Bioefficacy of a mangrove plant, *Sonneratia caseolaris* and a mangrove associate plant, *Hibiscus tiliaceus* against certain agricultural and stored product pests

Pathipati Usha Rani, Kurra Sandhyarani, Varahalarao Vadlapudi, Bojja Sreedhar ABSTRACT

The insect antifeedant and toxic activity of two marine plants. *Hibiscus tiliaceus* and Sonneratia caseolaris on the herbivorous insects, Spodoptera litura F. were tested in the laboratory. The crude extracts were further purified in a column and their purified fractions were assessed for their antifeedant and insecticidal activity. The leaves extracted separately with acetone yielded crude extract which showed significant antifeedant activity to the 2nd as well as 3rd instar larvae of S. litura. The topical application of the plant crude extracts resulted in causing toxicity to the lepidopteran pest which was not more than 50 per cent with both the plant treatments. The extracts had considerable effect on the S. *litura* metamorphosis in the form of a delayed pupal formation and morphogenetic abnormalities in pupae due to the larval treatment, which affected the percentage adult eclosion. The crude leaf extract possesses moderate insecticidal activity to three of the major stored product pests, the flour beetle, Tribolium castaneum H., the rice weevil, Sitophilus oryzae L. and the lesser grain borer Rhyzopertha dominica (F.). All eluted fractions from H. tiliaceus and S. caseolaris have shown excellent antifeedant activity on S. litura than crude extracts. Particularly hexane eluted fraction of the crude extracts of both the marine plants showed potent growth inhibitory activity. We infer that the marine dwelling plants H. tiliaceus and S. caseolaris extracts has good feeding inhibitor activity to S. litura and a moderate toxicity to three of the major stored product pests.

MS History: 09.08.2015 (Received)-17.010.2015 (Revised)-18.11.2015 (Accepted)

Key words: Toxic activity, *Hibiscus tiliaceus*, antifeedant activity, Metamorphosis, Inhibitor activity.

Citation: Pathipati Usha Rani, Kurra Sandhyarani, Varahalarao Vadlapudi and Bojja Sreedhar. 2015. Bioefficacy of a mangrove plant, *Sonneratia caseolaris* and a mangrove associate plant, *Hibiscus tiliaceus* against certain agricultural and stored product pests. *Journal of Biopesticides*, 8(2): 98-106.

INTRODUCTION

Mangrove plants are a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids, tannins (Nayak et al., 2014) that show different biological activities such as antibacterial, antifeedant, antifungal. molluscicidal. and pesticidal properties (Varahala Rao and Naidu, 2009). As several distinct chemicals are present in the extracts of various parts of the mangrove plants, they have been used for centuries as a popular method for treating several health disorders (Nayak et al., 2014). Of late, research interest on mangrove plant exploration for medical usage and their utility in drugs for several ailments such as cancer, aids, etc has been increased (Batsa and Periyasamy, 2013).

However, only a few number of reports are available on their agricultural use, particularly their utilization in pest management practices (Jeyasankar *et al.*, 2014; Kabaru and Gichia, 2001).

Coringa mangrove forests situated in the estuary of river Godavari supports rich mangrove vegetation with species like Rhizophora, Avicennia, Sonneratia, and Aegiceros and is the largest surviving patch of Mangrove forests in the state of Andhra Pradesh with more than 65 Mangrove tree species (ICMAM) citations). Among these, mangrove associates, Hibiscus and Sonneratia are the two important genuses. Hibiscus tiliaceus Linn. (Malvaceae) is a mangrove associate; which is commonly known as sea

Pathipati Usha Rani et al.,

hibiscus that occurs in the coastal environment and is also found within mangroves (Wong and Chan, 2010). Sea hibiscus is well adapted to grow in coastal environment in that it tolerates salt and water logging and can grow in quartz sand, coral sand and limestone. It is a fast-growing tree that reaches 15 m tall (Chan and Baba, 2009), and has several therapeutic uses such as cooling fever, soothing cough and removing phlegm, etc., whereas, flowers are used in treating ear infection and abscess and birth control in Asia and Africa (Rosa et al., 2006). Hibiscus genus are generally rich in a variety of bioactive molecules, such as lignanamides, phytosterols naphthalenes. tocopherols, polyphenols, carotenoids. flavonoids, anthocyanins, and long chain fatty esters (Holser et al., 2004). The plant is edible and almost all parts are consumed as vegetable in several regions of the world (Jariyah et al., 2014).

The genus Sonneratia consists of nine species in the tropical and subtropical regions worldwide (Wang and Chen, 2002). It is reported that Sonneratia caseolaris L. (Lythraceae) is a mangrove plant among the Sonneratia family and is traditionally used as an astringent and antiseptic, sprain poultices, in treating piles and also in arresting hemorrhage (Bandaranayake, 1998). Since none of these plants were explored for their insect pest control activity, here we aimed at studying their bioefficicay against a major agricultural pest, Spodoptera litura F, and three major stored product pests, Tribolium castaneum, Rhyzopertha dominica (F.) and Sitophilus oryzae L.

