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Scientific Note

A note on some epiphytic plants for their repellent activities against *Tribolium castaneum* adults

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Repellents, fumigants, feeding deterrents, growth inhibitors and insecticides of natural origin are rational alternatives to synthetic insecticides. Some plants draw a special attention for their low lethal and other environment-friendly activities (Talukder & Howse, 1995). Epiphytes or air plants that do not normally root in the soil but grow upon another living plant, draw attention as a source of bioactive potentials. Use of these plants in the traditional system of folk medicine is well known (Mallavadhani *et al.,* 2003). Epiphytes of Bangladesh remain untouched from investigation for their bioactive potentials. In this proposition six epiphytic plants viz. *Dendrophthoe falcate* (L.f.) Ettingsh. *Cuscuta reflexa* Roxb., *Drynaria roxburghii* (Bory) J. Sm., *Ficus lacor* Buch.-Ham., *Vanda roxburghii* R. Br. and *Loranthus longiflorus* Desr., were taken into consideration for evaluating their repellent activities.

Mallayadhani *et al.* (2006) mentioned that *D. falcata* has estrogen receptor binding activity. In the Ayurvedic medicine *C. reflexa* is said to be useful in diseases of eye and heart (Chopra *et al.*, 1956). *F. lacor* stem bark is used in gastric and ulcer (Bajracharya *et al*., 1978; Bhattarai *et al*., 2000; Pandey, 2001; Rai *et al*., 2004). Its milky latex of stem is used in typhoid and heavy fever, dysentery and boils (Oli, 2001). Decoction of buds is considered for ulcer and leucorrhoea (Chopra *et al*. 1956), gargle in salivation (Malla, 1994), boils, pimples and blisters (Manandhar, 1985). Dried buds of *F. lacor* are used to treat harsa (Nakarmi, 2001) and seeds are tonic in nature and used in treatment of stomach disorder (Bhatt, 1977). *Loranthus* species in semiparasitic plants are known to produce variety of bioactive compounds; i.e., sesquiterpene lactones for the treatment of schizophrenia (Okuda *et al*., 1987) and (+)-catechin, 3,4- dimethoxycinnamyl alcohol and 3,4,5-trimethoxycinnamyl alcohols for the antimicrobial and antifungal properties (Sadik *et al*., 2003). Many other chemical components such as triterpenoids from *L. grewinkii* (Rahman *et al*., 1973), and *L. falcatus* (Anjaneyulu *et al*., 1977), flavonoids from the leaves of *L. kaoi* (Lin & Lin, 1999) and from *L. europaeus* (Harvala *et al*., 1984), a cytotoxin from *L. parasiticus* (Zhou *et al*., 1993), and phenolics from *L. longiflorus* (Indrani *et al.*, 1985) have been reported so far. Other biological activities have also been reported, such as, antihypertensive effect (Obatomi *et al*., 1996), antiviral activity of *L. parasiticus* (Kusumoto *et al*., 1992), anti-diabetic properties of *L. bengwensis* (Obatomi *et al*., 1994). *V. roxburghii* contains heptacosane and octacosanol that show anti-inflammatory activity (Okuda *et al*., 1987). No information was available on the biological activity of *D. roxburghii.* However, previous workers investigated these plants giving emphasis mostly on the chemical constituents and their medicinal profile but a very few were given touch on agricultural openings, and information on their various biological activities is still scanty. Here repellent activity tests of these plants have been attempted to evaluate their efficacy as environment-friendly pest control agents.

Epiphytic plants *D. falcata* of family Loranthaceae, *C. reflexa* of family Convolvulaceae, *D. roxburghii* of family Polypodiaceae, *F. lacor* of family Moraceae, *V. roxburghii* of family Orchidaceae and *L. longiflorus* of family Loranthaceae were collected from Rajshahi and Chapai Nawabganj Districts and were identified at the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The materials were dried in a well-ventilated room under shade from 3 to 7 days and powdered in a hand grinder. The powdered materials were weighed and placed in separate conical flasks to add chloroform (30g $\times$ 100ml $\times$ 3 times) and filtration was done at 24 h interval. The output extracts were transferred to glass vials and preserved in a refrigerator at 4°C until used.

The insect used in this study was the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The stock culture was maintained in the laboratory at 30 ± 0.5ºC. The whole-wheat flour was sterilized at 60^0C for 36 hours in an oven. A standard mixture of whole-wheat flour and powdered dry yeast in a ratio of 19:1 (Zyromska-Rudzka, 1966) was used as food medium. In regular intervals the eggs were collected by sieving the food medium by two sieves of 500 and

250 mesh sizes for separating the adults and eggs, respectively following the methods of Khan and Selman (1981). A huge number of beetles were thus reared to get a regular supply of the newly formed adults.

The repellency test used was adopted from the method of McDonald *et al*. (1970) with some modifications by Talukder and Howse (1993, 1994). Half filter paper discs (Whatman No. 40, 9 cm diameter) were prepared and five doses (4.938, 2.469, 1.235, 0.617 and 0.309 µg cm^{-2}) of all the $CHCl_3$ extracts were applied separately onto each of the half-disc and allowed to dry out in the air for 10 min. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a Petri dish (9 cm dia.). Ten adult insects were released in the middle of each filter paper circle each dose was tested three times. Insects that settled on each half of the filter paper disc were counted after 1 h and then at hourly intervals for 5 h. The average of the counts was converted to percent` repellency (*PR*): *PR* = 2(C - 50), where C is the percentage of insects on the untreated half of the disc. Positive values expressed repellency and negative values attractant activity. The data were finally subjected to arcsin transformation for the calculation of ANOVA and interpretation for the repellency was made by the F values through 5, 1 and 0.1% levels of significance.

