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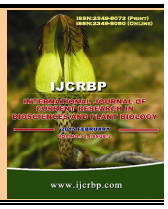

Original Research Article

Toxic Effect of *Glinus lotoides* (Aizoaceae) on Rice-Moth, *Corcyra cephalonica* Staintion (Lepidoptera: Pyralidae) Eggs

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A b s t r a c t

Hexane, chloroform and ethyl acetate extracts of *Glinus lotoides* were evaluated under laboratory conditions at 0.25, 0.50, 1.0 and 2.0% (W/V) concentrations against the *Corcyra cephalonica* eggs with two different food media (Rice flour and pearl millet flour). All the three extracts were combined with millet and rice flour and given to *C. cephalonica*. Preliminary phytochemical analysis was carried out for all the three extracts. The maximum egg mortality was caused by ethyl acetate extract (78.73%) in millet flour, followed by 75.42% in rice flour at 2% of treatment concentration. All the concentrations of the extracts applied were able to cause significant (p<0.05) ovicidal activity of the *C. cephalonica* eggs. The least value of LC_{50} was obtained with ethyl acetate extract in both rice and millet flour media (2.29% and 2.06%). There were concentration and exposure period dependent LC50 and LC90 levels and efficacy against *C. cephalonica* egg hatchability inhibition among all extracts, and levels increased significantly with increasing concentrations and exposure periods for all extracts. Regression equations and their probability values against lethal concentrations for all the extract revealed a significant and positive role in pest population inhibition. This study demonstrates the potential use of *G. lotoides* extracts for crop protection against the key pest of grains, *C. cephalonica* and suggests that additional bio-efficacy tests against different crop pests has to be pursued to determine the efficacy of *G. lotoides* extracts as a biopesticide.

K e y w o r d s

Corcyra cephalonica
Egg mortality
Glinus lotoides
Lethal concentrations
Phytochemicals

Introduction

Plant-based insecticides are friendly and safe alternative in managing pests and they can be incorporated in pest management programs (Ashamo and Akinneye, 2004; Ashamo, 2005). There is an increasing interest for natural pesticides derived from plants and microorganisms due to they

are assumed being safer than the synthetic pesticides (Isman et al., 2007; Shukla and Tiwari 2011). These concerns have resulted in a renewed interest in search for alternative control measures (Jbilou et al., 2006). Nearly more than 670 insect pests (beetles and moths) and 355 species of mites are estimated to attack stored product commodities (Rajendran, 2002; Ngamo et al., 2001).

In recent years, they are gaining increased attention for the development of new insecticides as they may be highly effective, rapidly biodegradable, less expensive than synthetic pesticides, readily available and probably easily applicable with simple techniques (Schmutterer, 1990; Kim et al., 2003). Therefore, plant based pesticides could be appropriate for pest control measures against Lepidopteran pests (Belmain et al., 2001). These botanical insecticides are of economic importance especially in developing countries. Also, there is a continuous need to search for new plant species with ideal insecticidal properties. Many plant based extracts and their constituents have been found to possess potential as alternative compounds to be used as pesticidal agents (Huang et al., 2000; Rajendran and sriranjini, 2008; Sahaf et al., 2008; Cosimi et al., 2009; Nerio et al., 2009).

Stored product insect pests cause losses ranging from 5-30% of the world's total agricultural production. Insect attack to stored product grains is severe because the insects have adaptations i.e., morphological, physiological and behavioral characters that suit them to affect storage grains. Extracts with ovicidal effects have been reported for the following insects, *Plutella xylostella*, *Helicoverpa armigera*; *Spodoptera frugiperda* (Lepidoptera: Noctuidae); *Bemisia tabaci* (Hemiptera: Aleyrodidae); *Tribolium castaneum* (Coleoptera: Tenebrionidae); *Diatraea saccharalis* (Tavares et al., 2010 and 2011; Souza and Vendramim, 2000; Das et al., 2006).

Glinus lotoides Linn., locally known as "Siruseruppadai" is an annual or short-lived perennial prostate herb growing in low land dry areas. It is classified under the family, Aizoaceae. The leaves are opposite or clustered at nodes and apparently verticillate, narrowly elliptical to surround. The plant contains stipules and the flowers are bisexual and clustered in axillary (Endale, 2000). *G. lotoides* is found mostly in tropical and subtropical regions such as India, Ethiopia, Sudan, Uganda, Egypt, Pakistan and South Africa. This plant has been used as antifungal agent and for fishing (Endale, 2000), and taenicidal activity (Endale et al., 1997 and 1998). The seeds of *G. lotoides* contain 10% crude saponin (Endale et al., 1998). Also, this plant contains fatty acids, glycosides of sitosterols, stigmasterol, flavonoids and waxes (Biftu et al., 1979). Abegaz and Tecle (1980) isolated biologically active triterpene glycoside from the seeds of the plant. Five triterpenoidal saponines (Glinusides A, B, C, D and E) have been isolated from the n-butanol fraction of *G. lotoides*. dicatamnoides (Hamed and El- Elmary, 1999). The taenicidal activity of *G. lotoides* has been attributed to saponins present in it (Endale et al., 1997 and 1998). However, previous reports on the use of *G. latoides* for insect control is scanty.

