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EVALUATION OF BIOEFFICACY OF *GLIRICIDIA SEPIUM* (JACQ.) STEUD. AGAINST RICE FIELD RAT, *BANDICOTA BENGALENSIS* GRAY & HARDWICKE

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ABSTRACT

Toxicity of ethanolic extract of *Gliricidia sepium* (Jacq.) Steud. leaves was evaluated against rice field rats, *Bandicota bengelensis* Gray and Hardwicke at 0.25%, 0.5%, 1.0% and 2.0% (2.5g a.i, 5.0g a.i, 10.0g a.i and 20.0g a.i, respectively per 1000g wheat flour and broken rice) in the laboratory. The quantity of bait consumed, hemorrhages (external and internal), pathological evidence and mortality were recorded in rats fed on *Gliricidia sepium* leaves extract. Feed consumption tests showed that bait ingestion was inversely proportional to concentration. *Gliricidia sepium* gave 100% mortality in 6.75 ± 1.99 days at 2.0%, 50% in 11.50± 2.40 days at 1.0%, 30% in 12.0±3.25 days at 0.5% and0% at 0.25% concentrations. The results demonstrated good acceptability and effectiveness of *Gliricidia sepium* against the rice field, rat *Bandicota bengalensis.*

Key-words:

INTRODUCTION

Rodent pests inflict severe damage to stored grains, buildings, insulations, electric wirings, wood, field crops, such as, wheat, rice, sugarcane etc (Sagar and Bindra, 1976; Robert, 1977; Lathiya, 1978; Beg *et al.,* 1979; Fulk and Lathiya, 1981; Shakunthala and Srikari, 1983; Shafi *et al.*, 1988; Rustamani and Wahab, 2005; Lathiya *et al.,* 2008). Rats eat and contaminate stored grains with their droppings, feces, urine and hair (Husain and Iqbal, 2002; Krasner, 2010). Invasion of rodent pests cause colossal losses amounting to millions of rupees annually to agricultural produce. They harbor a number of pathogens in their urine and feces that can be transmitted to both humans and their pets. Worldwide, rats and mice are known to spread over 35 diseases. Rodent-borne diseases can spread to humans through bite wounds, consuming food or water that has been contaminated with rodent feces and urine, coming in contact with contaminated water or even through breathing in germs that may be present in rodent urine or droppings (stirred in the air).

Many methods are used to overcome these conflicts, between man and rodents. It is impossible to eradicate rodents completely from the habitats; however, an integrated pest management approach can be followed. Most of the rodenticides may be toxic for human, livestock and other non-targeted animals (Brown *et al.,* 1988; Colvin *et al.,* 1988; Gray *et al.*, 1994; Parson *et al.*, 1996; Brakes and Smith, 2005). Plant materials have been used for pest control for centuries under traditional farming system. However, only few plant species are being used for pest control in various countries and that too are used for insects. There is a need to discover natural plant products for rodent management, feasible for traditional farming system. Rats are more sensitive to plant toxins than ruminants; some workers have used rat bioassays to test the presence of toxins in tropical pasture legumes including *Gliricidia sepium* (Bindon and Lamond, 1966, Strickland *et al.,* 1987). *Gliricidia sepium* (Fabaceae) *(*Chadhokar, 1982) is a leguminous, fast-growing, easily propagated, nitrogen-fixing tree used throughout the tropics for many purposes, such as, for fencing, shade, fodder, fuel wood, green manure, animal feed etc (Csurhes and Edwards, 1998; Adejumo and Ademosin, 1985; Gohl, 1981).The leaves of *G. sepium* have a high feeding value with crude protein comprising 20-30% of the dry matter, a crude fiber content of about 15% and in vitro dry matter digestibility of 60-65%. There are numerous reports of increases in weight and milk production in both large and small ruminants when *Gliricidia* forage is used as a supplement (Carew, 1983; Nochebuena and O'Donovan, 1986; Ash, 1990).*Gliricidia* originated from Central America, spread to many parts of the world; however, in Pakistan it was cultivated for the first time at Coastal Agricultural Research Station, SARC, PARC, Karachi (Solangi *et al.,* 2004). Preliminary studies on the major characteristics, agronomic features and nutrient value of *Gliricidia sepium* were carried out (Solangi *et al.,* 2010). Some studies have been carried out on nematicidal, insecticidal and antibacterial properties of ethanolic leaves extract of *Gliricidia*; the extract also proved as a good mosquito repellant *(*Nazli *et al.,* 2008).The toxic properties of the seeds, leaves and bark of *G. sepium* give rise to the generic epithet of this species (*Gliricidia*= mouse killer). Poison derived from it can be used for rodent management by mixing with grains. The present study

was under taken to evaluate toxicological effects of *Gliricidia sepium* against rice field rat. These preliminary findings may be useful in further studies on role of *Gliricidia sepium* in rodent pest management.