The repellent activity results of the six epiphytes against *T. castaneum* were promising since five test materials among six found strongly effective (Table 1 and 2). Except the *D. falcata* extract *C. reflexa, D. roxburghii, F. lacor, V. roxburghii* and *L. longiflorus* extracts were found potent and their repellency due to differences between doses were highly significant (P<0.001), however, differences between time intervals for all the epiphytic extracts were statistically non-significant (P >0.05). According to the intensity of repellency the extracts could be arranged in a descending order as: *V. roxburghii*>*L. longiflorus*> *D. roxburghii*> *C. reflexa*> *F. lacor.* These findings are supported by some previous workers, however none of the biological activities reported was on repellency by any of the six test plants. *D. falcata* has estrogen receptor binding activity (Mallayadhani *et al.,* 2006) that on the other hand offer male antifertility agents and properties for the inhibition of spermatogenesis. Methanol extracts of *C. reflexa* stem showed a broad spectrum of antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Bacillus licheniformis*, and fungi namely *Aspergillus niger* and *Trichoderma reesei* (Aggarwal & Dutt, 1935). The extract of *F. lacor* exhibited inhibition in adiorespirometry assay using ACTEC system with rifampin as positive control and the allied species *F. retusa* L., *F. lacor* Ham. and *F. cunia* Ham. ex Roxb. found potent for curing leprosy; *F. religiosa* L., *F. rumphii* Bl. and *F. heterophylla* L. for asthma; and all these materials are used in the folklore medicine of Puerto Rico (Kirtikar & Basu, 1935). The stembark of *F. lacor* contains ß-sitosterol, a-D-glucose and meso-inositol, the leaves contain petunidin di-glycoside and quercentin 3-galactoside and the fruits contain cyanidin rhamnoglycoside and polysaccharides, which have been found bioactive (Kirtikar & Basu, 1935). *Loranthus* species are known to produce variety of bioactive compounds (Okuda *et al*., 1987) and the antimicrobial and antifungal properties (Sadik *et al*., 2003).

Table 1. Percent repellency of *T. castaneum* adults by chloroform extracts of six epiphytic plants

Plant	Dose (µg cm^{-2})	Percent repulsion (PR)				
		1h	2h	3h	4h	5h
D. falcata	4.938	60	0	-93.4	-93.4	-73.4
	2.469	-26.6	-33.4	-33.4	-13.4	31.05
	1.235	-46.6	-26.6	-40	-33.4	-33.4
	0.617	20	20	13.4	33.4	26.6
	0.309	-6.6	-13.4	-20	-13.4	-20
C. reflexa	4.938	80	73.4	93.4	100	100
	2.469	100	93.4	93.4	93.4	93.4
	1.235	73.4	93.4	86.6	80	86.6
	0.617	6.6	-20	33.4	20	13.4
	0.309	80	86.6	66.6	53.4	80
D. roxburghii	4.938	100	86.6	73.4	100	100
	2.469	66.6	26.6	53.4	46.6	40
	1.235	93.4	100	100	93.4	93.4
	0.617	33.4	26.6	33.4	33.4	33.4
	0.309	66.6	26.6	33.4	40	26.6
F. lacor	4.938	33.4	46.6	46.6	40	33.4
	2.469	53.4	66.6	46.6	53.4	60
	1.235	46.6	60	73.4	53.4	66.6
	0.617	13.4	13.4	26.6	6.6	-6.6
	0.309	73.4	60	80	93.4	53.4
V. roxburghii	4.938	-6.6	-46.6	0	6.6	-6.6
	2.469	93.4	100	100	100	100
	1.235	40	53.4	40	40	53.4
	0.617	6.6	0	6.6	0	-13.4
	0.309	26.6	13.4	46.6	53.4	46.6
L. longiflorus	4.938	60	40	46.6	60	46.6
	2.469	-46.6	-40	-40	-53.4	-53.4
	1.235	-20	-33.4	-40	-40	-40
	0.617	-46.6	-26.6	-33.4	-33.4	-33.4
	0.309	86.6	86.6	100	93.4	86.6

A cytotoxin was reported from *L. parasiticus* (Zhou *et al*., 1993). *V. roxburghii* contains properties to show anti-inflammatory activity (Okuda *et al*., 1987). Except *D. falcata*, other 5 test plants offered strong repellent activity and it is good sign for the investigators working and looking for bioactive potentials in plant resources, since repelling from infestation and deterring from feeding are more acceptable than killing of the pest insects whenever environment friendly protection of crops and stored products is mostly considered.

Table 2. ANOVA results of repellency tests by chloroform extracts of epiphytes against *T. castaneum* adults

	Sources of variation (df)			F-ratio with level of significance	
Test materials	Between doses	Between time interval	Error	Between doses	Between time interval
D. falcata	4	4	16	3.267ns	1.380ns
C. reflexa	4	4	16	22.890***	0.210ns
D. roxburghii	4	4	16	28.961***	1.034ns
F. lacor	4	4	16	21.752***	0.888ns
V. roxburghii	4	4	16	37.538***	0.186ns
L. longiflorus	4	4	16	35.981***	1.114ns

(ns= not significant; *** = P<0.001

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