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Extraction, partial purification and pesticidal activity of plant lectin against major groundnut defoliator *Spodoptera litura* (Fab.) (Lepidoptera:Noctuidae)

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ABSTRACT

In the present study, pesticidal activity of lectin against different life stages of major groundnut defoliator *Spodoptera litura* has been studied under *in vitro* condition. Lectin was isolated from pulp of banana and partially purified by silica gel and mannose – sepharose 4B affinity chromatography and the total yield of affinity purified lectin was about 50 mg. Bio assay was studied against second and third instars of *S. litura* which reveals both the instars were susceptible to the lectin in concentration dependent manner. Distinct effect on lethal concentration 50 (LC 50), lethal time 50 (LT 50) against the larval instars, pupal, adult emergence and adult longevity was observed.

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Key words: Lectin, *Spodoptera litura*, pesticidal activity.

INTRODUCTION

Arachis hypogea, L. (Leguminaceae) is an important oil seed crop in India. It occupies 8.6 million hectares, of which 85 per cent is rainfed and 15 per cent is irrigated (Asensio *et al.,* 2003). India ranks first in area (7.26 million hectares) as well as in production of groundnut (7.85 million tones) followed by China (3.41 million hectares, 7.57 tones) (Enkerli *et al.,* 2005). The edible oil economy of the country primarily depends upon the groundnut production (Brar *et al.,* 2004). The state of Tamil Nadu in India grows about 1.1 thousand hectares of groundnut in three seasons (Mccoy *et al.,* 1988). Insect pests are the major constraints in groundnut production (Sahayaraj *et al.,* 2003; Sharma, 2004). More than 360 species of insects and mites were reported to attack the groundnut crop in the field and the pods in storage all over the world (Stalker and Cambpell, 1983). Among the various pests, *S. litura* is widely distributed throughout Asia and the Pacific islands. It is an important polyphagous pest reported to feed on 112 species of plants belonging to 44 different families. Sahayaraj *et al*. (2003) have reported that one *S. litura* larva per plant at seedling stage reduced the pod yield by 25.8 per cent. The early larval stages of *S. litura* feed on the leaves, flowers and pods of groundnut and reduce the production, whereas, the late larval stages feed on the pods in addition to the above mentioned parts. The defoliator population in groundnut ecosystem has been found to increase in number and intensity both during rainy and post rainy season, due to the destruction of natural control system, especially in fields where insecticides have been applied (Manjula *et al.,* 2004). The management of this pest using chemical insecticides is unsuccessful because of its insecticide resistance (Kennedy *et al.,* 2001).

Even though chemical pesticides are used to control the pest, the indiscriminate use of these chemical pesticides lead to various health hazards and insecticide resistance (Sharma, 2004). The development of pest control measures using biological methods has received increasing attention in recent years (Enkerli *et al*., 2004; Sahayaraj and Karthick Raja Namasivayam, 2008). Plant-based biopesticides (botanicals) and their metabolites are now being extensively used as a component of IPM (Padmaja, 2005). It has already been reported that plant species possessing pest control properties included 1005 species with antifeedent, 1297 species with repellent, 27 species with attractant and 31 species with growth inhibition properties (Babu

et al., 2001; Sahayaraj and Karthick Raja Namasivayam, 2011). Among the different plant species and metabolites, plant lectin from various plants is known to cause pesticidal effect against major insect pests. In the present study, plant lectin was isolated from pulp of banana and evaluated for the pesticidal activity against *Spodoptera litura.*

MATERIALS AND METHODS

Insect collection

The egg masses and larval instars of *Spodoptera litura* were collected from the groundnut field in an area around Vizhupuram and Thiruvallur district, Tamilnadu, India. Identification of the pest was studied using the criteria suggested by Wightman and Rao (1994). Collected larvae were maintained on groundnut leaves.

Isolation of plant lectin

Banana was purchased from retail market and kept in refrigeration until processing. The isolation and purification of lectin was done according to the modified method of Amin Sadeghi (2007). Pealed, over-ripped banana (1kg) was immersed in a solution of 25 mL acetic acid soaked overnight at 4°C, and homogenized with a domestic mixture. This was kept at 2°C for 24 hrs to remove the foam. The foam removed extract was poured through cheese cloth and adjusted to pH 3.0 with 1N acetic acid and centrifuged at 9000 rpm for 15 min. A supernatant was filtered through filter paper and loaded on the silica column and washed with forinate buffer. The bound protein eluded in a single step with 200 mL of 1N NaCl in forinate buffer. Lectin was isolated from protein fraction by affinity chromatography on immobilized mannose – sepharose 4B (2.6cm diameter,10 cm long, 50 mL bed volume) equilibrated with formate buffer containing 0.2M NaCl, the column was washed with the same buffer until the A_{280} fell acetic acid. Then the pH of the lectin solution was adjusted to 3.8 and solid NaCl was added to final concentration of 0.2M. After standing overnight in the cold, the lectin solution was cleared by centrifugation (9000 g for 15 min) and affinity chromatography, lectin was eluted with 0.1M mannose in formate buffer, dialyzed against appropriate buffers and frozen at 20°C until use. The total yield of purified lectin was about 50 mg.

