Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

ISSN 2320-7078
JEZS 2014; 2 (4): 221-226

Received: 30-06-2014
Accepted: 18-07-2014

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Assessment of repellency and insecticidal activity of *Ajuga parviflora* (Benth) and *Trichilia connaroides* (W&A) leaf extracts against stored product insects.

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Abstract
Repellency behavior and insecticidal activities of plant extracts of *Ajuga parviflora* and *Trichilia connaroides* were studied against stored product insects viz., the lesser grain borer *Rhyzopertha dominica* (F.), and the red flour beetle *Tribolium castaneum* (Herbst). The area preference method and dose-mortality was used to determine the repellency rate and insecticidal activity. The average repellency of the methanol, petroleum ether and ethyl acetate extract concentrate per cm^2 of both plant leaves totally achieved class II, III, class IV and class V repellency respectively at different intervals (2, 4, 8 hours) of time. The methanolic extract of *A. parviflora* and *T. connaroides* showed maximum repellency behavior compared to other solvent systems. This extract was also found to be more effective than the commercial product teflubenzuron (No: SZBB291XV) and also more effective than other solvent extracts mentioned above. Our results indicate that the repellency behavior was dose and extract dependent and dose-mortality of the methanolic extract varied depending on the insect species. Moreover, *T. castaneum* was susceptible to methanolic extract of *A. parviflora* and *R. dominica* was more susceptible to *T. connaroides* extract.

Keywords: repellency, mortality, *Ajuga parviflora, Trichilia connaroides, Rhyzopertha dominica, Tribolium castaneum*, Teflubenzuron.

1. Introduction
Commodities like cereal grains and pulses make up the major component of the world food supply. After harvest, these commodities are stored in large commercial elevators, where it can be invaded and infested by stored product pests and deteriorated by microorganisms. Although synthetic chemical pesticides have been commonly used to reduce these losses, there is a great risk of negative effects, the ecological consequences, environmental pollution [27], serious public health hazards and pesticide residues in food [4]. The continuous application and dependence on chemical pesticides has also resulted in potential toxicity hazards for non target organisms and users [2]. Furthermore, the continuous use of chemical pesticides for control on stored product pest has led to serious problems such as insecticide resistance [13]. To pull out the problems due to use of chemical pesticides, plant kingdom is thought to be potent against insect pests and become viable component of Integrated Pest Management (IPM).
In recent years, studies have been focused on plant materials and their bioactive chemical constituents as a rich source of natural substances which can be used to develop ecologically safer methods for insect control [14]. The main advantage of plant materials is that they are potentially less expensive, locally available, biodegradable, and sustainable; they can propagate easily, have no negative effects on the ecosystem [7]. Insecticidal activity of many plants against insect pests has been well documented by Jilani and Su, Susana *et al.* [6, 23]. The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity [26, 18], mortality [9], repellency [10, 28, 3, 5], anti-feedant, growth inhibitor, suppression of reproductive behavior and reduction of fertility and fecundity [17] on stored product insects. Natural plant products containing biodegradable compounds repel or inhibit insect feeding, and this property is highly species specific. Repellents are generally advantageous being specific and have low mammalian toxicity. Among the medicinal plants, several locally available species such as *Ambrosia tenuifolia* have been reported to be repellent and toxic to *Tribolium castaneum* [23].

There is an urgent need for new alternative approaches to control stored product insects by eco-friendly organic sources that are easily available, affordable, less toxic to mammals and less detrimental to the environment. The aim of this paper is to evaluate the repellent and insecticidal activity of *Ajuga parviflora* (Benth) (Lamiaceae) *and Trichilia connaroides* (W & A.) (Meliaceae). The different solvent extracts of *A. parviflora* and *T. connaroides* leaves were assessed against the lesser grain borer *Rhyzopertha dominica* (F.) and the red flour beetle *Tribolium castaneum* (Herbst) and results were compared with the synthetic repellent teflubenzuron.