Spodoptera litura (Fab.) (Lepidoptera: Noctuidae), is a notorious polyphagous pest distributed throughout the world. In India, S. litura severely damages several plants and the crop losses due to this pest vary between 10% and 30% in major crops (Ferry et al., 2004). product The stored pests, Tribolium castaneum (Herbst) (Flour beetle), Sitophilus oryzae L. (rice weevil) and Rhyzopertha dominica (F.) (Lesser grain borer) occur worldwide and cause serious problems to stored and processed grain

products by reducing their dry weight and nutritional value. The present work reports the insect antifeedant activity as well as the larval and pupal toxicity of the leaf extracts of two marine plants *H. tiliaceus* and *S. caseolaris* to *S. litura* and also fumigation toxicity against stored grain pests *T. castaneum*, *R. dominica*, and *S. oryzae*.

MATERIALS AND METHODS Insects and plant materials

Castor (Ricinus communis L.) plants of known variety (Kiran var.) were grown in the fields of the CSIR-IICT, Hyderabad, Telangana, India. The fresh castor leaves were collected daily and were used for rearing S. litura larvae and for the antifeedant bioassays. Spodoptera litura larvae used in this study were obtained from a laboratory culture maintained in the insectary at controlled laboratory conditions $(28 \pm 2^{\circ}C)$, 65 ± 5% RH and 16: 8 L: D photo period. Neonate larvae emerged from single egg mass on the same day was fed with fresh castor leaves. Healthy second and third instar larvae were used for the experiments. Tribolium castaneum, S. oryzae and R. dominica were grown in plastic containers separately in the insectary over 2 years without exposure to insecticide and in controlled conditions, $28\pm2^{\circ}$ C, and $65\pm5\%$ RH. The diet consisted of semi crushed Jowar (Sorghum bicolor) mixed with yeast (10: 1, w/w). The adult beetles of 1-5 d old were used in laboratory bioassays.

Collection and extraction of Plant materials Marine plant Hibiscus tiliaceus (Malvaceae) Linn and Sonneratia caseolaris (Lythraceae) were collected from Pedavalasa village, 10 km from coringa mangrove forest and near to Yanam, Kakinada, Andhra Pradesh, India. The leaves were separated and washed with water. The shade dried plant material (500 gms) was powdered mechanically using commercial electrical stainless steel blender and extracted with acetone in soxhlet apparatus (1000 mL) until exhaustion. The extract was concentrated in rotary evaporator under reduced pressure at 45°C, and the residue obtained was termed as crude leaf extract which was stored at 4°C till further use.

Fractionation by chromatography

The crude extracts Н. tiliaceus and S.caseolaris were chromatographed on a silica gel column (50 cm length and 4 cm diameter), with Hexane [(100%)](Fraction 1)].chloroform [(100%) (Fraction 2)],ethyl acetate [(100%) (Fraction 3)]; methanol [(100%) (Fraction 4)] as eluents. Each eluted material was further concentrated using a rotary vacuum evaporator (Heidolph Laborota 4000) to remove excess solvent and kept at -20°C till further use in bioassays.

Antifeedant Assay

Antifeedant activity of crude extracts of H. tiliaceus and S. caseolaris was assessed against the phytophagous pest, S. litura. The experiments were conducted in the laboratory using leaf-disc method (Devanand and Usha Rani, 2008). The method consists of exposing a known area of surface treated castor leaf disc to starved larvae which are in active feeding stage and measuring the quantity of leaf disc consumed. A small circular disc (21 cm^2) was cut from the fresh castor leaves. Crude extracts of *H. tiliaceus* and S. caseolaris at different concentrations (100 and 200 mg/ 21cm²) and purified fractions (1 mg/ 21cm²) were applied separately on the upper surface of the leaf disc with the aid of a micro pipette. After evaporating the solvent for about 30 sec at room temperature, leaf discs were kept in individual Petri plates (9 cm dia) lined with wet filter papers to prevent desiccation. In each petriplate single pre starved (for 3 hrs) 2nd and 3rd instar larvae of S. litura were introduced separately and the larvae were allowed to feed on treated discs for a period of 24 hrs. The leaf discs sprayed with acetone alone was the control.

All the bioassays were repeated three times and there were ten replicates per each trial. The leaf area consumed and larval mortality due to feeding if any were measured after every 24 hrs using leaf area meter (Area meter AM 300, ADC Bioscientific Ltd). After 24 hrs, the left over leaf were retrieved and the percentage feeding calculated according to the following formula. 100

The antifeedant index (AI) was calculated as $(C-T)/(C +T) \times 100$, where C is the consumption of control discs and T, the consumption of treated discs (Belles *et al.*, 1985).