MATERIAL AND METHODS

Collection and processing of plant

Gliricidia sepium (Jacq.) Steud. leaves were collected from Coastal Agricultural Research Station, SARC, PARC, Karachi. The leaves were preserved in wax-quoted paper bags and brought to the laboratory for biological assays.

Plant extraction

The fresh dried leaves of *Gliricidia sepium* (5kg) were ground and soaked in ethanol (10 L.). The filtrate was concentrated under reduced pressure at 40°C to a gum. This crude gum was used for activity purpose.

Collection of rats

Rice field rats, *Bandicota bangalensis* Gray and Hardwicke were live-trapped from rice fields, Thatta district, lower Sindh (24° 45′ N; 67° 55′ E) Pakistan. The rats were trapped by single catch traps, baited with pieces of guava and melon. The trapping was carried in night and trapped rats were collected early in the morning.

Acclimation of rats

The rats of approximate same size were sexed, weighed and caged individually in laboratory for 15 days. Sub-adults, pregnant and lactating females were discarded from the trials. The rats were fed on mixed grain diet, containing rice, millet, wheat and maize during acclimation period and between the trials. Water was provided *ad libitum*.

Preparation of bait

Gliricidia bait was formulated by mixing *Gliricidia* leaves extract 2.5g a.i (0.25 %), 5.0g a.i. (0.25%), 10.0g a.i. (1.00%) and 20.0g a.i. (2.00%) per 1000g wheat flour and broken rice (equal quantity). For comparative evaluation, bait without *Gliricidia*was prepared with a mixture of wheat flour and broken rice. The bait material was blended in an electric mixing machine by adding water. The stiff dough formed was rolled on board by roller and was cut in small pieces by a sharp knife. Bait was fan-dried and stored in plastic bags.

Experimental Design

Ten rats (five male and five female) were used in all trials beside control (one male and one female). The rats were weighed and caged singly, starved for four hours (before the start of each test) and offered 20g bait for each concentration for five days. Bait eaten (g), active ingredient ingested (mg/kg body weight) and mortalities were recorded. The behaviour and health of the rats was monitored continuously. Autopsy examination of dead rats was carried out to know the death cause.

Statistical analysis

The results were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range test and Fisher's least significant difference (LSD) test at P=0.05 (Zar, 2009).

RESULTS AND DISCUSSION

Gliricidia sepium, ethanolic leaves extract was tested in five doses 2.5g a.i. (0.25%), 5.0g a.i. (0.50%), 10.0g a.i. (1.00%) and 20.0g a.i. (0.20%) against field rats, *Bandicota bengalensis*. *Gliricidia* was given in bait form. The bait proved to be palatable; however the bait acceptance was inversely proportional to the dose, offered. By the increase of dose intake of the bait was reduced, however the intake of female rats was more than the male rats, except the dose 0.25% (Fig. 1). All the doses proved very effective against the rats. Rats became sick and sluggish after the *Gliricidia* bait intake (Fig. 1 and 2). Autopsy examination revealed internal bleeding (Fig. 5, 6, 7 and 8), which may confirm the presence of anticoagulant compounds in *Gliricidia*. These findings are in line with (Ahn, 1990) and Ahn *et al*. (1989) findings, in which they found depressed intakes, weight loss and fetal deaths in rats by offering diet containing 20% dried *Gliricidia* leaf. Sotelo *et al.*(1986) reported *Gliricidia* bark, leaves and seeds as rat poison in some countries; he reported a thermo stable toxin in seeds which killed mice within one week of feeding. Similarly in these trials, *Gliricidia sepium* leaves extract proved it as a very significant (P<0.05) anti-