2. Materials and Methods

Experiments on the efficacy of leaf extracts of *A. parviflora* and *T. connaroides* were carried out during October-December-2013. For this purpose *R. dominica*, and *T. castaneum* insect cultures were maintained in the growth chamber in the laboratory at a temperature of 27±2 ^{0}C, 12:12 (L:D) h and with 70±5% RH. *A. parviflora* (Lamiaceae) is a genus of about 40–50 species of annual and perennial herbaceous with most species native to Europe, Asia, and Africa, growing in open fields throughout sub-tropical and temperate regions. *T. connaroides* (Meliaceae) is commonly found along margins and openings of evergreen to semi-evergreen forests up to 2000 m. Distributed in Indo-Malaysia region; in the Western Ghats, India and is also endemic to Nepal.

2.1 Collection of plant leaves

Fresh plant leaves of *A. parviflora* were collected from Institute of Himalayan Bio-resource Technology campus Palampur, Himachal Pradesh, and *T. connaroides* from Indian Council of Medical Research, Regional Centre, Belgaum campus, Karnataka, India during August-October 2013. 500 g of *A. parviflora* and *T. connaroides* leaves were washed and air dried in the shade for 2 days and lyophilized. Further dried leaves were ground to powder using electric grinder. The resulting powder was passed through a 25-mesh sieve to obtain a fine powder. This powder was soaked in 1 liter of different solvents viz., methanol (polar), petroleum ether (60-80 ºC) (non polar) and ethyl acetate (medium polar) and stirred for 30 minutes using a magnetic stirrer and then filtered through Whatman No. 1 filter paper [21] . The solvents from the pooled filtrate were concentrated to dryness using a rotary flash evaporator at 50±5 ^{0}C. The yield of final crude extracts was recorded (6.2 gms from *A. parviflora*, 3.8 gms from *T. connaroides*), and preserved in sealed bottles in a refrigerator at 5 ^{0}C until used for insect bioassays. Sample specimens of these plants were deposited in the form of herbarium in the Food Protectants and Infestation Control Department, CSIR-Central Food Technological and Research Institute under the following codes *A. parviflora* P003 and *T. connaroides* B004.

2.2 Rearing of test insects

R. dominica and *T. castaneum* were reared for the present study. A small population of these insects was obtained from the entomology laboratory stock, Food Protectants and Infestation Control Department, CSIR-CFTRI, Mysore. The insects were cultured on whole wheat and wheat flour inside a growth chamber at 27 ± 2 ^{0}C, 12:12 L:D h and with 70 ± 5% RH [21] .

50 pairs of 1-2 day old adults were placed in a jar containing 250 gms of food media in glass containers covered by muslin cloth. Maximum of 7 days were allowed for mating and oviposition. Parent stocks were then removed and food media containing eggs were incubated in temperature/humidity controlled cabinet (27 ± 2 ^{0}C and RH 70 ± 5%) in darkness to obtain same aged insects [21]. Thus, subsequent progenies (7 days old) of the insects were used for all experiments.

2.3 Repellency bioassay

Repellency assay was carried out using area preference method described by Tapondjou *et al.* [25]. Test solutions were prepared by dissolving different volumes of the plant leaf extract with acetone (0.02 µl, 0.04 µl, 0.08 µl of the extracts per cm^2). Each Whatman filter paper circle (18 cm in diameter) was cut into two halves to fit into glass petri dish, (18 cm in diameter) and each plant extract was applied to a half filter paper as uniform as achievable using a pipette. The other half was treated only with acetone and it served as control. The plant-extract-treated and acetone-treated halves were air-dried to evaporate the solvent completely for 10 minutes. Each treated strip was attached lengthwise, edge to edge with cellophane tape. A filter paper was placed on the bottom of the glass petri-dish. Thirty unsexed adult insects of *T. castaneum* (7 days old) and *R. dominica* (7 days old) were released separately at the center of each filter paper disc. The petri dishes were covered and placed in dark at 27 ± 2 ^{0}C with relative humidity of 70 ± 5%. The number of *T. castaneum* on treated and untreated portions of the experimental paper halves were counted for each dish after 2, 4, 8 hours exposure. Percentage repellency (PR) for a given treatment time was obtained using the formula: PR = [(Nc-Nt) / (Nc+Nt)] ×100, where Nc= the number of insects in the untreated (control) and Nt = treated areas, respectively with positive values of PR indicate attractancy [8]. Along with this standard repellent teflubenzuron from Sigma-Aldrich batch no: SZBB291XV was used as a positive control [22] utilizing the same experimental conditions as the extracts. The averages were then assessed to different class using the following scale. Percent repulsion >0.01 to<0.1= class 0; 0.1-20 = class I; 20.1-40 = class II; 40.1 to 60 = class III; 60.1to 80=class IV; 80.1-100= class V (Mc Govern *et al.* 1977) [11]. Five replicates were performed for each test concentration of plant leaf extract and also for positive control.