Contact Toxicity of the Mangrove Leaf Extracts

The experiments were conducted to evaluate the contact toxicity of the mangrove plant extracts. For this, the topical application method as described by Usha Rani and Rajasekarreddy (2009)was employed. Different concentrations (100 and 200mg) of crude extracts in acetone and purified fractions $(2\mu L)$ were applied on to the dorsal thoracic region of second and third instar larvae of S. litura using a micro syringe. The larvae treated similarly with acetone as control. After the application the solvent was evaporate and allowed to the larvae transferred to plastic containers (500 mL capacity) having fresh castor leaves as food. The experiments were conducted in replicates. There were five replicates for each treatment and each treatment contained 10 larvae. The bioassays were conducted at laboratory temperature of $28\pm2^{\circ}$ C, and relative humidity of 65±5%. Per cent mortality was calculated according to Abbott (1925).

Fumigant toxicity of the extracts

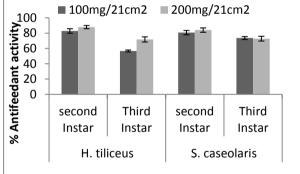
The fumigation toxicity of the leaf extracts of H. tiliaceus and S. caseolaris plants were evaluated against the three stored product pests according to a method described by Usha Rani and Rajasekharreddy (2010). Filter paper cut into small strips were treated separately with each test extract (50, 100, 150 and 200 mg $/100\mu$ L) and purified fractions (50 μ L) and were hung from the underside of the lid of a plastic container (100 mL capacity) having 20 gms diet of each pest insect. About 20 adults, 2 to 5-d old of each species of stored product pests, were released into the containers followed by closing the lid tightly. This prevented direct contact of the insects with the test compound. Solvent treatment was considered as controls. All tests were carried out at 28±2°C temperature, and 65±5% relative humidity. After 24 hrs of treatment,

the containers were opened and checked for the dead insects. Mortality was ensured by probing insect body with a slender paintbrush. Dead insects were counted every 24 hrs up to 72 hrs of treatment. There were five replicates per treatment while the tests were repeated 3 times on different dates to avoid any day-today variation if any.

RESULTS AND DISCUSSIONS Insect antifeedant activity of the mangrove

plants Antifeedant property of *H. tiliaceus* and *S. caseolaris* leaf extracts was assessed by comparing the percentage leaf area consumed in the treated leaves with that of the control. Reduced food intake by *S. litura* was observed in both the plant extracts treated leaf discs. The highest per cent antifeedant activity was noted in the extract of *H. tiliaceus* (df, 4, 45; F=26.7, P=<0.001) treated castor leaves followed by *S. caseolaris* (df, 1,14; F=39.9, P=0.001) treated leaves with second instar larvae at 100mg/21cm² concentration (Fig 1). **Fig 1.** Percent antifeedant activity of *H.*

tiliaceus and *S. caseolaris* extracts on the second and third instar larvae of *S. litura*.



Values are mean of the five replicates of three trials \pm standard error (ANOVA followed by TUKEY test performed, P< 0.001).

Interestingly both the mangrove plants caused almost similar present of antifeedant activity and there was not much difference (Fig 1) in the area fed by 2nd instar larvae in both the plant treatments. However, the efficiency of the mangrove plants decreased with the in age. increase At the dosage of $100 \text{mg}/21 \text{cm}^2$ the third stadium larvae could consume a limited area (df, 9, 90; F=4.74, P = < 0.001). Also the difference between the antifeedant efficiency of S.

caseolaris and H. tiliaceus towards the larvae was not very significant (df, 3,16; F=36.8, P = < 0.001 at 200 mg/21cm². There was a drastic difference in consumption between 2nd and 3rd instar S. litura larvae on H. tiliaceus treated leaf at this dosage. The data also indicated clearly that antifeedant activity had increased to 87.8 and 84 percent with increase in concentration against second instar larvae. It appeared that the marine plants, S. caseolaris and H. tiliaceus had good feeding inhibitory activity towards the pest, S. litura (Fig 1). The hexane eluted fraction (79.3 percent) of the plant *H. tiliaceus* followed by methanol (76.5 percent) and ethyl acetate (60.7 percent) showed reduced feeding rate on S. *litura* at $1 \text{ mg}/21 \text{ cm}^2$ concentrations (Table 1). Antifeedant activity of hexane fraction of caseolaris showed maximum feeding S. deterrent activity (74.6 %) when compared to other fractions such as methanol (53.6 %) and ethyl acetate (42.3 %) (Table 1).

Table 1. Percent antifeedant activity of *H. tiliaceus* and *S. caseolaris* fractions on third instar larvae of *S. litura*.

| Fractions | $1 \text{ mg/}21\text{cm}^2$ | | | | |
|-----------|------------------------------|----------------|--|--|--|
| | H. tiliaceus | S. caseolaris | | | |
| Hexane | 79.3±0.5 | 74.6±1.7 | | | |
| Ethyl | 60.7 ± 0.3 | 42.3 ± 0.2 | | | |
| acetate | 00.72 0.5 | 12.5 ± 0.2 | | | |
| Methanol | 76.5±1.1 | 53.6 ± 0.9 | | | |

Values are mean of the five replicates of three trials \pm standard error (ANOVA followed by TUKEY test performed, P<0.001).