coagulant by giving 100% and 50% mortality at 2.0% (in 5-8 days) and 1.0% (in 7-15 days) respectively (Table-1 and Fig. 3 and 4). Hochman (1966) observed the rodenticidal properties of *Gliricidia* by reporting the presence and conversion of coumarin (a constituent of phenolic fraction) into the hemorrhagic agent dicoumerol in rats fed leaves the plant. Similarly in our studies *Gliricidia* leaves extract served as a rodenticide by causing hemorrhages and pathological symptoms in rats (Table2). It appears that there is no relation in bait intake and time to death; however, the time to death was inversely proportional to the concentration used. *Gliricidia sepium* at 2.0%concentration gave 100% mortality with hemorrhage (bleeding from mouth and nose) and pulmonary distress, sluggishness at all doses. These findings are in accordance with the findings of Saxena and Sharma (1984), Mathur and Prakash (1980 a, b) and Soni (1981), who worked on the efficacy of brodifacoum against *F. pennanti*, *M. hurrianae* and *R. rattus.* They noticed poisoning symptoms such as pulmonary distress, sluggishness and hemorrhage (bleeding from mouth, nose and anal region) two days after feeding.

Table 1. Toxicological effect of *Gliricidia sepium* on *Bandicota bengalensis*

a.i*/ 1000g (conc. %)	No. of rats (M/F)	Mean body weight		Mean bait intake**		Mean a.i consumed/ kg body weight		No. of death	Days to death	
		M	F	M	F	M	F		Mean	Range
2.5 (0.25)	10 (5/5)	202.30 ± 5.53	219.24 ± 10.20	11.30 ± 1.19	9.26 ± 0.96	139.64 ± 3.46	105.59± 3.61	0/10	–	–
5.0 (0.50)	10 (5/5)	238.45 ± 8.73	285.40 ±9.97	9.61 ± 1.16	10.42 ± 1.04	201.50 ± 5.78	182.55± 3.62	3/10	12.00± 3.24	5-17
10.0 (1.00)	10 (5/5)	292.20 ± 6.00	225.15 ± 7.30	7.50 ± 0.70	8.40 ± 0.82	256.70 ± 4.94	373.08± 11.23	5/10	11.50± 2.40	7-15
20.0 (2.00)	10 (5/5)	317.41 ± 6.85	287.60 ± 11.98	3.70 ± 0.39	5.30 ± 0.85	233.14 ± 8.96	368.60± 8.85	10/10	6.75 ±1.99	5-8
Control (0)	10 (5/5)	275.92 ± 4.00	294.62 ± 12.90	13.15 ± 1.15	14.85 ± 1.50	-	-	0/10	-	-

*Active ingredient; **Bait offered=20 g; M = male; F = Female.

Table 2. Pathological and Hemorrhagic effect of *Gliricidia sepium* on *Bandicota bangalensis.*

Weight of a.i/ 1000g (% conc.)	No. of rats (M/F)	No. of sick rats*	No. of hemorrhagic rats	
			External	Internal***
2.5 (0.25)	10 (5/5)	1/10	-	-
5.0 (0.50)	10 (5/5)	4/10	0/10	3/10
10.0 (1.00)	10(5/5)	7/10	1/10*	4/10
20.0 (2.00)	10 (5/5)	10/10	6/10**	8/10
Control(0)	10 (5/5)	-	-	-

*Bleeding from nose. ** Bleeding from mouth and nose; *** Bleeding observed from liver and spleen.

Two factor ANOVA was performed for the data set pertaining to mean body weight, mean bait intake and mean a.i. consumed per kg body weight (Table 3, 4, 5). In case of mean body weight concentration was found highly significant ($p < 0.001$) while sex was found to be non-significant but interaction of concentration and sex was also significant ($p < 0.001$). The data of mean bait intake demonstrated that concentration was significant ($p<0.001$) but sex and interaction of concentration and sex were found non-significant, while data set of mean a.i consumed per kg of the body was revealed that concentration, sex and interaction of concentration and sex were highly significant ($p < 0.001$).

The findings may be a step towards confirmation of *Gliricidia sepium* as a toxic plant for development of safe, economical and eco-friendly substitute of conventional rodenticides and could be used for effective management of rodent pests in urban and field situations. Further study is recommended to identify the most toxic part of *G. sepium* used as bio-pesticide.

Table 3.Two factor ANOVA for the results of Mean body weight(factor 1 concentration, factor 2 Sex).