2.4 Mortality bioassay

For percentage mortality calculation, different concentrations (4%, 8% and 12%) of residue obtained by methanol extraction of the leaf were prepared using acetone. Solution of teflubenzuron standard was also prepared by dissolving in acetone at the same concentration. Both the solutions were applied on to the Whatman No 1 filter circle in each of the petri-plate (6 cm diameter) and allowed to stand for about 10 minutes in a hood for the solvent to evaporate. Control was prepared by treating the filter paper with acetone only. 30 unsexed insects (7 days old *T. castaneum* and 7 days old *R. dominica*) were released separately into each treated and control petri-plate. Dead insects were counted after 24 hours of exposure. The mortality (%) was calculated in the treated filter papers and compared with control.

2.5 Statistical analysis

The mean number and standard deviation of insects on the treated and untreated area of the filter paper was calculated for average repellency rate. The Duncan's Multiple Range Test (DMRT) was used to compare percentage of mortality and mean repellency at different intervals of time using SPSS-10 software ($p<0.05$).

3. Results and Discussion

The results revealed a definite impact of plant extracts obtained using different solvents on the overall repellency and percentage mortality of the test insect. The results of repellency rate assays of different extracts at different concentrations and time are presented in Table 1 and 2. The methanol extracts of *A. parviflora* and *T. connaroides* leaves exhibited strong repellent effect on both *R. dominica* and *T. castaneum* at all the doses showing above class III at different intervals compared to the other solvent extracts. However, high repellency effect of *A. parviflora* to *T. connaroides*. Percentage and rate of repellency was found to vary with the type of extract and also with the insect species used in the bioassay. Among all the treatments tested, 0.080 μg cm^{-2} concentration of methanolic *A. parviflora* extract exhibited the strongest repellent effect on *T. castaneum* with the mean repellency of 81.5 (Table 1) i.e. class V and *R. dominica* showed 70.6 (Table 2) i.e. class IV repellency. The Ethyl acetate extract of *A. parviflora* and *T. connaroides* leaf extracts exhibited least repellent activity against *T. castaneum* and *R. dominica* exhibiting class III and class II while petroleum ether recorded moderate repellency rate with class III and IV as presented in Table 1 and 2.

Table 1: Percentage and Repellency class of *Ajuga parviflora* leaf extracts on stored product insects

