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Antifeedant effect of crude extracts prepared from four plants on a household pest, the rubber plantation litter beetle, *Luprops tristis* Fabricius (Tenebrionidae: Coleoptera)

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Abstract Antifeedant (AF) activities of crude extracts from *Cymbopogon citratus, Clerodendron infortunatum, Gliricidia sepium,* and *Zingiber officinale* were studied against different developmental stages (pre dormancy adult stage, 4th instar larval stage and post dormancy adult stage) of a nuisance rubber plantation litter beetle, *Luprops tristis* . The methanol, petroleum ether and aqueous extracts of each plant at 0.005, 0.01, 0.02, 0.04 and 0.08 mg/ml (w/v) were used in this study. Bioassays were conducted using the leaf disc no-choice method. As an antifeedant *C. infortunatum* proved to be the most potent against all developmental stages of *L.tristis* with AF% between 90-95 at 0.08 mg/ml and between 80-85 at 0.04 mg/ml. *G. sepium* caused significant feeding reduction at the highest dosage (AF% between 60-65 at 0.08 mg/ml). *C. citratus,* and *Z. officinale* did not significantly reduce consumption at any dosage. There was a significant long-linear dosage response effect of increasing dosage with decreasing consumption for all extracts ($p<0.05$).

Key words: Antifeedant effect, crude extracts, *Luprops tristis, Cymbopogon citratus, Clerodendron infortunatum, Gliricidia sepium, Zingiber officinale*

Introduction

Household insects are a part of the total complex of pests that are of direct concern to man and his immediate environment. As people have improved their homes, they have unwittingly made them increasingly favorable environments for insects. *Luprops tristis*, the rubber plantation litter beetle, is a potential household pest for farming communities in the rubber plantation tracts of Kerala. Their massive seasonal invasion into residential buildings makes them the most dreaded beetles to people living in the vicinity of rubber plantations. The continued presence and attraction of these beetles towards light,

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following overnight invasion into buildings is a frustrating nuisance for local people. Clusters of several hundreds to thousands crawl into the living rooms and fall off into beds and food from ceilings, and when disturbed, they release an irritating odoriferous phenolic secretion that causes burn to the skin (Sabu *et al.,* 2008) It becomes a pest of man as it interferes with his welfare and convenience. The invasion and aggregation of this beetles causes considerable annoyance to most householders along rubber plantation belts. Because of their adaptable nature they are one of the more difficult pest to control. On this account alone, measures for their suppression are always worthwhile.

Fifty years of sustained struggle against harmful insects using synthetic and oil-derivative molecules has produced perverse secondary effects. The diversification of the approaches inherent in IPM is necessary for better environmental protection (Regnault-Roger, 1997). Although effective synthetic insecticides are available, there is global concern about their negative effects such as development of resistance by insect species, pest resurgence, residual toxicity, environmental pollution, toxicity to non target organisms and increasing cost of application of presently used synthetic pesticides (Talukdar *et al.,* 2000; Soon *et al.,* 2001; Kostyukovsky *et al.,* 2002; ogendo *et al.,* 2003; Rahman *et al.,* 2006; Haridasan and Gokuldas, 2009; Govindarajan *et al.,* 2011; Pavela, 2011). This awareness has created worldwide interest in the development of alternative strategies, including the re-examination of using plant derivatives against important insect pests. Terrestrial plants produce a bewildering array of natural products-terpenoids, phenolics, alkaloids-likely exceeding 100,000 novel chemical structures that could be exploited for the discovery of new insecticides or for novel structures that could serve as lead compounds in insecticide development (Isman and Akhtar, 2007). Pesticides of plant origin are gaining increased attention and interest among those concerned with environment friendly, safe and integrated pest management approaches. Plant-derived materials are more readily biodegradable. They may be easily and cheaply produced by farmers and small-scale industries as crude, or partially purified extracts. (Shaaya *et al.,* 1997; Keita *et al.,* 2001; Tapondjou *et al.,* 2002; Valsala and Gokuldas, 2004; Baskar and Ignacimuthu, 2012).

The role of plant allelochemicals in plant herbivore interaction is well known. Although some of these phytochemicals act as phago stimulants, the majority of allelochemicals examined appear to function primarly in plant defense, acting as insect antifeedants, growth regulators, or toxins (Jermy, 1966; Bernay and Chapman, 1977). In view of the ecotoxicity of synthetic insecticides, antifeedants offer considerable promise as components of emerging integrated pest management (IPM) due to their capacity to reduce feeding by insects (Kumari *et al.,* 2003). Reduction or complete inhibition of

feeding using organic derivatives, crude plant extracts and pure allelochemicals as antifeedants has been demonstrated in several orders such as Lepidoptera, Coleoptera, Hemiptera and Orthoptera (Andres and John, 2011). Therefore, antifeedants constitute a useful element for integrated pest management strategies because they can prevent insect herbivory by making the food less palatable. Furthermore, antifeedants are usually safer alternative to deter insects than conventional synthetic pesticides owing to their low toxicity, specificity, effectiveness at small concentration and lack of impact on non target organisms.

