarium*, *A. calamus*, and *P. pinnata*. Evidently, some of the main chemical ingredients,á - asarone , â – asarone (Mazza, 1985a,b; Schmidt and Streloke, 1994) present in the fractions of *A. calamus* probably diffused into the eggs and affected vital physiological and biochemical processes associated with embryonic development in all those eggs that failed to hatch. These findings in a broad sense, agree with the earlier findings of Sarmah *et al* (1999) and Deka *et al* (1997 and 1998 a,b,c), who observed high mortality following exposure of eggs of *O.coffeae* and eggs of *Helopeltis theivora* to some plant extracts.

Plant extracts contain many active compounds in addition to those that are inactive. Some of these may act synergistically to enhance a specific bioactivity, whereas some may act antago-

Table 3. Post embryonic developmental data of *Oligonychus coffeae* following their egg exposure to different fractions of *Xanthium strumarium, Acorus calamus* and *Pongamia pinnata* during embryogenesis

Solvent fraction	Concentration (%)	*Mortality (%)		*Adult emergence (%)		**Mean L-N duration	**Total PED duration
		L	N	M	F		
ACPE	Control	2	5	23	70	1.7 ± 0.65	7.1 ± 0.84
	0.5	68	32	0	0	-	-
	1.0	84	16	0	0	-	-
	2.0	-	-	-	-	-	-
	5.0	-	-	-	-	-	-
ACAE	0.5	71	29	0	0	-	-
	1.0	38	62	0	0	-	-
	2.0	52	48	0	0	-	-
	5.0	71	29	0	0	-	-
ACME	0.5	18	27	12	43	2.6 ± 0.62	7.7 ± 0.92
	1.0	27	54	7	12	2.8 ± 0.12	8.4 ± 0.34
	2.0	71	29	0	0	-	-
	5.0	79	21	0	0	-	-
XSPE	Control	10	5	35	50	1.5 ± 0.50	7.0 ± 1.00
	0.5	25	26	12	37	1.8 ± 0.25	7.2 ± 0.65
	1.0	24	19	25	32	1.8 ± 0.56	7.6 ± 0.72
	2.0	55	29	6	10	2.4 ± 0.84	7.1 ± 0.55
	5.0	60	25	5	10	2.3 ± 1.25	7.6 ± 0.74
XSAE	0.5	28	30	14	28	2.2 ± 0.25	7.5 ± 0.62
	1.0	38	21	11	30	2.8 ± 0.84	7.9 ± 0.18
	2.0	49	28	6	17	2.5 ± 1.62	8.1 ± 1.64
	5.0	64	21	5	10	2.6 ± 0.50	8.4 ± 0.33
XSME	0.5	19	26	16	39	1.9 ± 0.16	7.3 ± 0.62
	1.0	30	20	18	32	2.1 ± 0.35	7.8 ± 0.57
	2.0	62	28	3	7	2.2 ± 0.42	8.3 ± 0.68
	5.0	71	29	0	0	2.5 ± 0.80	8.6 ± 0.18
PPPE	Control	7	4	32	57	1.4 ± 0.98	6.8 ± 0.76
	0.5	12	14	24	50	1.5 ± 0.22	7.3 ± 0.92
	1.0	18	11	29	42	1.6 ± 0.15	7.4 ± 0.15
	2.0	24	29	18	29	1.6 ± 0.62	7.2 ± 0.64
	5.0	26	31	12	31	1.5 ± 0.54	7.5 ± 0.36
PPAE	0.5	20	12	25	43	1.5 ± 0.76	7.0 ± 0.85
	1.0	12	23	27	38	1.6 ± 0.35	7.4 ± 0.41
	2.0	33	55	10	35	1.8 ± 0.14	7.4 ± 0.76
	5.0	34	28	16	22	1.8 ± 0.75	7.3 ± 0.25
PPME	0.5	17	26	23	34	1.8 ± 0.45	7.8 ± 0.94
	1.0	24	19	16	41	1.8 ± 0.34	7.9 ± 0.65
	2.0	31	27	11	31	1.9 ± 0.15	8.0 ± 0.36
	5.0	28	35	12	25	1.9 ± 0.26	8.1 ± 0.82

nistically to mask certain activities. A similar trend was reported by Leatemia and Isman (2004b)in terms of antifeedant activity with the crude aqueous seed extracts of *A. squamosa* at a concentration of 10%, which was much higher than the 0.01% aqueous emulsion of Azatin (3% azadirachtin) and 0.01% aqueous emulsion of neem seed oil (1% azadirachtin) against fourth-instar *P. xylostella* (Perera *et al*., 2000). This may be the reason why some/all of the fractions of *X. strumarium* and *P. pinnata* showed lesser or no ovicidal action against red spider mite.