Laboratory bioassay on *S. litura*

The second and third instar larvae of *S. litura* were selected for bioassay studies. 20 larvae in each instar (second and third) of *S. litura* and *H. armigera* were sprayed with 1, 10, 100 and 1000 mg/mL of the respective polymer coated plant extract using ULV (Ultra Low Volume) sprayer. The treated larvae were introduced into the plastic container (34mm X 21mm) provided with moist cotton swab covered with tissue paper at the bottom of the container to provide humidity. The containers were covered with meshed lid to provide aeration to the larvae. Another 20 larvae of each instar treated with distilled water only served as control. The containers were incubated at room temperature 28 ± 0.5 ^{o}C in an incubator (Remi BOD incubator, Mumbai, India). Daily observation on larval mortality was recorded for a period of 10 days. The total larval and pupal durations, adult longevity, and the adult emergence were recorded.

The LT_{50} of the dose of Polymer coated plant extract to kill the different larval instars was assessed in hours followed Blever and Hostetter (1971).

$$\mathbf{LT_{50} = a + e\ c - b\ /d}$$

Where, a = the no of hours from the initiation of the test until the reading made just before the 50% value was recorded; b= the total number of larvae dead at the reading just before 50% value was recorded; c = 50% of the total number tested; d = the no of larvae dying in 24 hrs period during the 50% mortality was reached and e = the number of hours between mortality counts. The dose mortality data were subjected to probit analysis (Finney, 1962) for $LC_{50.}$

RESULTS AND DISCUSSION

Isolation of lectin

Crude extract from pulp of banana was prepared by homogenization of 1gm of tissue) in 10mL of 0.2mL of NaCl clearly reveals the Pesticidal activity against both the tested pests. After the crude extraction of lectin and concentration on a cation exchange column, the lectin reminded in solution and could be purified by affinity chromatography on immobilized mannose. The overall yield of affinity purified lectin was 50mg/kg crude pulp of banana.

Pesticidal activity of lectin

All the tested instars of *Spodoptera litura* were susceptible to all the tested concentration of lectin.

Maximum mortality was recorded in second instar in all the tested concentration except 10µg/mL. 100% mortality was recorded in 75 and 100 µg. 80% and 75% mortality was recorded in 50 and 25 µg/mL (table 1). In the case of 3rd instar larvae maximum mortality was recorded in 100 µg/mL which reveals 85.0 followed by 65.0 in 75 µg/mL concentration. 30.0 and 50.0 % mortality was recorded in 10 and 25 µg/mL of lectin (Table 1).

Table 1. *Mortality of Spodoptera litura larval instars treated with lectin.*

Lectin Concentration (µg/mL)	**Mortality (%)**	
	2nd instar	**3rd instar**
10	50.0	30.0
25	75.0	50.0
50	80.0	60.0
75	100.0	65.0
100	100.0	85.0

LT_{50} and LC_{50} of larval instars

The present mortality and LT_{50} of 2nd and 3rd instars are *Spodoptera litura* was presented in Table 2. The LT_{50} increased as the larvae grew older as well as the increase in the concentration of lectin used. As the instars advanced a decrease in mortality and increase in time and initial mortality was recorded. In value of LT_{50} for second instar larvae ranged from 1061 to 5.27 as the concentration of lectin was increased from 10 µg/mL t0 100 µg/mL. Respective value for third instar larvae of *S. litura* were 2.17 and 6.27 (table 2).

Table 2. Effect of lectin on LT_{50} of *Spodoptera litura.*

Lectin Concentration (µg/mL)	**LT_{50}**	
	2nd instar	**3rd instar**
10	1.61	2.17
25	2.13	3.07
50	3.17	3.57
75	4.21	4.51
100	5.27	6.27

The result of LC_{50} value determination through probit analysis was presented in table 2. Among the various estimate of regression based probit analysis, the chi-square test of the bioassay showed homogeneity of the test population which is a reflection of a good fit of the observed and expected response. From the table 3 it is very clear that the LC_{50} values of different larval instars of *Spodoptera litura* in response to lectin showed an increased trend in the LC_{50} value, when the age of larva was advanced. The median lethal concentration of 2nd and 3rd instar of *S. litura* was 4523.77mg and 13473.80mg respectively. Similar finding of Pesticidal activity of plant lectin isolated from *Viscum album* against *Apamea sordens* Hufn. and *Pyrausta nubilalis* (Keburia *et al.*, 2010), Soybean against *Helicoverpa armigera* (Sonali Shukla *et al.*, 2005), garlic against *Spodoptera litura* (Amin Sadeghi *et al.*, 2007) have been reported. Further study under microplot and field condition is in progress which would suggest the possible utilization of lectin as an effective biopesticidal agent against *Spodoptera litura.*

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Table 3. Toxicity of tested samples against *Spodoptera litura* second and third instar larvae.

LC_{50} (mg)	95% Confidence limit		LC_{90} (mg)	95% Confidence limit		Chi square value
	Lower	Upper		Lower	Upper	
Second instar larvae						
71.01	31.12	184.59	4523.77	1128.90	63898.54	0.036*
23699.88	2273.39	25468.00	5344184.00	64944.65	10053370.00	0.810*
15343.20	2214.94	57868423168.00	703534.31	21087.68	761807901.29	0.563*
2149.05	451.77	132843.00	845946.50	29095.87	3181850.82	0.658*
Third instar larvae						
172.21	72.04	578.47	13473.80	2590.73	397025.40	0.004*
49726.13	-	-	2758214.50	-	-	0.238*
-	-	-	-	-	-	-
5026.23	575.64	261078.10	13528330.00	90845.60	1045829.00	0.464*

LC_{50} and LC_{90} values are expressed as percentage (n=24); * $\chi 2$ values are significant at $P \leq 0.05$ levels

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