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RESEARCH ARTICLE

BIO-EFFICACY OF *ACORUS CALAMUS* EXTRACTS AGAINST TEA MOSQUITO BUG, *HELOPELTIS THEIVORA* WATERHOUSE

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ABSTRACT

Studies were carried out at Tocklai Tea Research Institute, Jorhat, Assam during 2011-2012 to assess the bioactivity of water, acetone and methanol extracts of *Acorus calamus* in terms of antifeedant, repellent, ovicidal, and field bioefficacy against *Helopeltis theivora.* The results were compared with a neem formulation, Neemazal-F 5% (Azadirachtin5%EC) and Thiamethoxam 25WG. Responses in terms of reduction of feeding spot, number of adult reached to the treated shoots, egg hatchability and reduction of shoot infestation in the field varied according to different solvent extract and concentration. All the extracts exhibited significant bioactivity compared to control. Among all the solvent extracts, acetone extract exhibited the highest bioactivity followed by methanol and water extracts. Effect was concentration dependent. The number of feeding spot was reduced significantly over control to the tune of 62-81% in acetone extract followed by 58-79% in methanol and 46-68% in water extract compared to 74% in Azadirachtin5%EC. All the extracts exhibited 25-52% reduction in egg hatchability and 88-100% repellency at the tested concentrations.

The most effective fraction i.e. acetone extract was further fractionated with silica gel column (60-120 mesh) chromatography by successively eluting with step-wise gradient of different solvents. Fraction III was found to be the most active fraction among those partitioned from the acetone extract and possessed 85.2% antifeedant activity at 0.5% concentration.

The acetone extract exhibited the highest reduction of shoot infestation (52-61%) under field condition followed by methanol (42-55%) and water extracts (30-51%) compared to 50-57% in Azadirachtin5%EC and 87-97% in Thiamethoxam 25WG. So the extract of *Acorus calamus* can be utilized effectively as one of the component of IPM for the management of tea mosquito bug in organic as well as conventional tea plantations.

INTRODUCTION

Tea, *Camellia sinensis* L (O) Kuntze is the most widely consumed non alcoholic beverage all over the world, and regarded as a miraculous drink with numerous rejuvenating properties. In India tea occupies area more than 5.07 lakhs hectares with an annual production of about 1207 million kg made tea of which Assam contributes 610.97 mkg (Sundar, 2014). Insect pests infestation is one of the major constraints of tea production in North East India and Tea Mosquito Bug, *Helopeltis theivora* Waterhouse (Miridae: Heteroptera) is considered as one of the major pests. It causes damage to the harvestable shoots (two leaf and a bud) and remains active from March to November causing severe damage to the tea bushes (Sarmah and Phukan, 2004). Both nymphs and adults cause damage by sucking the sap of the young leaves, buds and tender stems and also by laying eggs on the tender stems, midrib and petioles. Badly affected leaves are deformed and curl up. Use of synthetic pesticides has been the main management approach to combat this pest during recent decades. The outbreak of this pest during recent years is assumed to be the consequences of frequent and indiscriminate use of synthetic chemicals especially pyrethroid group of insecticides by the tea planters. Further over reliance on chemical insecticides lead to various problems like pesticides residue in made tea, environmental pollution, etc. The hazardous effects of synthetic insecticides and stringent regulatory measures necessitate measures for reduction of pesticide load in tea and search for some safer alternatives. Many plant products had been reported to possess good pesticidal properties against various crop pests (Pandey *et al.* 1977, Akhtar and Isman 2004, Sarmah and Bhola 2014). Environmental, economic and social benefits of using botanical pesticides are gaining importance and have been well

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documented. Our interest has been to identify an effective plant having pesticidal properties, which is abundantly available in tea ecosystem that might prove alternative or complimentary to the present strategies.