The present study has, therefore been undertaken to study the antifeedant effect of four plants against rubber plantation litter beetle, *Luprops tristis* under laboratory conditions, so that information thus gathered may be utilized for the management of this pest under field conditions.

Materials and methods

Insect collection

The different life cycle stages of *Luprops tristis*, were used for antifeedant bioassay experiment. All stages were maintained at optimum conditions of temperature (27$\pm$ 0.5^{o}C) and relative humidity (70$\pm$5%) in clay vessels half filled with soil and litter collected from rubber plantations.

Preparation of test extracts

Plant materials used to prepare extracts for assaying antifeedant bioactivities against different life cycle stages of *Luprops tristis*, are presented in Table 1. Fresh leaves or rhizome of the four plants were collected locally during October-December, washed and air dried in shade for 7 days. Dried leaves/rhizome were pulverised using an electric grinder. The powdered materials were then sealed in plastic jars and stored at 4^{o}C. Extracts of each powdered materials were prepared in different solvents (methanol, petroleum ether and water). Fifty grams each of the powered plant materials were mixed with 200 ml of solvents taken in a conical flask and the mixture was agitated on an automatic shaker for 24 h at room temperature keeping the flask tightly covered. The extract was filtered through Whatman No. 1 filter paper by negative pressure using a Buchner funnel and a suction pump. The filtrates were allowed to dry in a hot air oven maintained at 40°C. The weight of the dried residue was determined. After ascertaining the final weight of the residue, 10% stock solution was prepared in appropriate solvents. Required concentrations (0.005, 0.01, 0.02, 0.04, 0.08 mg/ml) of each extracts were

prepared from stock solution by diluting with the respective solvents and were stored in air-tight glass containers.

Table 1. Plant materials used for assaying antifeedant effect against different life cycle stages of *Luprops tristis*

Scientific name	Family	Tissue used	Yield (%)		
			A	B	C
Clerodendron infortunatum	Verbenaceae	Leaves	4.40	1.28	1.18
Gliricidia sepium	Papilionaceae	Leaves	8.70	3.34	2.46
Cymbopogon citratus	Graminaceae	Leaves	4.90	1.42	1.24
Zingiber officinale	Zingiberaceae	Rhizome	3.64	1.38	1.20

Methanolic extract, B) Petroleum ether extract, C) Water extract
(Yield (%) =Dry weight of extract ÷ Dry weight of test plant × 100)

Feeding deterrence bioassay

Experiments to evaluate the feeding deterrent effects of extracts in methanol, petroleum ether and water of four plants on different developmental stages of *Luprops tristis* were done in the laboratory by feeding deterrence test using wilted tender rubber leaf discs of *Hevea brasiliensis* (All stages of *L. tristis* show significant preference for wilted tender rubber leaves; Sabu *et al.*, 2009). Five concentrations [0.005, 0.01, 0.02, 0.04, 0.08 mg/ml (w/v)] of each extract were tested on each life cycle stages of the test insect. Bioassay was conducted by no-choice method. Leaf discs (2×2 cm) were soaked in different concentrations of all the extracts for 5 min. Controls were treated with corresponding solvents alone, and all the leaf discs (treated and control) were allowed to dry at room temperature for 10 min. Post-dormancy adults, 4th instar larvae and pre-dormancy adults, pre- starved for one day were released (10 adults/larvae in each set up) on to the treated and control leaf discs placed in perforated plastic jars (500 ml) with a thin layer of soil at the bottom, and they were allowed to feed for a period of 24 hr. Six replicates were maintained for each treatment. The leaf area consumed was assessed in both treated and control set up using transparent millimeter-square graph paper. The antifeedant activity percentage (%AF) was calculated by the following formula

%AF=100 - (Leaf area consumed in treated/Leaf area consumed in control) ×100

Antifeedant experiments on post-dormancy adults, larvae and pre-dormancy adults were conducted during 3rd week of January, 2nd week of March and 3rd week of March respectively.

Statistical analysis

The data were subjected to a three-way analysis of variance (ANOVA) in a completely randomized block design. Significant differences between treatments were determined using Least Significant Difference (LSD) test ($p<0.05$), through an SPSS v 16.0 software package in Microsoft Windows 7 operating system.