Insect toxicity of the mangrove plant leaf extracts

The topical application of *S. caseolaris* and *H. tiliaceus* plant leaf extracts at 100 mg concentration caused larval mortality in different ranges (Table 2). The treated larvae were reduced in size (Fig-1) and lethargic in nature when compared to those in the control. However, majority of the treated 2^{nd} instar larvae, about 70% in *H. tiliaceus* and 60% in *S. caseolaris*, metamorphosed into normal pupae, whereas 76% *H. tiliaceus* and 63% *S.*

Bioefficacy of two mangrove plants against certain pests

JBiopest 8(2):98-106 (2015)

102

caseolaris treated third stadium larvae at a concentration of 100 mg.

Table 2. A morphogenetic effect of *H. tiliaceus* and *S. caseolaris* extracts on the 2^{nd} and 3^{rd} instar larvae of *S. litura*.

| | H. tiliaceus | | S. caseolaris | | H. tiliaceus | | S. caseolaris | | |
|------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|--|
| Parameters | 100 mg | | | | 200 mg | | | | |
| | 2 nd | 3 rd | |
| (%) mortality | 29.6±0.3 | 23.0±0.4 | 40.2±0.2 | 36.0±0.4 | 43.0±0.4 | 42.0±0.3 | 50.0±0.4 | 42.6±0.3 | |
| (%) pupation | 69.8±0.3 | 75.6±0.3 | 60.0±0.4 | 62.6±0.3 | 56.0±0.4 | 56.0±0.4 | 50.0±0.4 | 56.0±0.4 | |
| (%) No. of deformed pupae | 5.0±0.4 | 6.0±0.2 | 4.4±0.3 | 4.4±0.3 | 8.0±0.2 | 5.4±0.3 | 8.6±0.3 | 4.6±0.3 | |
| (%)No. of emerged adults | 16.0±0.2 | 17.0±0.2 | 14.0±0.2 | 4.4±0.3 | 8.8±0.2 | 12.0±0.4 | 6.0±0.4 | 12.6±0.4 | |
| (%)No. of adults deformed | 1.6±0.1 | 1.6±0.2 | 1.8±0.2 | 1.4±0.1 | 2.0±0.2 | 1.8±0.2 | 1.6±0.1 | 1.4±0.1 | |

Values are mean ± SE. ANOVA followed by TUKEY test performed; all the values are significantly different at P< 0.001.

When an increased dose (200 mg) was applied topically to both the 2^{nd} and 3^{rd} instar larvae there was a slight increase in the mortality rate of the *S. litura* larvae and the rate of successful pupation was decreased by 15-20 % with both plants. *S. caseolaris* and *H. tiliaceus* plant leaf extracts at this dose interfered with the larval molting process leading to deformed pupae. Furthermore, adult moths which emerged from these pupae showed malformations in the wings (Table 2). The treatment of the stored grain pests with the test plant extracts resulted in moderate to excellent level of insecticidal properties (Table3).

Table 3: Fumigation toxicity of the *H. tiliaceus* and *S. caseolaris* leaf extracts against stored grain pests 24, 48 and 72 hours exposure.