Source	SS	df	MS	F	P
Main Effect					
Concentration	48979.3	4	12244.8	0.8625	0.0000***
Sex	192.47	1	192.47	0.492	0.4867
Interaction					
Concentration × Sex	20764.9	4	5191.23	13.2914	0.0000***
Error	15622.7	40	390.5696		
Total	85559.5	49			

$LSD_{0.05}$ (Conc) = 17.862 $LSD_{0.05}$(Sex) = 11.297

Table 3a.(Cont.).Results of Duncan's multiple range tests. Means followed by different letters are significantly different ($P<0.05$) for factor concentration.

Rank	Treatment	Mean	n	Non- significant range
1	4	302.64	10	a
2	5	286.05	10	a
3	2	262.2	10	b
4	3	258.76	10	b
5	1	210.2	10	c

Table 3b.(Cont.).Results of Duncan's multiple range tests. Means followed by different letters are significantly different ($P<0.05$) for factor concentration.

Rank	Treatment	Mean	n	Non- significant range
1	1	265.944	25	a
2	2	262.02	25	a

Table 4.Two factor ANOVA for the results of Mean bait intake(factor 1 concentration, factor 2 Sex).

Source	SS	df	MS	F	P
Main Effect					
Concentration	493.71	4	123.428	26.517	0.0000***
Sex	6.53	1	6.5301	1.4029	0.243
Interaction					
Concentration × Sex	33.822	4	8.455	1.8166	0.1446
Error	186.181	40	4.654		
Total	720.248	49			

$LSD_{0.05}$= 1.9500 $LSD_{0.05}$= 1.2332

Table 4a. (Cont.).Results of Duncan's multiple range tests. Means followed by different letters are significantly different (P <0.05) for factor concentration.

Rank	Treatment	Mean	n	Non- significant range
1	5	14.33	10	a
2	1	10.965	10	b
3	2	10.3901	10	b
4	3	8.256	10	c
5	4	4.82	10	d

Table 4b. (Cont.).Results of Duncan's multiple range tests. Means followed by different letters are significantly different (P <0.05) for factor concentration.

Rank	Treatment	Mean	n	Non- significant range
1	2	10.1156	25	a
2	1	9.39	25	a

Table 5.Two factor ANOVA for the results of Mean a.i consumed/ kg body weight(factor 1 concentration, factor 2 Sex).

Source	SS	df	MS	F	P
Main Effect					
Concentration	684693.8	4	171173.4	897.93	0.0000***
Sex	19802.7	1	19802.7	103.88	0.0000***
Interaction					
Concentration × Sex	62948.34	4	8.455	82.553	0.0000***
Error	7625.186	40	4.654		
Total	775070	49			

$LSD_{0.05}$ (conc) = 12.479 $LSD_{0.05}$(sex) = 7.8926

Table 5a. (Cont.).Results of Duncan's multiple range tests. Means followed by different letters are significantly different (P <0.05) for factor concentration.

Rank	Treatment	Mean	n	Non- significant range
1	3	314.998	10	a
2	4	300.689	10	b
3	2	192.46	10	c
4	1	122.46	10	d
5	5	0	10	e

Table 5b. (Cont.).Results of Duncan's multiple range tests. Means followed by different letters are significantly different (P <0.05) for factor Sex.

Rank	Treatment	Mean	n	Non- significant range
1	2	206.03	25	a
2	1	166.228	25	a

Fig.1and 2. Sick and sluggish rats after *Gliricidia* bait intake.

Fig. 3and 4. Dead rats after *Gliricidia* bait intake.

F ig. 5 and 6. Autopsy examination of dead rats after *Gliricidia* bait intake.

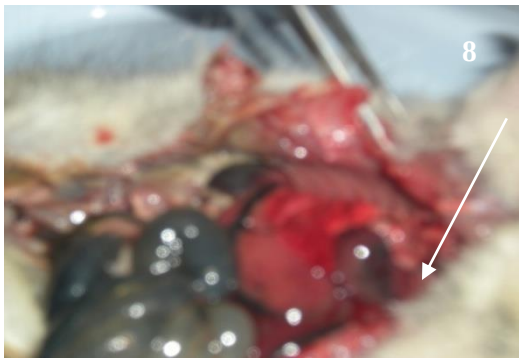

Fig.7 and 8. Bleeding from liver and spleen of dead rats after *Gliricidia* bait intake.

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