Acorus calamus L., Araceae is a semi aquatic perennial herb which is growing abundantly as a weed in marshy places in North East India, locally known as "Boch". This herb has been used as traditional Chinese and Indian prescriptions for its beneficial effects on memory disorder, learning performance, and anti-aging and anticholinergic activity. Moreover, *Acorus* rhizome and its main active principle α and β-asarone possess a wide range of pharmacological activities such as sedatives, CNS depressant, anti-inflammatory, antidiarrheal effects (Mukherjee *et al*., 2007). Insecticidal (Chandel *et al*. 2007), antifeedant & repellent (Pandey *et al*. 1977; Yan-Jing *et al*. 2006) and ovicidal (Yasodha and Natarajan 2007) properties of *A. calamus* were well established in mustard, cabbage, radish, brinjal, and cotton pests. . The objective of this study was to explore the insecticidal, ovicidal, antifeedant and repellent properties of *A. calamus* against *H. theivora* and effective utilization of this weed as a natural source for pest management programme.

MATERIALS AND METHODS

Preparation of plant extracts

Preparation of aqueous extract

The rhizome of *Acorus calamus* were collected locally from Jorhat area, Assam during March-April, shade dried and powdered to 20 mesh size. The powdered plant material was mixed separately with distilled water to prepare 2, 4, 6 and 8% water extract.

Preparation of chemical solvent extract

Solvent extract was done by following the method of Sarmah and Bhola, 2014. This extract was then dissolved in methanol to prepare 20 per cent stock solution from which different concentrations ranging from 1 to 4% were prepared in distilled water.

Fractionation of acetone extract through column chromatography

The acetone extract of *Acorus calamus* was fractionated using silica gel column (60-120 mesh) chromatography (Sarmah and Bhola, 2014). Fractions so obtained were dissolved in acetone and 0.5 per cent solution in distilled water was prepared and evaluated to test the biological activity against tea mosquito bug.

Bio-efficacy test

Antifeedant properties of the extract against tea mosquito bug were assessed by following the method of Sarmah and Bhola, 2014**.** Repellent effect of the extract was tested by following the method of Pandey *et al*. 1977 with slight modification. Wooden insect rearing box of size 75cm x 75cm x 45cm was used for this study. Data on the number of adults which have reached treated and control shoots as well as number of feeding spots induced was recorded. Effect of plant extracts on eggs, were assessed by following the method described by Sarmah and Bhola, 2014**.** Each treatment was replicated six times in a completely randomized design. Data were analyzed by using SPSS software for analysis of variance.

Field experiments were conducted at Borbhetta Experimental Tea Estate, TTRI, TRA, Jorhat, Assam, India in Randomized Block Design (RBD) with three replications against tea mosquito bug during 2011-2012. Sixty tea bushes of genotype TV9 (a susceptible Tocklai variety) were selected for the study with a spacing of 105 x 60 cm. Each plot was separated by two rows of tea bushes. Four concentrations (2, 4, 6 and 8%) of aqueous extracts and four concentrations (1, 2, 3 and 4%) of solvent extracts (acetone and methanol) were used to evaluate the efficacy of the extracts. A commercial neem formulation Neemazal-F 5% (Azadirachtin 5%) at 0.0033% concentration, one systemic insecticide Thiomethoxam 25 WG (Actara 25WG) 0.0063% concentration and one untreated control were included for comparison. Bushes were sprayed twice at an interval of seven days with hand operated Knapsak sprayer using NMD/ 60450 nozzle.100g shoots were taken after harvesting from each plot after through mixing; counted the number of infested and healthy shoots and calculated per cent shoot infestation. Pre treatment observation was taken immediately before treatment. Post treatment observations were taken up to 4^{th} week after first application of treatment at weekly interval.