| | Mortality (%) mean ± SE | | | | | | | | | |
|----------|---|---|--|--|---|--|--|--|--|--|
| | Days of the treatment | | | | | | | | | |
| Dose(mg/ | 1 | | | 2 | | | 3 | | | |
| cm^2) | Т. с | <i>R. d</i> | S. 0 | Т. с | <i>R. d</i> | S. 0 | Т. с | <i>R. d</i> | S. 0 | |
| 50 | 12.5±0.9 | 10±0.2 | 12.8±0.9 | 14.6±0.4 | 10.3±0.3 | 15.2±0.3 | 12.6±0.4 | 12.3±0.3 | 18.1±0.3 | |
| 100 | 30.5±0.3 | 20.4±0.4 | 22.4±0.9 | 30.1±0.4 | 25.4±0.3 | 29.5±0.3 | 25.1±0.4 | 25.6±0.3 | 31±0.3 | |
| 150 | 60±0.9 | 70.3±0.8 | 29.8±0.6 | 60.3±0.3 | 69.3±0.4 | 29.9±0.3 | 71±0.3 | 1.3±0.4 | 34±0.3 | |
| 200 | 89±0.6 | 84.4±0.5 | 49.8±0.9 | 92.9±0.7 | 83.7±0.8 | 59±1.8 | 93.9±0.7 | 84 ±0.8 | 62±1.8 | |
| 50 | 0.8±0.2 | 10.7±0.3 | 1.4±0.3 | 5.3±0.5 | 14.5±0.5 | 4.5±0.5 | 6.3±0.5 | 16.5±0.5 | 4.9±0.5 | |
| 100 | 1.7±0.9 | 10.6±0.3 | 1.5±0.3 | 7.1±0.4 | 17.9±0.4 | 5.4±0.34 | 7.4±0.4 | 20.9±0.4 | 7.5±0.34 | |
| 150 | 31.6±1.6 | 20.9±0.4 | 10.1±0.3 | 34.9±0.5 | 25.3±0.3 | 10.2±0.3 | 35.9±0.5 | 25.3±0.3 | 12.2±0.3 | |
| 200 | 50.4±0.6 | 29.9±0.3 | 11.3±0.3 | 59.6±0.8 | 30.1±0.3 | 15.2±0.5 | 60.5±0.8 | 32.2±0.3 | 18.2±0.5 | |
| | cm ²) 50 100 150 200 50 100 150 200 | $\begin{array}{c} \mathbf{cm}^2 \\ \hline \mathbf{r} \\ \hline 50 \\ 12.5 \pm 0.9 \\ \hline 100 \\ 30.5 \pm 0.3 \\ \hline 150 \\ 60 \pm 0.9 \\ \hline 200 \\ 89 \pm 0.6 \\ \hline 50 \\ 0.8 \pm 0.2 \\ \hline 100 \\ 1.7 \pm 0.9 \\ \hline 150 \\ 31.6 \pm 1.6 \\ \hline 200 \\ 50.4 \pm 0.6 \end{array}$ | $\begin{array}{c c} \mathbf{cm}^2 & \overline{\textbf{T. c}} & \overline{\textbf{R. d}} \\ \hline 50 & 12.5 \pm 0.9 & 10 \pm 0.2 \\ \hline 100 & 30.5 \pm 0.3 & 20.4 \pm 0.4 \\ \hline 150 & 60 \pm 0.9 & 70.3 \pm 0.8 \\ \hline 200 & 89 \pm 0.6 & 84.4 \pm 0.5 \\ \hline 50 & 0.8 \pm 0.2 & 10.7 \pm 0.3 \\ \hline 100 & 1.7 \pm 0.9 & 10.6 \pm 0.3 \\ \hline 150 & 31.6 \pm 1.6 & 20.9 \pm 0.4 \\ \hline 200 & 50.4 \pm 0.6 & 29.9 \pm 0.3 \\ \hline \end{array}$ | $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | Dose(mg/ cm ²) T. c R. d S. o T. c 50 12.5±0.9 10±0.2 12.8±0.9 14.6±0.4 100 30.5±0.3 20.4±0.4 22.4±0.9 30.1±0.4 150 60±0.9 70.3±0.8 29.8±0.6 60.3±0.3 200 89±0.6 84.4±0.5 49.8±0.9 92.9±0.7 50 0.8±0.2 10.7±0.3 1.4±0.3 5.3±0.5 100 1.7±0.9 10.6±0.3 1.5±0.3 7.1±0.4 150 31.6±1.6 20.9±0.4 10.1±0.3 34.9±0.5 200 50.4±0.6 29.9±0.3 11.3±0.3 59.6±0.8 | $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | $\begin{array}{ c c c c c c c c c c c c c c c c c c c$ | $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | |

(T.c = T. castaneum, R. d = R. dominica, S. o= S. oryzae), Values are mean ± SE. mean values are significantly

different at P< 0.001 (ANOVA, Tukeys test performed). The data presented in (Table 3) shows that both plants were significantly (H. tiliaceus, df, 2,51; F= 5.56; P=<0.001) (S. caseolaris df,2,51; F=7.65, P=<0.001) more toxic to all tested stored grain pests at a dosage of 200mg/cm² after 24, 48, 72 hrs times of exposure. Tribolium castaneum was the most sensitive insect (df. 11,108; F=2.72, *P*=<0.001) followed by *S. oryzae* (df, 11, 108; *P*=<0.001) and *R*. F=2.40.dominica (df,11,108; F=2.40, P=<0.001 value should be included) with H. tiliaceus after 24 hrs treatment. The treatments of both the plant

extracts at a dosage of 50 (*H. tiliaceus*, df, 2,51; F=39.7; P=<0.001) (*S. caseolaris* df,2,51; F=122.8, P=<0.001) and 100 mg (*H. tiliaceus*, df, 2,51; F=10.5; P=<0.001) (*S. caseolaris* df,2,51; F=69.3, P=<0.001) (*S. caseolaris* df,2,51; F=69.3, P=<0.001) failed to show significant toxic effects on the test insects, while a dosage of 200mg was considerably good. Among both the marine plants *H. tiliaceus* produced more than 85 percent toxicity to almost all the three pests and this toxicity had increased 3-days after the pests exposure to the compounds. However, the mangrove plant, *S. caseolaris* though a

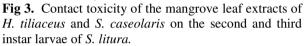
good antifeedant to *S. litura*, failed to show any toxicity to stored pests even after 72hrs post treatment.

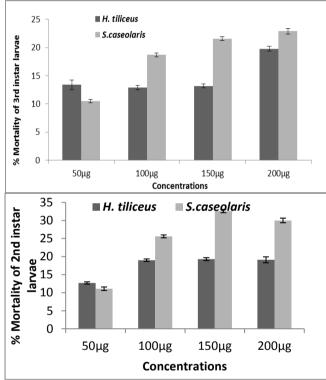
Mangroves have received much attention in recent years for their possession of potentially useful natural chemicals. Mangroves and a few of mangrove associated plants due to their unique property of salt tolerance also consist of a unique range of biochemicals having immense medicinal potential (Patra and Mohanta, 2014).