Corcyra cephalonica (Staintion) is a major pest of stored cereals and cereal products in India as well as in other tropical and subtropical regions of the world (Cox et al., 1981; Allotey and Kumar, 1985; Piltz, 1977). Its larval stages cause appreciable loss to rice, sorghum, currants, gram, milled products, cocoa beans, peanuts, cottonseed, linseed, raisins, chocolates, army biscuits and nutmeg (Piltz, 1977; Allotey, 1991). Information is available pertaining to the effect of specified plant components (Pathak and Krishna, 1992) on the *C. cephalonica* egg hatchability. Nathan et al. (2006) compared to the development stages of *C. cephalonica* with different food media. The objective of this study was to determine the ovicidal effects of different solvent extracts on the eggs of *C. cephalonica* to examine the possibility of using *G. lotoides* extracts in the control of *C. cephalonica* pest.

Materials and methods

Plant material

The fresh leaves of *Glinus lotoides* were collected from Arakkonam block, Vellore district, Tamil Nadu, India. Plant leaves were washed with tap water and dried at room temperature for one week in a shady place. The dried plant materials were subsequently powdered with electric blender. Ground plant material (500 g) was soaked with 1.5 liter hexane solvent in 2 liter conical flask. The flask covered

with aluminum foil and gently shaken with periodic duration. After 72 h the suspensions was filtered through filter paper and the extract was collected. Subsequently chloroform and ethyl acetate were used as solvents and extracted. The collected extracts were kept in aerated room. All the extracts were stored at 4ºC until further use.

Insect

A standard laboratory culture of the rice moth, *Corcyra cephalonica* was maintained in plastic trays (30×30×5cm), containing previously cleaned and partially milled rice and millet flour in the ratio of 1:1 (w/w) with maize seeds. Insects were grown at 27±1°C, 75±5% RH with a 14:10 h light: dark cycle as described by Osman, (1986) with some modifications. The food media were sterilized in an autoclave before experimentation. The subcultures and the experiment were maintained under the same conditions. *C. cephalonica* larvae (16±1-day old) were used in the experiments. All the cultures in plastic containers (28×18×18 cm) were held in trays with guards submerged in water to prevent insects from crawling into them (Allotey and Azalekor, 2000).

Egg collection

Egg-laying apparatus (Allotey, 1985; Allotey and Goswami, 1990) consisting of plastic container was used as the oviposition cage for *C. cephalonica*. Each container containing 10 pairs of newly emerged adults was inverted over a glass Petri-dish lined with filter paper at the bottom. The filter paper provided a rough surface for oviposition. The eggs laid on the filter paper by the moths were collected from the filter paper with a camel hair brush. Uncollapsed eggs (examined under stereo microscope) were used for ovicidal activity.

Ovicidal activity

Normal eggs (0-12 h old) were collected and placed in Petri dish according to the procedure described by Zambare et al. (2012) with minor modification. Four different concentrations of extracts (0.25, 0.50, 1.0 and 2.0%) were prepared and mixed with rice (*Oryza sativa* L.) and maize (*Zea mays* L.) flours separately. Each flour material was allowed to evaporate solvent and 15 eggs were introduced for each test concentration. Each experiment was replicated five times along with appropriate control. The numbers of unhatched eggs were counted after 96 h and the rate of mortality was calculated. Percent mortality was calculated according to Abbot (1925):

$$\text{Abbot corrected mortality (\%)} = \frac{\text{\% unhatched eggs in treatment} - \text{\% unhatched eggs in control}}{100 - \text{\% unhatched eggs in control}} \times 100$$

Phytochemical analysis

Preliminary phytochemical analysis was performed following the method of Harborne (1984) to check the presence of various phytochemicals in hexane, chloroform and ethyl acetate extracts. Presence or absence of different phytochemicals *viz*., alkaloids, flavonoids, triterpeniods, tannins, phenols, saponins, quinines, anthraquiones, coumarins and steroids were checked by qualitative tests

Statistical analysis

The percent ovicidal activity was analysed using one way ANOVA. Significant differences between the treatments were determined using Tukey's multiple range test ($p \leq 0.05$). LC_{50} and LC_{90} values were calculated with using appropriate values (Finney, 1971).