Chronic effects of plant extracts on embryogenesis of red spider mite resulted from the volatile components of *X. strumarium*, *A. calamus*, and *P. pinnata* acting on the developing embryo or the neonate larva inside the egg, rather than being carried over as active material into the larva itself and subsequently disrupting the processes of post-embryonic development. Further tests would be required to differentiate between these two possibilities. The chemical reactivity of many of the major components of different plant extracts especially *A. calamus* suggests that the former explanation may be more likely (Gurusubramanian and Krishna, 1996; Marimuthu *et al*., 1997).

Plant extracts and essential oils are potentially useful for many insects control because many of them are selective to pests, and have little or no harmful effects on nontarget organisms and the environment (Isman, 2000; Leatemia and Isman, 2004b). They act in many ways on various types of pests and can be applied to plants or stored products in the same way as other conventional insecticides (Desmarchelier 1994; Isman 2000; Choi *et al.,* 2003; Leatemia and Isman, 2004a). Many plant extracts and essential oils are known to possess ovicidal, repellent, and insecticidal activities against various insect species (Saxena 1989; Desmarchelier 1994; Isman 2000; Choi *et al.,* 2003; Leatemia and Isman, 2004a,b). Additionally, some plant-derived compounds can be highly effective against insecticide-resistant insect pests (Lindquist *et al*., 1990; Ahn *et al*., 1997). In the current study, potencies varied according to plant, type of fraction, dose, and developmental stage of the insect. Promising ovicidal activity was noticed only in *A. calamus* and not in *X. strumarium* and *P. pinnata* (Table 1). However, potent adulticidal activity against adult stages of *O. coffeae* was observed with all the solvent fractions of *X. strumarium*, *A. calamus*, and *P. pinnata* between 1 and 5 % concentration (Table 4). Cent per cent adult mortality was observed at 5 % concentration of all the fractions of *X. strumarium*, *A. calamus* and *P. pinnata* in contrast to aqueous emulsions of petroleum ether and acetone extract registered the same trend even at 1 % concentration (Table 4). Tables 2 and 5 summarise the results of a two-way analysis of variance for reduction in egg hatchability and adulticidal activity with different solvent fractions and concentrations of *X. strumarium*, *A. calamus* and *P. pinnata* and varied significantly (p=0.05) with respect to different variables (Solvent and concentration) and their interactions. From the above it was noticed that methanol and acetone fractions of *X. strumarium* , and petroleum ether and acetone fractions of *A. calamus* and *P. pinnata* exhibiting marked variations in mortality of adult mites, which revealed the fact that all the plant extracts showed only slight variations in terms of adult mite mortality and exposure time (24 and 48 h) indicating short persistence and photodegradable nature of plant extracts (Weinzierl, 1998). These plant extracts might be good candidates for naturally occurring *O. coffeae* control agents. Elucidation of the mode of action of chemicals is of practical importance for mite control because it may give useful information on the appropriate formulation types. Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols, aldehydes, and terpenoids, especially monoterpenoids, and exhibit fumigant activity (Coats *et al*., 1991; Ahn

Table 4. Acaricidal action of different solvent fractions of *Xanthium strumarium, Acorus calamus* and *Pongamia pinnata* against adults of *Oligonychus coffeae* at 24 and 72 h.

