

INSECTICIDAL, NEMATICIDAL AND ANTIBACTERIAL ACTIVITIES OF *GLIRICIDIA SEPIUM*

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Abstract

Gliricidia sepium is an exotic plant belonging to the family *Fabaceae*. This plant has been cultivated in Pakistan for the first time. For the preliminary studies, Ethanol extract of leaves has been used to check the efficacy on parasitic nematodes, clinical pathogens and mosquito's repellent activity. Nematicidal property of extract was observed in different concentration against *Meloidogyne incognita* nematode showing 60% mortality. Mosquito's repellent activity has been studied against *Aedes aegypti*, the maximum repellency was 78% compared with the citronella oil, the repellency was 74%. Using well diffusion method the antibacterial activity of ethanol extract against *E.coli*, *S.aureus*, *Pseudomonas* spp., *S.typhi*, *Klebsillia* spp., showed the best result against *Escherichea coli*.

Introduction

Insect's transmitted disease remains a major source of illness and health hazard worldwide. Mosquitoes alone transmit disease to more than 700 million people annually (Taubes, 1997). Malaria alone kills 3 million each year including 1 child every 30 seconds (Shell, 1997). Although mosquitoes-borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk (Fradin & Day 2002). A person normally acquires malaria only through the bite of an infective female *Anopheles* mosquitoes which has previously obtained the plasmodia from a malaria patient. *Aedes aegypti* mosquitoes transmit many serious diseases like Dengue fever and Yellow fever which has recently struck Pakistan and has almost turned up to an epidemic proportion. This mosquito thrives in urban and suburban neighborhood because backyard containers, buckets, water cans, etc., offer ideal breeding conditions for them. Control of such diseases is becoming increasingly difficult because of increasing resistance approach to pesticides (Ranson *et al.*, 2001). An alternative approach for mosquito control is the use of natural products of plant origin.

Plant materials have been used for pest control for centuries currently, however, only a few plant species are being used for pest control in various countries and that too primarily under traditional farming systems. *Gliricidia sepium* a leguminous tree belongs to the family *Fabaceae* (Chadhokar, 1982). *Gliricidia*, which literally means "Rat poison" originated in central America and its plantation, has spread to many parts of the world specifically South Asia. The plant is used for fuel wood, animal feed, green manure, shade, living fences and as support plants (Csurhes & Edwards, 1998). The leaves of *G. sepium* has 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% (Adejumo & Ademosin, 1985; Gohl, 1981).

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There are numerous reports of increases in weight gain and milk production in both large and small ruminants when *Gliricidia* forage is used as a supplement. Nochebuena & O'Donovan, (1986), reported that for Tabasco sheep in Mexico, both intake and dry matter digestibility increased when this plant was used as a supplement, up to 30% of the diet with grass hay. Interestingly, *Gliricidia* means Mouse or Rat killer, which is derived from its bark & leaves which when cooked with grain can be used as poisonous bite for rodent. Though poisonous to rodents and insects, the leaves contain 3-4% dry weight of nitrogen and small amount of phosphorus, potassium, calcium and magnesium, so they can be used as excellent green manure and fodder. The present studies were focused on the ability of the plant or root to decrease soil nematodes population and control insects and fungi. Researches have been conducted on both the antifungal and antimicrobial properties of *Gliricidia* plant extracts.

There are many different species of root feeding nematodes. They attack a wide range of plants including many common vegetables, fruits, trees and ornamental plants. Poisonous effect of nematicides on beneficial soil, micro organisms, human health and environmental pollution (Reimann *et al.*, 2001), due to these problems scientists prefer other method of control such as biocontrol and organic amendment (Alam & Jairpuri 1990). *Gliricidia* is a useful tree that will be of immense help in increasing the yield of crop in Pakistan. The temperature 20.7-29.2°C at native site. It is largely deciduous during the dry season, which runs from January to the first rains in May. In area where sufficient moisture prevails, however, the tree does not become leafless (Seibert, 1987). The leaves can be used as fodder for cattle that improves their health and increases milk yield. It is an ideal for fattening cattle fold during Eidul Azha; the valuable feed resources improve the weight.

Material and Methods

Plant collecting and processing: *Gliricidia* plant leaves were collected from Coastal Agricultural Research Station, SARC, PARC, Karachi. All the leaves samples were preserved in wax-quoted paper bags and brought to the laboratory for biological assays.

Plant extraction: The fresh dried plant of *Gliricidia sepium* (5kg) was ground and soaked in ethanol (commercial, doubly distilled 50 lit). The filtrate was concentrated under reduced pressure at 40°C to a gum. This crude gum was used for activity purpose.

Rearing of the test insects: Laboratory-reared female mosquitoes *Aedes aegypti* of 4-5 days old were placed into separate laboratory cages measuring 1x1ft. The temperature was maintained at 27°C and 85% relative humidity.