The percent reduction of shoot infestation was calculated by the following formula.

$$\%\ \text{Reduction of shoot infestation} = \frac{\text{Pretreatment infestation} - \text{Post treatment infestation}}{\text{Pretreatment infestation}} \times 100$$

Table 1 Antifeedant activity of solvent extracts of *A. calamus* on adult *Helopeltis theivora*

Treatment	Conc.	Antifeedant activity after 48h: Aqueous		Acetone		Methanol	
		Feeding spot(FS)	% reduction of FS over control	Feeding spot(FS)	% reduction of FS over control	Feeding spot(FS)	% reduction of FS over control
Acorus calamus	1% (2%)*	75.83±2.97	46.15	51.83±3.40	62.35	56.67±2.81	58.83
	2% (4%)*	60.83±2.73	56.80	40.67±2.49	70.45	45.00±2.38	67.31
	3% (6%)*	52.00±3.11	63.07	32.83±2.27	76.15	37.83±2.67	72.52
	4% (8%)*	43.83±2.54	68.87	25.67±2.98	81.35	28.67±2.36	79.17
Neemazal-F 5%	0.0033%	36.50±1.71	74.08	36.50±1.71	73.48	36.50±1.71	73.48
Control	-	140.83±3.1	-	137.67±5.88	-	137.67±5.88	-
C.D. P=0.01		6.92		6.31		6.74	
CV (%)		6.98		7.28		7.36	

( )* = Concentration tested in case of aqueous extracts; Each figure represents the mean number of feeding spots ± SD (n=6)

Table 2 Antifeedant activity of column chromatography fractions of acetone extract of *Acorus calamus* on adult *Helopeltis theivora*

Solvent used	Fractions (0. 5%)	No. of feeding spots after 24 hrs
Hexane	I	-
Ethyl Acetate/Hexane (1: 3)	II	15.2±1.17
Ethyl Acetate/Hexane (1: 1)	III	10.8±1.17
Ethyl Acetate/Hexane (3: 1)	IV	17.2±0.75
Acetone	V	20.2±1.33
Methanol/Acetone (1:3)	VI	25.2±1.33
Methanol/Acetone (1:1)	VII	30.2±1.60
Methanol	VIII	35.4±1.36
Control		72.8±1.72
C.D. P=0.01		2.9

Each figure represents the mean number of feeding spots ± SD (n=6); '–' mark represents fraction not extracted

All the data were subjected to RBD analysis of variance and significant differences between means were determined by comparing critical differences at 5% probability level.

RESULTS AND DISCUSSION

Antifeedant and repellent activity of aqueous, acetone and methanol extracts of *Acorus calamus* against adult tea mosquito bug was significant. The results were presented in table 1-3. Among the extracts acetone extract exhibited the highest antifeedant activity (62-81%) after 48 hours of treatment followed by methanol extract (58-79%) and aqueous extract (46-68%). Neemazol F 5%, a commercial neem formulation @ 0.0033% recorded 73% antifeedant activity which was comparable with 2% extract of acetone & methanol and 4% aqueous extract of *A. calamus* (Table 1). Further fractionation of the most biologically active fraction i.e. acetone extract of *A. calamus* through silica gel column chromatography revealed that the fraction III separated with the solvent ethyl acetate and hexane @ 1:1 ratio exhibited the highest reduction of 85.16% feeding spot after 24 hours of treatment at 0.5% concentration (Table 2). Good repellent activity was exhibited by all the solvent extracts of *A. calamus* and the highest being with

Table 3 Repellent activity of solvent extracts of *A. calamus* on adults *Helopeltis theivora*