PE	Petroleum ether fraction					Acetone fraction					Methanol fraction				
	C	0.5	1.0	2.0	5.0	C	0.5	1.0	2.0	5.0	C	0.5	1.0	2.0	5.0
Xanthium strumarium (24 h)	0.0 ± 0.0 (0.57)	13.2 ± 1.08 (21.30)	22.1 ± 1.19 (28.04)	59.8 ± 3.99 (50.65)	98.0 ± 1.19 (81.87)	0.0 ± 0.0 (0.57)	48.0 ± 1.52 (43.85)	74.7 ± 1.52 (59.80)	89.1 ± 1.11 (70.72)	97.3 ± 1.12 (80.54)	0.0 ± 0.0 (0.57)	54.5 ± 1.87 (47.58)	79.6 ± 2.25 (63.15)	88.8 ± 1.20 (70.45)	100.0 ± 0.0 (84.74)
Acorus calamus (24 h)	0.0 ± 0.0 (0.57)	80.9 ± 0.78 (64.08)	100.0 ± 0.0 (84.74)	100.0 ± 0.0 (84.74)	100.0 ± 0.0 (84.74)	0.0 ± 0.0 (0.57)	80.0 ± 0.93 (63.44)	100.0 ± 0.0 (84.74)	100.0 ± 0.0 (84.74)	100.0 ± 0.0 (84.74)	0.0 ± 0.0 (0.57)	36.7 ± 2.11 (37.29)	59.1 ± 0.62 (50.24)	72.7 ± 1.12 (59.50)	94.6 ± 1.52 (76.56)
Pongamia pinnata (24 h)	0.0 ± 0.0 (0.57)	48.2 ± 1.69 (43.97)	60.5 ± 2.29 (51.06)	72.1 ± 1.22 (58.12)	82.4 ± 1.970 (65.20)	0.0 ± 0.0 (0.57)	52.1 ± 0.860 (46.20)	68.0 ± 1.52 (55.55)	76.3 ± 0.99 (60.87)	85.4 ± 0.56 (67.54)	0.0 ± 0.0 (0.57)	7.4 ± 1.06 (15.79)	42.6 ± 2.71 (40.40)	69.1 ± 1.23 (56.23)	82.6 ± 0.93 (65.35)
Xanthium strumarium (48h)	0.0 ± 0.0 (0.57)	13.2 ± 1.21 (21.30)	22.1 ± 1.19 (28.04)	65.3 ± 2.87 (53.90)	100.0 ± 0.0 (84.74)	0.0 ± 0.0 (0.57)	54.7 ± 1.52 (47.70)	76.7 ± 1.34 (61.14)	89.8 ± 0.91 (71.37)	98.0 ± 1.19 (81.87)	0.0 ± 0.0 (0.57)	57.8 ± 1.43 (49.49)	84.3 ± 1.30 (66.68)	90.9 ± 0.80 (72.74)	100.0 ± 0.0 (84.74)
Acorus calamus (48 h)	0.0 ± 0.0 (0.57)	82.3 ± 1.11 (65.12)	100.0 ± 0.0 (84.74)	100.0 ± 0.0 (84.74)	100.0 ± 0.0 (84.74)	0.0 ± 0.0 (0.57)	82.0 ± 1.19 (64.90)	100.0 ± 0.0 (84.74)	100.0 ± 0.0 (84.74)	100.0 ± 0.0 (84.74)	0.0 ± 0.0 (0.57)	36.7 ± 2.11 (37.29)	62.6 ± 1.86 (52.30)	78.0 ± 1.52 (62.03)	100.0 ± 0.0 (84.74)
Pongamia pinnata (48 h)	0.0 ± 0.0 (0.57)	50.2 ± 1.45 (45.11)	60.5 ± 2.29 (51.06)	85.0 ± 1.33 (67.21)	100.0 ± 0.0 (84.74)	0.0 ± 0.0 (0.57)	53.4 ± 0.95 (46.95)	68.0 ± 1.52 (55.55)	79.8 ± 1.21 (63.29)	100.0 ± 0.0 (84.74)	0.0 ± 0.0 (0.57)	7.4 ± 1.06 (15.79)	70.0 ± 0.93 (56.79)	80.5 ± 1.81 (63.74)	100.0 ± 0.0 (84.74)

Mean (± SE) of 5 observations; Data in parentheses are arc sine transformed values; PE – plant extract; C- Control.

et al., 1998; Kim and Ahn, 2001; Kim *et al*., 2003). Fumigant activity of *A. calamus* against *Callosobruchus phaseoli* has been reported (Rahman and Schmidt, 1999). Our study demonstrated that *A.calamus* was very effective against all stages of *O. coffeae* (egg, larva, nymph and adult) as ovicidal, adulticidal and growth inhibitory . These results indicated that the mode of delivery of *A. calamus* was largely caused by action in the vapor phase: they may be toxic through penetration via the respiratory system.

Understanding the susceptibility to chemicals for different developmental stages of *O. coffeae* is important for red spider mite control. Differential susceptibility of developmental stages of insects to chemicals has been well described by Harris (1972), Eda (1985) and Choi *et al* (2003). The insensitivity might be attributable to an increase in body weight, a decrease in penetration, or biochemical and physiological changes in insect itself (Harris 1972, Eda 1985). *Trialeurodes vaporariorum* (Mele *et al*., 1992), *Spodoptera litura* and *Dysdercus koenigii* (Gurusubramanian and Krishna, 1996), and *Earias vittella* (Marimuthu *et al*., 1997) eggs were found to be more susceptible to volatiles of natural plant species in an *in vitro* bioassay. In our study, the stage most susceptible to the test fractions of *A. calamus* was both egg and adults but only adults to *X. strumarium* and *P. pinnata*, possibly due to decreased detoxicative metabolism. Therefore, the extract of *A. calamus* should be targeted at both egg and adult stages and *X. strumarium* and *P. pinnata* towards only adult stages of this pest.