Result and discussions

The results of antifeedant effect (AF%) of crude extracts of four plants in methanol, petroleum ether and water against pre dormancy adult stage, 4th instar larval stage and post dormancy adult stage at the concentrations of 0.005, 0.01,0.02, 0.04, 0.08 mg/ml (w/v) are given in Table 2, 3, and 4 respectively.

Table 2. Antifeedant effect of A) *C.infortunatum*, B) *G.sepium*, C) *C.citratus*, and D) *Z.officinale* on pre-dormancy adult beetles
(values are mean ±SD, n=6)

Concentration (mg/ml)	**Antifeedant effect (AF%)**											
	Methanolic extract				Petroleum ether extract				Aqueous extract			
	A	B	C	D	A	B	C	D	A	B	C	D
0.005	18.87 ±3.14	6.79 ± 4.91	9.25 ± 5.92	3.28 ± 7.08	12.81 ±4.73	3.75 ± 12.18	3.72 ±7 .36	0.57 ± 2.69	13.11 ±3.58	1.53 ± 8.66	6.35 ± 4.51	6.63 ±7.57
0.01	33.81 ±3.90	9.71 ± 4.34	10.69 ±1.86	4.84 ± 6.36	21.72 ±12.1 5	4.95 ± 9.28	6.38 ±7 .55	4.45 ± 11.36	22.53 ±5.23	6.15 ± 7.08	9.42 ± 4.40	11.35 ±5.0 7
0.02	51.06 ±4.01	19.76 ±4.40	13.44 ±1.77	9.16 ± 4.73	47.61 ±9.79	15.24 ±4.16	10.83 ± 3.19	7.69 ± 6.32	45.36 ±1.91	20.97 ±6.82	11.31 ±8.38	15.12 ±7.4 2
0.04	85.39 ±1.48	40.49 ±5.83	15.30 ±5.49	13.78 ±5.19	81.40 ±7.93	32.80 ±6.70	12.35 ± 3.75	16.27 ±5.17	77.82 ±3.86	38.65 ±2.34	13.54 ±5.70	17.69 ±9.1 2
0.08	95.24 ±2.03	60.49 ±5.45	20.66 ±5.26	18.07 ±4.40	91.00 ±5.22	60.07 ±4.38	17.95 ± 5.04	18.03 ±4.26	89.53 ±3.33	63.70 ±9.03	17.61 ±7.38	20.70 ±6.5 6

Solvent effect; f=0.308, df=2, p>0.05. Concentration effect f=22.966, df=4, p<0.05. Plant effect f=37.240, df=3, p<0.05.

Table 3. Antifeedant effect of A) *C.infortunatum*, B) *G.sepium*, C) *C.citratus*, and D) *Z.officinale* on 4th instar larvae
(values are mean ±SD, n=6)

Concentration (mg/ml)	Antifeedant effect (AF%)											
	Methanolic extract				Petroleum ether extract				Aqueous extract			
	A	B	C	D	A	B	C	D	A	B	C	D
0.005	22.58 ±5.03	3.75 ± 9.67	2.41 ±1 .88	4.68 ± 1.87	17.50 ±1.62	3.42 ± 2.21	4.57 ± 3.39	4.22 ± 4.09	17.56 ±3.47	4.41 ± 2.00	3.10 ± 4.52	6.63 ± 2.10
0.01	32.52 ±5.37	8.00 ± 10.03	5.04 ±5 .52	8.01 ± 1.10	30.82 ±5.35	8.15 ± 5.14	6.95 ± 1.52	6.71 ± 1.18	33.47 ±3.04	9.85 ± 4.30	5.93 ± 4.38	9.81 ± 3.36
0.02	52.93 ±5.13	20.27 ±3.64	9.29 ±5 .32	12.86 ±5.57	50.88 ±1.55	18.21 ±0.80	9.58 ± 2.37	12.62 ±1.01	53.57 ±1.88	19.76 ±3.96	9.09 ± 5.73	11.57 ±6.69
0.04	84.60 ±3.59	41.00 ±5.45	10.42 ± 2.35	14.20 ±5.88	84.23 ±2.09	38.73 ±5.05	13.59 ±2.70	13.86 ±4.87	84.44 ±1.17	43.36 ±2.89	12.66 ±4.25	14.15 ±5.75
0.08	94.62 ±2.81	64.13 ±6.39	18.18 ± 10.23	21.34 ±1.98	94.13 ±1.42	62.81 ±3.55	24.49 ±3.47	22.59 ±3.31	93.55 ±1.44	63.49 ±2.78	23.29 ±1.91	21.57 ±4.84