Results

The experiments were conducted for evaluating phytochemical and ovicidal activities of leaf extracts of *G. lotoides* tested against *C. cephalonica* eggs. The phytochemical investigation of these crude extracts revealed the presence of phytochemicals as shown Table 1. Saponins, anthraquinones and coumarin were present in hexane extract of *G. lotoides.* In the case of chloroform extract of the plant showed that the positive results of alkaloids, steroids, flavonoids, terpenoids and quinines. Ethyl acetate extract showed positive for the presence of alkaloids, tannins, flavonoids, terpenoids, anthraquinones and coumarines phytochemicals. The eggs of *C. cephalonica* treated with different concentrations of *G. lotoides* caused ovicidal activity resulting in failure to hatch eggs.

The ovicidal activity of *G. lotoides* extracts tested against *C. cephalonica* eggs in two different food materials (Table 2). Significant differences in toxicity of extracts to the eggs were observed. Highest egg mortality was registered at higher concentration (2%) of ethyl acetate extract to the level of 78.73% for millet flour diet and 75.42% for rice flour diet respectively, and the least egg mortality of 2.27 % was observed at 0.25 % hexane extract treatment in rice flour diet and 4.34 % egg mortality observed in millet flour diet. All the treatments showed ovicidal activity in a dose dependent manner.

Table 1. Preliminary phytochemical analysis of crude extracts of *Glinus lotoides.*

Solvent extract	Phytochemicals									
	Alk	Ste	Sap	Tan	Fla	Ter	Ant	Qui	Phe	Cou
Hexane	-	-	+	-	-	-	+	-	-	+
Chloroform	+	+	-	-	+	+	-	+	-	-
Ethyl acetate	+	-	-	+	+	+	+	-	-	+

(**Alk:** Alkaloids, **Ste**: Steroids, **Sap**: Saponins, **Tan**: Tannins, **Fla**: Flavonoids, **Ter**: Terpenoids, **Ant**: Anthraquinones, **Qui**: Quinones, **Phe**: Phenolics and **Cou**: Coumarines); (+ present and – absent)

Table 2. Ovicidal activity (%) of crude extracts of *Gilnus lotoides* against *Corcyra cephalonica.*

Extract	Concentration (%)	Ovicidal activity (%)	
		Rice flour	Pearl millet flour
Hexane	0.25	2.27±0.14b	13.04±1.17
	0.50	6.81±0.62	17.39±0.52
	1.00	13.63±0.65	23.91±1.63
	2.00	20.45±1.06	26.08±0.98
Chloroform	0.25	4.54±0.44bc	4.34±0.86
	0.50	6.81±1.06	10.86±0.26
	1.00	18.18±1.27	19.56±1.48
	2.00	25.00±2.01	23.91±1.94
Ethyl acetate	0.25	9.09±0.84	19.56±1.10
	0.50	20.45±1.26	34.78±1.71
	1.00	34.09±1.61	52.17±2.53
	2.00	75.42±1.92	78.73±2.14
Control		1.09±0.33a	2.18± 0.83a

Within the column, mean ± SD followed by the same letter do not differ significantly using Tukey's test, $p \leq 0.05$.

The determination of LC_{50} and LC_{90} for the above experiments was derived by probit analysis and the data pertaining to it were depicted in tables 3 and 4. The statistical data regression line and 95% confidence limits were also calculated. Data presented in Table 3 demonstrated the LC_{50} and LC_{90} value for the tested all these extracts against *C. cephalonica* eggs under laboratory conditions. The LC_{50} value of all the three extracts ranged from 2.29 to 10.47 % for rice flour diet and millet flour diet 2.06 to 22.75 % respectively. Among the treatment, ethyl acetate extract was found to be more pronouncing than the other extracts for both diets. Lowest LC_{50} and LC_{90} values were observed in ethyl acetate treatment of two diets.

The data obtained revealed that ethyl acetate extract was the most significant (LC_{50} = 2.29% and LC_{90} = 19.64%) followed by chloroform extract (LC_{50} = 9.56% and LC_{90} = 46.19%) in rice flour diet and the same trend was observed in millet diet for ethyl acetate extract (LC_{50} = 2.06% and LC 90 = 9.31%) and chloroform extract (LC_{50} = 10.10% and LC_{90} = 56.72%). Highest LC_{50} and LC_{90} values were noticed in hexane extracts 10.47 % and 45.47 % in rice flour diet and 22.75 % and 75.05% in millet flour diet respectively. Chi- square values were significant $p \leq 0.05$ level. The order of impact on egg mortality, the extracts under present study could be arranged in the following descending order i.e., ethyl acetate > chloroform > hexane.