All the test fractions of *X. strumarium*, *A. calamus*, and *P. pinnata* were potent especially at higher concentrations(2 and 5%) in relation to killing adult mites (Table 4). Petroleum ether fraction of *A. calamus* and methanol fraction of *X. strumarium* were more potent in reducing the egg hatchability than other two fractions (Table 1) strongly suggesting that most of the active principles were extracted in the aforementioned respective fractions. Prijono *et al* (1997) and Leatemia and Isman (2004b) demonstrated that ethanolic and acetonic extracts of *Annona squamosa* were 6-30 and 11-17 times more potent than crude aqueous seed

Table 5. Two-way analysis of variance for acaricidal activity at different solvent fractions and concentrations of *Xanthium strumarium*, *Acorus calamus* and *Pongamia pinnata* against *Oligonychus coffeae*

Plants	Source	SS	DF	MS	SEM ±	CD (0.05)	CV %
Xanthium strumarium (24 h)	Total	64672.43	74	873.95	-	-	-
	Factor A	4213.67	2	2106.83	1.73	4.08	0.24
	Factor B	56996.08	4	14249.02	1.28	3.02	0.40
	Interaction A x B	2965.18	8	370.64	1.01	2.38	6.12
Xanthium strumarium (48 h)	Total	67376.82	74	4790.53	-	-	-
	Factor A	4310.03	2	2155.01	1.31	3.09	0.18
	Factor B	59071.32	4	14767.83	1.01	2.38	0.31
	Interaction A x B	3686.11	8	460.76	0.80	1.89	4.69
Acorus calamus (24 h)	Total	75605.74	74	1008.07	-	-	-
	Factor A	6006.14	2	3003.07	0.98	2.31	0.11
	Factor B	66628.07	4	16657.01	0.76	1.79	0.19
	Interaction A x B	2795.65	8	349.45	0.60	1.41	2.98
Acorus calamus (48 h)	Total	77431.14	74	1046.36	-	-	-
	Factor A	4576.89	2	2288.44	0.83	1.96	0.09
	Factor B	69514.12	4	17378.53	0.64	1.51	0.16
	Interaction A x B	3214.50	8	401.81	0.51	1.20	2.47
Pongamia pinnata (24 h)	Total	43826.81	74	592.25	-	-	-
	Factor A	1526.20	2	763.10	1.30	3.07	0.21
	Factor B	39940.05	4	9985.01	1.00	2.36	0.35
	Interaction A x B	2055.68	8	256.96	0.79	1.86	5.38
Pongamia pinnata (48 h)	Total	64448.26	74	870.92	-	-	-
	Factor A	537.75	2	268.87	1.73	4.08	0.16
	Factor B	60993.50	4	15248.37	0.76	1.79	0.27
	Interaction A x B	2686.65	8	335.83	0.47	1.11	4.06

Factor A - Between different solvent fractions; Factor B – Between different concentrations;

AxB – Between different solvent fractions and concentrations.

extracts respectively.

Results of this and earlier studies indicate that many plant extracts could be useful as potent candidate for *O. coffeae*. Paneru *et al* (1997), Rahman and Schmidt (1999) and Leatemia and Isman (2004b) found that there were geographical variations in bioactivity of the powder and oil vapours of *A. calamus* and crude seed extracts of *Annona* species respectively. For practical use of these native botanicals as novel phyto-chemicals, further research should be performed on several issues, including geographical variations in bioactivity, safety of these crude plant extracts for human health, changes in the quality of tea treated with crude aqueous extracts (e.g., colour, flavour, odour, brightness, strength and texture), effects on natural enemies such as predatory insects : larvae and adults of St*ethorus gilvifrons, Verania vincta, Jauravia quadrinotata, Scymnus* sp. (Coccinellidae), Staphylinid beetle, *Chrysoperla carnea* (Neuroptera) and predatory mites: *Agistemus hystrix* (stigmaeidae), *Exothorhis caudata* (Eupallopslidae), *Cunaxa* sp. (Cunaxidae) , and formulations for improving the miticidal potency and stability.

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Mean (± SE) with three observations. Ten larvae were used at the start of each test. Percentage values pertaining to larval/nymphal mortality and male/female adult emergence are derived from absolute data obtained for 10 larvae. * Larval-nymphal mortality and adult emergence values are significant at $p<0.05$ (t-test) whereas **larval-nymphal duration and total post embryonic development duration values are not significant at $p<0.05$ (t-test).

ACPE – *Acorus calamus* Petroleum Ether Extract; ACME - *Acorus calamus* Methanol Extract; ACAE - *Acorus calamus* Acetone Extract; XSPE – *Xanthium strumarium* Petroleum Ether Extract; XSME - *Xanthium strumarium* Methanol Extract; XSAE - *Xanthium strumarium* Acetone Extract; PPPE – *Pongamia pinnata* Petroleum Ether Extract; PPME - *Pongamia pinnata* Methanol Extract;
PPAE - *Pongamia pinnata* Acetone Extract; L- Larva; N- Nymph; L-N- Larval –Nymphal; M-Male; F- Female; PED – Post Embryonic Development.