Solvent effect; f=0.023,df=2, p>0.05. Concentration effect f=28.924,df=4, p<0.05; Plant effect f=51.517,df=3, p<0.05

Table 4. Antifeedant effect of A) *C.infortunatum*, B) *G.sepium*, C) *C.citratus*, and D) *Z.officinale* on post-dormancy adult beetles
(values are mean ±SD, n=6)

Concentration (mg/ml)	Antifeedant effect (AF%)											
	Methanolic extract				Petroleum ether extract				Aqueous extract			
	A	B	C	D	A	B	C	D	A	B	C	D
0.005	14.20 ±6.32	6.76 ± 4.00	5.90 ± 3.39	5.75 ± 2.50	14.56 ±2.78	3.15 ± 2.95	2.69 ± 2.37	3.80 ± 2.99	11.21 ±3.00	4.97 ± 9.08	4.42 ± 3.98	3.23 ± 5.53
0.01	27.17 ±3.14	9.71 ± 7.30	7.17 ± 10.50	6.76 ± 2.50	24.53 ±6.08	6.39 ± 1.29	6.13 ± 1.23	1.41 ± 2.02	25.24 ±3.24	7.36 ± 8.59	2.38 ± 2.74	6.76 ± 2.65
0.02	48.31 ±4.78	20.68 ±3.16	12.62 ±7.63	12.23 ±.34	49.57 ±3.30	19.15 ±1.80	9.16 ± 2.00	4.56 ± 5.13	47.71 ±4.85	22.64 ±9.53	3.74 ± 3.26	10.78 ±3.46
0.04	81.66 ±2.62	41.94 ±2.11	13.04 ±2.13	11.55 ±8.24	81.06 ±1.63	37.26 ±1.95	12.60 ±3.08	6.74 ± 2.95	80.82 ±1.91	41.42 ±1.95	5.10 ± 3.77	15.96 ±5.46
0.08	95.64 ±2.14	67.05 ±4.17	25.78 ±3.82	18.28 ±9.26	93.38 ±2.96	63.37 ±3.99	22.96 ±2.31	19.28 ±4.04	92.70 ±0.72	64.35 ±3.76	10.20 ±3.28	22.46 ±6.16

Solvent effect; f=0.037, df=2, p>0.05. Concentration effect f=26.704, df=4, p<0.05; Plant effect f=39.193,df=3, p<0.05.

Higher AF% normally indicated decreased rate of feeding. All crude extracts showed significant antifeedant activity against pre dormancy adult beetles (f=37.240,df=3, p<0.05), 4th instar larvae (f=51.517,df=3, p<0.05) and post dormancy adult beetles(f=39.193, df=3, p<0.05). Comparison among solvents indicates that, no significant difference between the type of solvents used (f=0.308, 0.023, 0.037 and p= 0.737, 0.978, 0.964 for pre dormancy adult stage, 4th instar larval stage and post dormancy adult stage respectively. df=2 in all cases), suggesting that using any one of the solvents renders no difference. Significant difference in feeding deterrence between different concentrations of each extracts has been noticed against pre dormancy adults (f=22.966, df=4, p<0.05), 4th instar larvae (f=28.924, df=4, p<0.05) and post dormancy adults

($f=26.704$, $df=4$, $p<0.05$). Andifeedant activity was dose dependent in all cases. In the order of effectiveness as an antifeedant the extracts under present study could be arranged in the following ascending order, *C. infortunatum*> *G. sepium*> *C. citratus* > *Z. officinale*.