Treatment	Conc.	Repellent activity of plant extract: Aqueous		Acetone		Methanol	
		ψAdults reached to the treated shoots	# No. of spots/adult	ψAdults reached to the treated shoots	# No. of spots/adult	ψAdults reached to the treated shoots	# No. of spots/adult
Acorus calamus	1% (2%)*	6.00±1.29	3.75±1.41	2.33±0.47	2.00±0.58	3.50±0.96	4.00±0.76
	2% (4%)*	3.50±1.26	1.75±0.80	0.50±0.50	0.50±0.76	2.83±0.69	2.42±0.67
	3% (6%)*	2.00±0.82	1.50±0.96	0.00±0.00	0.00±0.00	1.67±0.47	1.33±0.62
	4% (8%)*	0.50±0.76	0.50±0.76	0.00±0.00	0.00±0.00	1.00±0.58	0.92±0.73
Neemazal-F 5%	0.0033%	0.00±0.00	0.00±0.00	0.17±0.37	0.00±0.00	0.00±0.00	0.00±0.00
Control	-	38.00±2.38	11.00±2.18	47.00±1.15	10.00±0.96	41.00±1.00	10.17±1.18
C.D. P=0.01		2.08	1.94	0.99	0.96	1.22	1.30

( )* = Concentration tested in case of aqueous extracts; ψ Each figure represents the number of adults reached to the treated shoots ±SD (n= 6) after 6h; # Each figure represents the number of feeding spots ±SD (n= 6) after 6hours.

Table 4 Ovicidal activity of solvent extracts of *A. calamus* against *Helopeltis theivora*

Treatment	Conc.	Aqueous: % egg hatched #	Aqueous: % reduction of egg hatching over control	Acetone: % egg hatched #	Acetone: % reduction of egg hatching over control	Methanol: % egg hatched #	Methanol: % reduction of egg hatching over control
Acorus calamus	1% (2%)*	50.67±2.98	27.61	45.33±4.42	35.24	52.00±5.16	25.71
	2% (4%)*	46.67±3.77	33.32	40.00±3.27	42.85	46.67±2.98	33.32
	3% (6%)*	42.67±4.42	39.04	36.00±3.27	48.57	42.00±3.83	40.00
	4% (8%)*	40.67±3.59	41.90	33.33±3.77	52.38	39.33±3.59	43.81
Neemazal-F 5%	0.0033%	35.33±2.75	49.52	36.00±4.27	48.57	36.00±4.27	48.57
Thiomethoxam 25WG		5.33±1.89	92.38	4.00±3.27	94.28	4.00±3.27	94.28
Control	-	70.00±3.83	-	70.00±3.83	-	70.00±3.83	-
C.D. P=0.01		6.70		7.53		8.44	
CV (%)		9.43		11.90		12.10	

( )* = Concentration tested in case of aqueous extracts; # Each figure represents % egg hatched ±SD (n= 6).

Table 5 Field bio-efficacy of solvent extracts of *A. calamus* against tea mosquito bug

Treatments	Conc. (%)	Aqueous: Pre treatment (%) shoot inf.	Aqueous % reduction of shoot infestation over pre treatment shoot infestation: I Spray 7Days	I Spray 14Days	II Spray 7Days	II Spray 14Days	Acetone: Pre treatment (%) shoot inf.	Acetone % reduction of shoot infestation over pre treatment shoot infestation: I Spray 7Days	I Spray 14Days	II Spray 7Days	II Spray 14Days	Methanol: Pre treatment (%) shoot inf.	Methanol % reduction of shoot infestation over pre treatment shoot infestation: I Spray 7Days	I Spray 14Days	II Spray 7Days	II Spray 14Days
Acorus calamus	1 (2)*	54.48	33.15	35.20	32.08	30.24	55.17	53.32	55.19	54.19	52.41	53.64	43.25	47.18	45.33	41.98
	2 (4)*	55.13	42.30	43.44	41.68	40.14	53.64	55.25	57.12	56.18	54.23	52.09	48.16	51.00	50.23	45.21
	3 (6)*	56.88	45.49	46.53	44.62	42.42	53.78	56.39	59.55	58.12	55.16	53.50	53.06	54.13	53.27	50.39
	4 (8)*	55.08	50.21	51.14	49.16	48.22	52.35	59.17	61.79	60.11	57.15	54.42	54.06	55.43	55.09	52.33
Neemazal-F 5%	0.0033	55.50	55.85	57.20	56.30	50.91	55.50	55.85	57.20	56.30	50.91	55.50	55.85	56.30	57.20	50.91
Thiomethoxam 25 WG	0.0063	55.25	89.53	96.18	97.59	87.33	55.61	89.53	96.18	97.59	87.33	55.61	89.53	97.59	96.18	87.33
Control (untreated)	-	54.80	1.58	0.65	-2.62	-1.49	54.80	1.58	0.65	-2.62	-1.49	54.80	1.58	-2.62	0.65	-1.49
S.Em ±			1.31	1.07	1.13	1.12		1.53	0.42	1.36	1.05		1.30	1.33	1.35	1.26
CD(P=0.05)			3.81	3.12	3.28	3.27		4.44	1.22	3.94	3.04		3.77	3.86	3.92	3.66