Insect species	Solvent	Concentration of extract μgcm^{-2}	*Average repellency (%) after treatment (hours)			Mean	Repellency Class
			2	4	8		
T. castaneum	Methanol	0.020	69.3 ± 1.8^a	71.4 ± 2.3^b	75.3 ± 3.2^c	72.0	IV
		0.040	72.4 ± 2.6^a	83.3 ± 2.4^b	86.2 ± 3.4^c	80.6	V
		0.080	77.4 ± 2.7^a	79.4 ± 2.8^b	87.7 ± 3.2^c	81.5	V
	Petroleum ether	0.020	50.8 ± 3.8^a	53.2 ± 4.1^b	60.4 ± 5.2^c	54.8	III
		0.040	63.2 ± 2.9^a	66.3 ± 3.7^b	68.2 ± 6.8^b	65.9	IV
		0.080	69.1 ± 3.9^a	68.4 ± 4.1^b	69.4 ± 4.4^b	68.9	IV
	Ethyl acetate	0.020	21.4 ± 4.2^a	29.2 ± 3.6^b	38.6 ± 3.5^c	29.7	II
		0.040	28.4 ± 4.6^a	34.3 ± 4.2^b	39.4 ± 3.3^c	34.0	II
		0.080	36.4 ± 4.4^a	40.4 ± 4.1^b	42.3 ± 4.0^b	39.7	II
R. dominica	Methanol	0.020	50.1 ± 2.6^a	54.4 ± 4.7^b	90.3 ± 3.2^c	64.9	IV
		0.040	62.4 ± 2.9^a	66.1 ± 4.2^b	51.3 ± 3.1^c	59.9	III
		0.080	74.1 ± 4.6^a	79.4 ± 4.1^b	58.4 ± 2.8^c	70.6	IV
	Petroleum ether	0.020	44.4 ± 3.2^a	48.8 ± 3.3^b	69.4 ± 2.3^c	54.2	IV
		0.040	52.7 ± 3.9^a	54.1 ± 3.2^b	35.4 ± 5.6^c	47.4	III
		0.080	60.4 ± 3.5^a	62.3 ± 3.1^b	69.4 ± 2.3^c	64.0	IV
	Ethyl acetate	0.020	26.1 ± 6.2^a	29.7 ± 5.7^b	35.4 ± 5.6^c	30.4	II
		0.040	38.1 ± 5.1^a	42.4 ± 5.4^b	47.2 ± 6.2^c	42.5	III
		0.080	41.1 ± 4.3^a	44.2 ± 5.8^b	48.1 ± 5.3^c	44.4	III

values are averages of five replicates and mean ± SD; Means within same rows followed by same letter aren't significantly different ($p<0.05$)

Table 2: Percentage and Repellency class of *Trichilia connaroides* leaf extracts on stored product insects

Insect species	Solvent used	Concentration of extract μgcm^{-2}	*Average repellency (%) after treatment (hours)			Mean	Repellency class
			2	4	8		
T. castaneum	Methanol	0.020	44.4 ± 2.4^a	48.6 ± 3.4^b	50.8 ± 4.1^b	47.9	III
		0.040	49.1 ± 2.6^a	54.4 ± 3.3^b	58.1 ± 4.5^c	53.8	III
		0.080	60.4 ± 3.2^a	62.5 ± 3.1^b	63.4 ± 3.9^b	62.1	IV
	Petroleum ether	0.020	39.3 ± 1.3^a	40.1 ± 1.4^a	41.2 ± 1.8^a	40.2	III
		0.040	41.4 ± 1.8^a	42.4 ± 1.9^a	42.3 ± 2.1^a	42.0	III
		0.080	51.2 ± 2.4^a	53.1 ± 2.6^b	54.4 ± 2.4^b	52.9	III
	Ethyl acetate	0.020	44.9 ± 4.1^a	43.8 ± 2.8^a	46.3 ± 2.3^c	45.0	III
		0.040	48.4 ± 5.1^a	47.1 ± 3.9^b	49.1 ± 2.9^c	48.2	III
		0.080	41.3 ± 6.1^a	50.3 ± 4.4^b	52.4 ± 3.4^b	48.0	III
R. dominica	Methanol	0.020	61.8 ± 3.3^a	71.3 ± 3.2^b	75.3 ± 4.6^c	69.4	IV
		0.040	75.2 ± 4.5^a	80.2 ± 3.1^b	89.1 ± 4.8^c	81.6	V
		0.080	90.1 ± 2.4^a	94.2 ± 4.8^b	96.4 ± 4.1^b	93.5	V
	Petroleum ether	0.020	26.1 ± 3.1^a	24.3 ± 2.8^b	23.4 ± 2.9^b	24.6	II
		0.040	40.1 ± 3.2^a	46.7 ± 2.9^b	39.7 ± 1.9^{ac}	42.1	II
		0.080	41.2 ± 4.1^a	46.3 ± 3.6^b	48.5 ± 3.9^b	45.3	III
	Ethyl acetate	0.020	40.6 ± 4.6^a	42.3 ± 3.2^b	41.4 ± 4.$^{a b}$	41.4	III
		0.040	43.4 ± 4.3^a	45.6 ± 5.2^b	48.5 ± 5.8^c	45.8	III
		0.080	50.1 ± 2.3^a	55.4 ± 4.1^b	56.4 ± 3.0^c	53.9	III

values are averages of five replicates and mean ± SD; Means within same rows followed by same letter aren't significantly different ($p<0.05$)