Discovery of novel toxins and/or antifeedants from plant extracts has been recently emphasized as a potential method for the development of "ecologically safe pesticides" (Weires and Riedl, 1991). In this study, the analysis of antifeedant effect of each extract, regardless of solvent used, shows that *Clerodendron infortunatum* exhibits remarkable antifeedant effect with AF% in the range of 90-95 and 80-85 at 0.08 and 0.04 mg/ml respectively. This result indicated the presence of more active chemical constituents in it. The active principles present in the plant inhibit feeding behavior or make the food unpalatable resulting in feeding deterrence. These results confirm the findings of several workers who had demonstrated the toxic and highly phagodeterrent action of several clerodendron spp against a wide range of insect pests. Earlier, Munkata (1975) has reported that after the discovery of insect antifeeding substances from *Clerodendron*, constituents of Verbenaceae plants have interested us for screening of insect antifeedants. The structure and stereochemistry of clerodin, a diterpenoid bitter principle isolated from the Indian bhat tree *C.infortunatum*, were established using X-ray analysis (Sim *et al.,* 1961; Barton *et al.,*1961). *C. tricotomum,* a representative member of the Verbenaceae family, is reported to possess feeding deterrent activity against the larvae of *Prodenia litura* due to the presence of clerodendrin A and clerodendrin B in the leaves (Kato *et al.,* 1972). Later, Hosozawa *et al.* (1974) isolated *a* new antifeedant, 3-epicaryoptin, from *C. calamitosum,* and clerodendrin A from *C. cryptophyllum.* Two feeding inhibitors, a diterpene hydroquinone and a flavone , were isolated from *C. siphonenthus,* were found to inhibt the feeding of adult *Sitophilus oryzae* (Srikumar *et al.,*1989) . Roy *et al.,* (2009) studied the antifeedant and insecticidal activities of *C. infortunatum* on eggs, nymph and adults of tea mosquito bug, *Helopeltis theivora*. They observed high antifeedant activity in all the concentrations of different solvent extracts and the feeding spots of *H. theivora* in tea foliage were reduced in the tune of 38.13-87.24% over untreated control. Antifeedant and growth inhibitory effects of various neo-clerodane diterpenoids having a furofuran moiety, isolated from *Clerodendron* spp., were studied by Kumari *et al.* (2003) against *Earias vitella* and *Spodoptera litura*. They reported that the compounds clerodendrin B, 3-epicaryoptin, 15-hydroxyepicaryoptin, and clerodin were effective antifeedants against *E. vitella* and *S. litura.* 3-Epicaryoptin isolated from the leaves of *C. inerme,* mixed in housefly larval diet, is responsible for growth inhibition and antifeedant activities in housefly and mosquito (Pereira *et*

al.,1990). In our study, irrespective of the solvent used for extraction, *G. sepium* showed promising antifeedant activity in the range of 60-65% AF value at highest dosage against all developmental stages of the test insect. The antifeedant activity of *G. sepium* is also evident from the studies conducted by Flores *et al.,* (2008) on *Bemisia tabaci*, an important virus vector on a number of crops worldwide. Their studies had revealed that *G. sepium* exert a very good phagodeterrence effect on *B. tabaci.* Mortality of *B. tabaci* adults was observed in plants treated with either the crude extract or the fractions of *G. sepium*, which was always dose-independent, may be attributed either to an indirect effect of strong deterrence, causing heat stress, energy depletion or dehydration (Veierov, 1996). The observed effects are probably explained by the specific chemicals present in *G. sepium* foliage, which includes a wide array of compounds, such as terpenoids, flavonoids, arilpropanoids and isoflavonoids, some of which may have deterrent activity. Evaluations of toxicity, antifeedant, growth-regulatory activity of the methanol extract of *G. sepium* leaves were carried out against the bug, *Dysdercus koenigii*, *Achaea Janata* , and *Spodoptera litura*. In this study, at certain doses a strong antifeedant activity was evident against the lepidopteran insects (Parvathi *et al.,* 1999).

In comparison to *C. infortunatum*, and *G. sepium*, *C. citratus* and *Z. officinale* showed no significant antifeedant effect against all developmental stages ($p=0.899$, $p= 0.761$and $p=0.941$ for pre dormancy adult stage, 4th instar larval stage and post dormancy adult stage respectively) even at high dosages, indicating that the active principle may not contain any strong phagodeterrents. Natural antifeedants are mainly plant substances of various chemical groups. Particularly effective insect antifeedants are triterpenes (Van beek and Groot, 1986), sesquiterpene lactones and alkaloids (Nawrot *et al.,* 1986), cucurbitacines, quinines and phenols (Norris, 1986). Of the four plant extracts tested, *Clerodendron infortunatum* may be a valuable source of natural antifeedant against *L. tristis*. The possible antifeedant single components or mixtures of the components showing synergistic effects can be isolated, purified and tested for their activity against the different developmental stages of of *L. tristis* . The current findings suggest that the extracts from *C. infortunatum*, and *G. sepium* can be fractionated and the fractions and the single components of further purification procedures be tested for antifeedant and toxicity effects against *L. tristis* . The use of plant materials may be a safe, cost-effective and eco-friendly method for suppression of pest population and thereby by provide protection against pest infestation among low-resource poor farmers who live in traditional tile roofed residential buildings and thatched sheds in rubber plantation tracts of Kerala. However, this alternative often does not provide effective check against *Luprops* beetles unless the development of a

formulation suitable for application in rubber litter layers. Today, the environmental safety of an insecticide is considered of paramount importance.

An insecticide does not have to cause high mortality to target organisms in order to be acceptable (Schmutterrer, 1994). Antifeedant and growth inhibiting activity can therefore be incorporated into other insect control techniques in the strategy of integrated pest management (IPM).

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