This phenomenon instigated us to explore a mangrove plant, S. caseolaris and a mangrove associate plant, H. tiliaceus for insect control activity which can lead to environmentally safer pest control measures. Both the test plants possess insect antifeedant properties against the major agricultural pest, S. litura. Antifeedant is defined as a chemical that inhibits feeding without killing the insect directly, while the insect remains near the treated foliage and dies through starvation. Since both the plants prevented pest feeding at higher doses tested, we presume that the plant leaves possess chemicals that can act upon S. litura larval gustatory receptors which leads to the feeding inhibition on the surface treated castor plant leaf. It also appears that in both the cases 2nd instar larvae are more susceptible than the 3rd instars. The feeding inhibition was more in second instar larvae. The third instars appeared to overcome the treatment effects and could consume a slightly more quantity of leaf than the 2nd instars. Screening botanical extracts for deleterious effects on insects is one of the approaches used in the search for novel insecticides (Isman et al., 2001). The results obtained with the test plant extracts indicate a positive role that these plants can play in the management of the notorious pest in the world. Santhanam et al. (2014) also studied the effect of pentacyclic triterpenoids and a linear alkane from the milky mangrove tree, Excoecaria agallocha L. on the larva of Helicoverpa armigera Hubner. Extract of E. agallocha showed growth inhibition of late 2nd and early 3rd instars larvae of *H. armigera* and caused 50% mortality of early third stadium instars larvae. Previously a few investigators had screened the plant extracts for their

103

antifeedant activity against the third instar larvae of *S. litura* (Arivoli and Samuel, 2013; Jaipal Singh choudhary *et al.*, 2014; Tukiran, 2013). None of the compounds acted as postingestive toxins which is evident during the antifeedant assays. At the lower doses employed leaf consumption had occurred, but even after the ingestion larvae remained the same without any toxic symptoms. Only the cuticular penetration of the extracts into the insect body through topical application caused larval mortality.





Bars indicate the means (\pm SE) are different significantly at P<0.0001 (ANOVA, Tukey's test), N=30.

The crude acetone extracts of *S. caseolaris* and *H. tiliaceus* plants were also evaluated for their toxic property against an agricultural as well as three stored grain pests. The crude leaf extract possesses moderate level of toxicity to *S. litura* larvae. Even at a high dose tested i:e 200mg, only less than 50 percent larval mortality was obtained. Among the plants, *S. caseolaris* caused higher larval mortality rate than *H. tiliaceus* against both the second and third instar larvae of *S. litura*. This larval

mortality with crude extract of H. tiliaceus and S. caseolaris may be due the presence of active toxic group in the leaves of mangrove plants. Similar results with both topical and leaf applications of crude aqueous extract of Lantana camara leaves were observed to be highly effective in controlling the lepidopteran pest S. litura by causing heavy mortality (Deshmukhe al., 2008). Similarly et insecticidal activities of many plants and their compounds against different groups of insect pests have been reported previously (Rajam, 1991; Supratman et al., 2001; Leatemia, and Isman 2004; Jeyasankar, 2012). The reason for the activity is due to the fact that the insecticidal property present in the selected plants have compounds that may arrest the various metabolic activities.

The present investigation revealed that column eluted fractions of H. tiliaceus and S. caseolaris exhibited maximum activity on 3rd of S. litura larvae at lower instar concentrations compared to crude. In both the fractions of H. tiliaceus and S. caseolaris, we found that hexane eluted fraction is the most potent growth inhibitor. This indicated that the active principles present in the plants inhibit larval feeding behavior or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence (Jeyasankar et al., 2010)

Fumigation is one of the major chemical methods to control stored-product insect infestations and currently, phosphine and methyl bromide are being used worldwide (Duangsamorn Suthisut et al., 2011). Bioactivity of phytochemicals against stored product pests depends upon several factors such as the chemical composition of the crude extracts and varied susceptibility of target species (Usha Rani et al., 2011). No reports are available previously on the insecticidal activity of the mangrove and mangrove associated plant S. caseolaris and H. tiliaceus against the stored grain pests. However, results of some of the previous research work may be comparable with the present findings. In our study we found that *H. tiliaceus* and *S.* leaf extracts produced caseolaris high volatility, which is a desirable characteristic

104 for insecticidal preparations that can act as fumigants for the control of stored product pests *T. castaneum*, *R. dominica* and *S. orvzae*. The observed mortality percentage

pests T. castaneum, R. dominica and S. oryzae. The observed mortality percentage was increased with increase in time intervals after treatment and also with increase in dose. Similarly, Adeniyi et al. (2010) also reported the insecticidal activity of Bryophyllum pinnatum and Eucalptus globules against rice weevil. They have found that toxic effects of extracts were proportional the to the concentration and that higher concentrations had stronger effects. Ethanolic extract of melgota, Macaranga postulata was tested for repellency, insecticidal activity against rice weevil, S. oryzae (Rahman et al., 2006). Since both the test plants consist no mammalian toxicity, it is ideal to utilize these plants for the control of the major stored products in fumigation method.