The results of the repellency rate of tested plant methanolic extracts at higher dose and longer hours showed that *T.*

castaneum was very sensitive to *A. parviflora* with mean 81.5; class V (Table 1) than *T. connaroides* with mean 62.1; Class IV (Table 2). This is in contrast with *R. dominica* (mean value 70.6; class IV Table 1) from *A. parviflora* when compared to *T. connaroides* with a mean value of 93.5; class V as given in Table 2.

Standard repellent teflubenzuron was less effective with class III repellency, and the mean repellency was 50.3 to 59.0 for *T. castaneum* and 56.8 to 65.4 at 0.020 µl/cm^2, 0.040 µl/cm^2 and 0.080 µl/cm^2 for *R. dominica* with class IV, but not class V repellency (Table 3). These results indicated that repellency depends upon the dosage and time and also upon different solvents used for preparing extracts.

Table 3: Percentage and repellency class of Teflubenzuron on stored product insects

Insect species	Concentration of extract µgcm^{-2}	*Average repellency (%) after treatment (hours)			Mean	Repellency Class
		2	4	8		
T. castaneum	0.020	44.3 ± 5.1^a	46.4 ± 5.1^b	60.3 ± 3.1^c	50.3	III
	0.040	48.4 ± 6.1^a	47.2 ± 4.1^b	62.3 ± 3.9^c	52.6	III
	0.080	54.3 ± 3.4^a	56.3 ± 2.1^b	66.4 ± 4.1^c	59.0	III
R. dominica	0.020	56.8 ± 2.3^a	59.3 ± 2.8^b	65.4 ± 2.9^c	60.5	IV
	0.040	60.8 ± 4.1^a	62.3 ± 3.9^b	67.1± 3.3^c	63.4	IV
	0.080	68.7 ± 4.9^a	71.1 ± 4.7^b	79.4 ± 4.2^c	73.0	IV

values are averages of five replicates and mean ± SD; Means within same rows followed by same letter aren't significantly different (p<0.05)

The effect of percentage mortality along with standard deviation with various concentrations of methanolic leaf extracts on adult *T. castaneum* and *R. dominica* in comparison with teflubenzuron is given in Table 4. Our results showed that increase in concentration tended to affect the percentage mortality. There was an increase in mortality rate (12%>08%>04%) compared to the control. Methanolic extract of *A. parviflora* showed high mortality of 12.8% at higher dose on adult *T. castaneum* and lower mortality of 9.6% in *R. dominica* (Table 4). These results were in contrast to those obtained in methanolic extracts of T. connaroides for *R. dominica*: 11.4% when compared with *T. castaneum*: 7.21%. Further it was found that percentage mortality of insects due to teflubenzuron was moderate among both *T. castaneum* and *R. dominica* with a mean value of 5.21% and 7.82% respectively at higher dosage.

Table 4: Percentage mortality of plant extracts and Teflubenzuron on stored product insects