Mosquitoes repellent activities: The duration of protection provided by each product was tested by means of arm-in-cage studies, in which volunteers insert their repellent treated arms in to a cage with a fixed number of unfed mosquitoes as a described by Granett & Starnes, (1960). 0.2 ml of the *Gliricidia* leaves ethanol extract was applied evenly over a hand and a part of a forearm so as not leave any area uncovered. The treated area of a hand was then exposed for 5 minutes at half an hour intervals in a cage containing 50 unfed mosquitoes, *Aedes aegypti* of 4-5 days old. The time elapsing between the treatment and the first confirmed bite was determined as the maximum effective repellent time. Results obtained with the *Gliricidia* extract were compared with the standard repellent Citronella oil.

Nematicidal activities: Measured quantities of plant leaves extract was dissolved in water to make different dilution, G/1, G/2, G/3, G/4 and control. To determine the nematicidal effect of plant extract 100 freshly hatched second stage juveniles of *Meloidogyne* spp, were transferred to different Petri dishes. Measured amount of all dilution were added and only distilled water was taken as control. Each treatment was replicated thrice. The Petri dishes were kept at room temperature $28\pm 2^\circ\text{C}$ for 45 hours. The number of active and Inactive nematodes was confirmed, by keeping them in distilled water for 24 hrs, percentage mortality was calculated.

Antibacterial analysis

Preparation of samples: The concentrated residues were suspended in an emulsifier, polyethylene glycol 400 (PEG-400) which has no inhibitory effect on fungal and bacterial growth. The organic extracts were dissolved in this solvent. Aliquots of this solution (5% extract solution) were used to test for antibacterial activity against pathological cultures of *E. coli*, *S. aureus*, *Pseudomonas* spp., *S. typhi*, *Klebsillia* spp.

Well diffusion method: 24 hrs fresh broth cultures (media; nutrient broth, merk) were used. Seeding of media (Muller hinton-Merk) with test cultures were initially done and then well were dug in media. Extract were filled in well and plates were incubated at 37°C for 48 hrs. The clear zone was measured against the test culture.

Results and Discussion

The antimicrobial activity of *Gliricidia sepium* (Leaf ethanol extract) was assayed invitro by well diffusion method against clinical isolates of *E. coli*, *S. aureus*, *Pseudomonas*, *S. typhi*, *Klebsillia* sp. Summarized the microbial growth inhibition by ethanol extract (Table 1). The plant extract was particularly active against *E. coli*. The sequence of antibacterial activity of plant extract can show as 1.5cm, 1.3cm, 1.2cm, 1.1cm, and 1.0cm of zone of inhibition on plate assay. The highest activity was noted against *E. coli* bacteria. The reason for different sensitivity could be due to morphological difference between micro-organisms. The result obtained might be considered sufficient for further studies for isolation and identification of active principle & for the evaluation of possible antimicrobial activity of other extract from other parts of the plant.

Ethanol leaf extract of *G. sepium* was tested for its nematicidal property at different concentration against *Meloidogyne incognita* nematodes. It has been observed that G/2 dilution is highly significant showing 60% mortality against control and G/3, G/4 (Table 2). Sixty percent mortality of nematodes in above mentioned plant extract can be attributed to the presence of some toxic chemical in them. Although 97% mortality in G/1 dilution, but may be this concentration toxic for plant would be effect on growth yield.

Repellent activity of *G. sepium* (ethanol extract) has been studied by testing it against adult Mosquito *Aedes aegypti* (L.). Results were compared with the citronella oil. The maximum repellency percentage afforded by the repellent *G. sepium* was 78%, whereas with citronella oil, the repellency percentage was 74% respectively (Table 3). As a result of the present studies it may be concluded that the ethanolic leaves extract of *Gliricidia sepium* is non-irritating and longer lasting than the standard repellent.

In this context, it is worth mentioning that research in this field would open door of future exploitation of our natural heritage and its commercialization in modern era.

Table 1. Antibacterial activity of ethanol extract of leaves of *Gliricida sepium*.

S. No.	Strains	Plant part used	Inhibition zone (cm)
1.	<i>E. coli</i>	Leaf extracts	1.5
2.	<i>S. aureus</i>	--	1.1
3.	<i>Pseudomonas</i> spp.	--	1.2
4.	<i>S. typhi</i>	--	1.0
5.	<i>Klebsillia</i> spp.	--	1.3

Table 2. Effect of *G. sepium* extracts on nematode (meloidogyne) mortality number of mortality (initial no; of nematodes 50).

S. No.	Concentration of extract	Plate 1	Plate 2	Plate 3	Plate 4	Mean	Percentage %
1.	G/0	02	01	04	01	02	4
2.	G/1	50	48	46	50	48.5	97
3.	G/2	30	33	28	29	30	60
4.	G/3	17	21	10	13	15.25	30

Table 3. Efficacy of *G. Sepium* (ethanol extract) as Repellent against Mosquitos *Aedes egypti*.

S. No.	Name of repellent	Dose ml	Replicates	No. of insects released in each cage	No. of insects repelled in each cage	Mean	Repellency percentage
1.	<i>Citronella Oil</i>	0.2 ml	1	50	41	37	74
			2	50	42		
			3	50	39		
2.	<i>G. sepinum</i>	0.2 ml	1	50	40	39	78
			2	50	38		
			3	50	39		
3.	Control	0	1	50	0	--	0
			2	50	0		
			3	50	0		

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