This is the first report on mangrove plants, H. tiliaceus and S. caseolaris which can be consider as crop protectants and for pest management. The results obtained suggest that potential use of marine plant extracts as both fumigant agents against T. castaneum, R. dominica, and S. oryzae adults. Hence, plantderived insect control agents play a very important role in Integrated Pest Management By considering the overall strategy. performance, and identifying the components from both crude and purified fractions of leaf extracts of H. tiliaceus and S. caseolaris, they may be utilized in the management of lepidopteran pests in future.

ACKNOWLEDGEMENTS

We are grateful to Ministry of Earth Sciences, New Delhi, India (MoES / 36 / OOIS / Extra / 18 /2013) for the research grant and to the Director, Indian Institute of Chemical Technology, Hyderabad, for providing the facilities.

REFERENCES

- Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, **18**: 265-267.
- Adeniyi, S.A., Orjiekwe, C.L., Ehiagbonare,J.E. and Arimah, B.D. 2010.Phytochemical screening and insecticidal

Pathipati Usha Rani et al.,

activity of leaf extracts of Bryophyllum pinnatum and Eucalptus globules against rice weevil (Sitophilus oryzae). International Journal of Biological and Chemical Sciences, **4(1)**: 241-246.

- Arivoli, S. and Samuel, T. 2013. Antifeedant activity. developmental indices and morphogenetic variations of plant extracts (Fab) against *Spodoptera* litura (Lepidoptera: Noctuidae). Journal ofEntomology and Zoology Studies, 1 (4): 87-96.
- Bandaranayake, W. M. 1998. Traditional and medicinal uses of mangroves. *Mangroves and Salt Marshes*, **2(3)**: 133-148.
- Belles, X., Camps, F., Coil, J. and Piulachs, M. D. 1985. Insect antifeedant activity of clerodane diterpenoids against larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera). *Journal of Chemical Ecology*, 11: 1439-1445.
- Chan, H.T. and Baba, S. 2009. Manual on Guidelines for Rehabilitation of Coastal Forests damaged by Natural Hazards in the Asia-Pacific Region. International Society for Mangrove Ecosystems (ISME) and International Tropical Timber Organization (ITTO), 66 **PP**.
- Choudhary, J.S., Srivastava, C. and Suresh, W. 2014. Screening for antifeedant activity of *Gymnema sylvestre* leaf extracts against *Spodoptera litura* f. (lepidoptera: noctuidae), *The Bioscan*, **9(2):** 633-638.
- Devanand, P. and Usha Rani, P. 2008. Fumigant action of Solanaceae plants against four major species of stored grain pests. Uttar Pradesh Journal of Zoology, suppl I, 165–173.
- Ferry, N., Edwards, M., Gatehouse, J. and Gatehouse, A. 2004. Plant–insect interaction: Molecular approaches to insect resistance (Edited by Sasaki T, Christou P), *Current Opinion in Biotechnology*, 15: 155–161.
- Holser, R.A., Bost, G. and Boven, M. 2004. Phytosterol composition of hybrid Hibiscus seed oils. *Journal of Agricultural and Food Chemistry*, **52**:2546–2548.
- ICMAM project Directorate and institute for ocean management, 2001. Critical habitat

information system for coring mangroves Andhra Pradesh, India, 1-31 **PP**.

- Isman, M.B., Wan, A.J. and Passreiter, C.M. 2001. Insecticidal activity of essential oils to the tobacco cutworm, *Spodoptera litura*. *Fitoterapia*, **72**: 65-68.
- Jariyah, Widjanarko, S. B., Yunianta, Estiasih, T. and Sopade, P. A. 2014. Pasting properties mixtures of mangrove fruit flour (*Sonneratia caseolaris*) and starches. *International Food Research Journal* **21(6):** 2161-2167.
- Jeyasankar, A. 2012. Antifeedant, insecticidal and growth inhibitory activities of selected plant oils against black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae). *Asian Pacific Journal of Tropical Disease*, 2: S347-S351.
- Jeyasankar, A., Chinnamani, T., Chennaiyan,
 V. and Ramar, G. 2014. Antifeedant activity of *Barleria buxifolia* (Linn.) (Acanthaceae) against *Spodoptera litura* fabricius and *Helicoverpa armigera* hübner (Lepidotera: Noctuidae), *International Journal of Natural Sciences Research*, 2(5): 78-84.
- Jeyasankar, A., Premalatha, S. and Kuppusamy, E. 2014. Antifeedant and insecticidal activities of selected plant extracts against Epilachna beetle, *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae). *Advances in Entomology*, **2(1)**: 14-19.
- Jeyasankar, A., Raja, N. and Ignacimuthu, S. 2010. Antifeedant and Growth Inhibitory Activities of Syzygium lineare Wall (Myrtaceae) Against *Spodoptera litura* Fab (Lepidoptera: Noctuidae), *Current Research Journal of Biological Sciences*, **2** (**3**): 173-177.
- Kabaru, J.M. and Gichia, L. 2001. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophorace ae) Lam. against three arthropods. *African Journal of Science and Technology*, **2**(2): 44-49.
- Leatemia, J.A. and Isman, M.B. 2004. Insecticidal activity of crude seed extracts

106

of *Annona* spp., *Lanium domesticum* and *Sandoricum koetjape* against Lepidopteran larvae. *Phytoparasitica*, **32**, 30-37.