Table 3. Lethal concentrations (LC_{50} and LC_{90}) values of *Glinus lotoides* against *Corcyra cephalonica* in rice flour media.

Extract	LC_{50}	95% Confidence limit		LC_{90}	95% Confidence limit		Chi square value	Regression value
		Lower	Upper		Lower	Upper		
Hexane	10.47	5.74	311.93	45.47	13.83	5205.35	0.043*	Y=2.94+0.49x
Chloroform	9.56	5.43	25.86	46.19	14.25	221.76	0.801*	Y=3.16+0.53x
Ethyl acetate	2.29	3.23	9.40	19.64	9.68	152.37	0.143*	Y=3.61+0.48x

*χ^2 values are significant at $p<0.05$ levels.

Table 4. Lethal concentrations (LC_{50} and LC_{90}) values of *Glinus lotoides* against *Corcyra cephalonica* in millet flour media

Extract	LC_{50}	95% confidence limit		LC_{90}	95% confidence limit		Chi square value	Regression value
		Lower	Upper		Lower	Upper		
Hexane	22.75	-	-	75.05	-	-	0.083*	Y=3.86+1.18x
Chloroform	10.10	5.53	211.195	56.72	15.80	5294.41	0.071*	Y=3.282+0.58x
Ethyl acetate	2.06	3.12	23.05	9.31	6.12	23.05	1.440*	Y=4.03+0.43x

*χ^2 values are significant at $p<0.05$ levels.

Discussion

Plants and plant products can provide influential insect control which requires a thorough evaluation of their potential activity against pests who need to protect their crops. Present study, two diets were used (rice and millet flour) as experimental media to compare the ovicidal activity of *G. lotoides*. Out of three different solvent extracts, ethyl acetate extract of *G. lotoides* exhibited maximum inhibition on egg hatchability, followed by chloroform extract, and the hexane extract brought least ovicidal activity at verifying concentrations. The ovicidal effect appears to be due to its inability to penetrate the chorion of the eggs (Don Pedro, 1989).

This is the prime report for phytochemical analysis and ovicidal activity in leaves of *G. lotoides*. In the present study, phytochemicals namely, alkaloids, tannins, flavonoids, terpenoids, anthraquinones and coumarines were found in ethyl acetate extract of *G.lotoides.* Already alkaloids (Baskar et al., 2010), tannins (Lingathurai et al., 2011), flavonoids (Baskar et al., 2012), terpenoids (Nathan et al., 2005), anthraquinones (Lingathurai et al., 2010) and coumarines (Baskar et al., 2010 and Vendan et al., 2010) in plants were reported to be toxic against many Lepidopteran pests. However, the present work is the first report on ovicidal activity on *C. cephalonica* eggs. These observations are in accordance with the studies conducted using different plant species for ovicidal activity. *G.lotoides* attributed these phytochemicals synergistically induce the inhibition of egg hatchability. The plant secondary metabolites and their volatiles can enter into eggs via aeropyles, the tiny holes of the chorion connected with the respiration of embryos (Chapman, 1982; Mill, 1985; Sehnul, 1985), leading to non–hatchability of eggs.

Ovicidal effect of seed extract of custard apple against *Corcyra cephalonica* and *Trogoderma granarium* was reported by Rao and Sharma (2007). They reported that LC_{50} values for hexane, ethyl acetate and methanol extracts respectively were 0.218, 0.113 and 0.285% against Lepidopteran insect pest, rice moth. Similar to present results Dwivedi and Garg (2003) reported ovicidal activity of flower extract of turmeric and *Lantana camara* against *C.cephalonica* eggs. They observed that ovicidal effect was dose dependent which may be due to its easy penetration through delicate covering of vitellin and chorion membrane thereby increasing the mortality rate. High percentage of egg mortality caused by the extract is assumed to be caused by the active ingredients present in them which might have disrupted blastokinesis and induced impaired larval hatching.

The findings of the present study are also in agreement with that of Khani et al. (2012) who reported that petroleum ether extracts from *Piper nigrum* and *Jatropha curcas* resulted strong ovicidal action towards *C. cephalonica*. The bioassay results

are encouraging that this extract can prevent egg hatchability through contact toxicity. Pathak and Pandey (2011) reported that on the efficacy of combination of neem and eucalyptus oil suppressed the *C. cephalonica* egg hatchability.

Further analysis to isolate the active compound for larval control is under way in our laboratory. More studies are needed to elucidate the ovicidal activity against a wide range of insect pests and the active compound responsible for the efficacy should be identified which could be used to control other insect pest species in the future.

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