Percentage Mortality ± SD						
	Ajuga parviflora		*Trichilia connaroides*		Teflubenzuron	
Conc	*T. castaneum*	*R. dominica*	*T. castaneum*	*R. dominica*	*T. castaneum*	*R. dominica*
4%	7.01 ± 0.86^b	3.20 ± 0.83^b	2.66 ± 0.89^b	4.03 ± 1.58^b	2.60 ± 1.51^b	3.10 ± 0.83^b
8%	10.4 ± 1.14^c	6.8 ± 1.30^c	4.41 ± 1.14^c	6.60 ± 2.30^c	3.40 ± 1.14^b	5.60 ± 1.51^c
12%	12.8 ± 1.78^d	9.6 ± 1.78^b	7.21 ± 1.48^d	11.4 ± 1.14^d	5.21 ± 1.09^c	7.82 ± 1.30^d
Control	0.20 ± 0.44^a	0.41 ± 0.54^a	0.10 ± 0.44^a	0.26 ± 0.54^a	0.41 ± 0.54^a	0.21 ± 0.45^a
F-value	152.7	59.1	35.9	47.5	37.8	15.8

values are averages of five replicates and mean ± SD; Means within same column followed by same letter aren't significantly different (p<0.05)

Currently, management of stored product pests using materials of natural origin is the subject of matter which has received much attention because of their low mammalian toxicity and less environmental hazard [15]. At present, botanicals constitute 1% of world insecticide market, despite the knowledge that plants constitute a rich source of bioactive chemicals and provide alternatives to regular insect control agents [9]. Several species from various plant families have been tested for their insecticidal potency [20, 1]. The present work revealed the effective repellent activity of three solvent extracts of *A. parviflora and T. connaroides* leaves along with a standard repellent Teflubenzuron. Significant repellent activity against *T. castaneum, R. dominica* adults was observed with crude methanol extract of *A. parviflora and T. connaroides* leaves, followed by petroleum ether and ethyl acetate as given in Table 1.

Effect of methanolic extract dose was found to be directly proportional to the time taken for the repellent behavior in the two beetles. This is in contrast to the results for petroleum ether and ethyl acetate and also for teflubenzuron standard repellent by Saeideh [22], where the repellent activity was uneven in time, dose and also repellency class was lower than the methanolic extracts. However, our findings suggest that there may be different compounds in different solvent extracts possessing different bioactivities. Previous work by Talukder and Howse [24] on repellent effect of different solvent extracts of Pitraj seed on *T. castaneum* showed that the acetone extract exhibited 88% and 93% repellency at 0.5% and 1% concentrations respectively. Our result is in contrast to the work of Jilani [6] who reported that petroleum ether extract of neem leaf acted as repellent to *T. castaneum.* Interestingly, repellent properties of the methanolic isolates were better than the standard repellent teflubenzuron, which showed modest repellency rate with class III and IV, but not class V for any of the stored-product insects which is in contrast to the work of Saeideh [22]. Further repellency behavior was more in case of *R. dominica* than *T. castaneum* when exposed to teflubenzuron at different intervals of time and dose. These findings receive support from the result of Karina Caballero-Gallardo *et al.* [8].

Based on the present findings, the tested plant extracts indicate potential in controlling and chasing stored product pests. These studies provide an interesting opportunity to develop bio-insecticides and repellent formulations based on the extracts from lesser known plants. Although this study has verified the scientific principle for use of *A. parviflora* and *T. connaroides* in controlling stored product insects, further research on mechanism of action of bioactive compounds extracted from above plants is necessary.

4. Conclusion

Assessment of repellent behavior by area preference method can be used for preliminary screening of plant products for their insecticidal activity on stored product insects. This will help to save time in identifying the insecticidal activity of certain endemic plants and their products. This should encourage the breeding or selection of plant varieties which exhibit the insecticidal activity. Our results suggest that extract of selected plants have potential for repellent behavior and insecticidal property. Our study also indicates that repellent behavior and mortality of stored product insects depended on several factors including plant species used, constituents of extracts, application rate and exposure time. Considering the side effects of synthetic pesticides, the present study showed that plant extracts which are eco and user friendly, play an important role in protection of storage commodities. Therefore, these extracts may be potential candidates for their use in the formulation of commercial repellents and insecticides that serve as effective control option, in the management of stored product insects responsible for huge loss of food commodities during storage.

5. Acknowledgement

The authors are grateful to Prof. Ram Rajasekharan Director, CSIR-CFTRI for the facilities and encouragement. The authors are also thankful to the Department of Science and Technology, SERB, New Delhi, India for financial support and fellowship to GBR.

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