- Nayak, B. K., Janaki, T. and Ganesan, T. 2014. Antimicrobial activity of *Avicennia marina* (Forsk) Vierh from back water area of Puducherry, India, *International Journal of ChemTech Research*, **6**(11): 4667-4670.
- Patra, J.K. and Mohanta, Y.K. 2014. Antimicrobial compounds from mangrove plants: A pharmaceutical prospective. *Chinese Journal of Integrative Medicine*, 311–320.
- Rajam, M.V. 1991 Insecticidal activity of inhibitors of polyamine synthesis on Spodoptera litura F. larvae. Indian Journal of Experimental Biology, 29, 881-882.
- Rosa, R.M., Melecchi, M.I., da Costa Halmenschlager, R., Abad, F.C., Simoni, C.R., Caramao, E.B., Henriques, J.A.,Saffi, J. and de Paula Ramos, A.L. 2006. Antioxidant and antimutagenic properties of *Hibiscus tiliaceus* methanolic extract. *Journal of Agricultural and Food Chemistry*, **54**:7324–7330.
- Santhanam, S.R., Subramanian, M., Meseret
 C. E. and Ajay, P. 2014.Pentacyclic
 Triterpenoids and a Linear Alkane from the
 Milky Mangrove Tree (*Excoecaria* agallocha L.) are toxic to the larva of
 Helicoverpa armigera Hubner.
 (Lepidoptera: Noctuidae), International
 Journal of Advanced Research, 2(6): 1-12.
- Supratman, U., Fujita, T., Akiyama, K. and Hayashi, H. 2001. Insecticidal compounds from *Kalanchoe daigre-montiana* X *tubiflora. Phytochemistry*, **58**: 311-314.
- Suthisut, D., Paul, G., Fields. and Chandrapatya, A. 2011. Fumigant toxicity of essential oils from three thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids, *Journal of Stored Products Research*, **47**:222-230.
- Tukiran. 2013. Bioinsecticide test of crude stem bark extracts of some meliaceous plants against *Spodoptera litura*, *Global Journal of Biology*, *Agriculture and Health Sciences*, **2**(**3**):28-31.

- Usha Rani, P., Venkateshwaramma, T. and Devanand. P. 2011. Bioactivities of *Cocos nucifera* L. (Arecales: Arecaceae) and *Terminalia* catappa L. (Myrtales: Combretaceae) leaf extracts as post-harvest grain protectants against four major stored product pests. *Journal of Pest Science*, **84**:235–247.
- Usha Rani, P. and Rajasekharreddy, P. 2009. Toxic and antifeedant activities of *Sterculia foetida* (L.) seed crude extract against *Spodoptera litura* (F.) and *Achaea janata* (L.). *Journal of Biopesticides*, **2(2)**: 161-164.
- Usha Rani, P. and Rajasekharreddy, Pala. 2010. Insecticidal activity of (2noctylcycloprop-1-enyl)-octanoic acid (I) against three coleopteran stored product insects from *Sterculia foetida* (L.). *Journal of Pest Science*, **83(3)**: 273-279.
- Varahalarao, V. and Naidu, K. C. 2009. Bioefficiency of Mangrove Plants *Lumintzera racemosa* and *Bruguiera gymnorhiza*, *Journal of Pharmacy Research*, **2(10)**:1591-1592.
- Wang, R.J. and Chen, Z.Y. 2002. Systematics and biogeography study on the family Sonneratiaceae. *Guihaia*. **22**:214-219.
- Xie, Y. S., Isman, M. B., Gunning, P., MacKinnon, S., Arnason, J. T., Taylor, D.
 R., Sanchez, P., Hasbun C. and Towers, G.
 H. N. 1994. Biological activity of extracts of *Trichilia* species and the limonoid hirtin against lepidopteran larvae. *Biochemical Systematics and Ecology*, 22: 129–136.
- Wong, S.K. and Chan, E.W.C. 2010. Antioxidant properties of coastal and inland populations of *Hibiscus tiliaceus*, *ISME/GLOMIS Electronic Journal*, 8(1): 1-2.

Pathipati Usha Rani ¹*, Kurra Sandhyarani ¹, Varahalarao Vadlapudi¹, Bojja Sreedhar²

*¹Biology and Biotechnology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad-500 007, India

²Inorganic and Physical Chemistry Laboratory, Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500607, Andhra Pradesh, India.

*Corresponding author

E-mail: usharani65@gmail.com