( )* = Concentration tested in case of aqueous extracts; Each figure is the mean of three replications acetone extract (95-100%) closely followed by methanol (93-98%) and aqueous extract (88-99%). However, neem formulation (Neemazol F5%) exhibited 100% repellency (Table 3).

Tewari and Moorthy (1985) reports from India that petroleum ether extracts of the rhizome of *Acorus calamus* at 0.1% concentration exhibit 100% protection of leaves of solanaceous and cucurbitecious vegetables against *Henosepilachna vigintioctopunctata*. Solvent extracts of *Pogostemon parviflorus, Pongamia glabra* and *Annona squamosa* leaf also exhibit good antifeedant activity against *Helopeltis theivora.*(Gogoi *et al*. 2003). Significant repellent action of pure ethanol extract of *Acorus calamus* is being observed by Yan-Jing *et al.* (2006) against stored-grain insects.

Hatching of eggs of tea mosquito bug was significantly reduced when exposed to different solvent extracts (Table 4). The highest ovicidal activity was exhibited by the acetone extract (35-52%) followed by methanol (25-43%) and aqueous (27-41%) extracts compared to 49% in Azadirachtin5%EC. The ovicidal activity of acetone extracts of *A. calamus* at 2-4% and methanol extract at 4% concentration were statistically at par with Azadirachtin5%EC. However, Thiamethoxam 25 WG, a systemic neonicotenoid insecticide exhibited the highest ovicidal activity of 92-94% at 0.00625% concentration. Present results are in agreement with the results of Roy *et al.* 2009 and earlier findings of Sarmah and Bhola, 2014 where they report that different solvent extracts of *Xanthium strumarium* and *Clerodendron infortunatum* possess significant ovicidal properties against tea mosquito bug.

All the solvent extracts of *A. calamus* exhibited significant reduction of tea mosquito bug infestation in the field and comparable with Azadirachtin 5%EC (Table 5). However Thiamethoxam 25WG treatment was found to be significantly superior among all the treatments. Acetone extracts exhibited the highest reduction of tea mosquito bug infestation (52.4-61.8%) followed by methanol (41.2-55.4%) and aqueous (30.2-51.1%) extracts (Table 5). Azadirachtin 5%EC (used as standard) showed 50.9-57.2% reduction of tea mosquito bug infestation and comparable with 2-4% concentration of methanol, 1-4% concentration of acetone and 8% concentration of aqueous extract. However, Thiamethoxam 25WG, a systemic insecticide exhibited 87.3-97.6% reduction of infestation and found to be significantly superior to all other treatments. Similarly Deka *et al.,* 2000 and Sarmah and Bhola, 2014 reported the effectiveness of different solvent extracts of *Clerodendrum inerme, Pongamia pinnata* and *Xanthium strumarium* against *Helopeltis theivora* under field condition. Effectiveness of Plant extracts under field conditions have also been reported by Sakthivel *et al*., 2007 against sucking pests of okra.

Thus the present study clearly indicates the significant bioactivity of different solvent extracts of *A. calamus* against tea mosquito bug and the extracts can be effectively utilized for the management of the same specially in the organic tea cultivation and under the umbrella of IPM.

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