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Pesticidal activity of endophytic fungal metabolites against major groundnut defoliator *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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ABSTRACT

The present study has been undertaken to evaluate the pesticidal activity of crude metabolites against major groundnut defoliator *Spodoptera litura* derived from the fungal isolates resides in the leaflets of common garden weed *Clerodendron inerme*. A total of 75 isolates belong to *Alternaria* sp, *Cladosporium* sp, *Paecilomyces* sp, *Rhizopus* sp, *Tricoderma* sp were isolated from leaflets of garden weed Clerodendron *inerme* by surface washing method and the isolated fungi were grown in liquid media. Ethyl acetate extract was prepared from the filtrate of the respective fungal organism and the pesticidal activity was studied. Pesticidal activity of the metabolites extracted from *Cladosporium* sp revealed distinct pesticidal activity against all the life stages of *S. litura* as dose dependent manner.

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INTRODUCTION

Endophytes are microorganisms that grow within plants without causing any obvious symptoms of infection or disease (Lugtenberg *et al.*, 2001, Ge *et al.*, 2007).Some of the endophytes are thought to protect their host from attack by fungi, insect and mammals by producing secondary metabolites. Interact closely with their host plants, and could therefore potentially be used as biological control agents in sustainable crop production. Endospore forming Bacillus comprising more than 60 species of quite different phenotypes have properties that make them suitable to be developed as biocontrol agents, such as good stress resistance and producing low molecular weight fungal toxic compounds (Abang *et al.*,2009).An endophyte is a bacterial (including actinomycetes) or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and/or intra cellular healthy tissues of the host plant, typically causing no apparent symptoms of disease. Endophytes are being accepted as an important source of novel bioactive secondary metabolites that can be excellent new starting points for the development of novel pharmaceuticals and/or agrochemicals. Endophytes are well known as producer of antibiotics and other biologically active substances of higher commercial value, such as vitamins, alkaloids, plant growth factors, enzymes and enzyme inhibitors (Karthick Raja Namasivayam *et al.*, 2010, Thyagarajan and Karthick Raja Namasivayam, 2011). They play major role in physiological activities of host plants influencing enhancement of stress, insect, nematode and disease resistance. Endophytes can also accelerate plant growth and nitrogen fixing capabilities of host plants (Bills *et al.*, 2002, Bhilabutra *et al.*, 2007). These endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites (Knight *et al.*, 2003).

Among the different microbial groups, fungi as endophytes are widely studied. Endophytic fungi associated with agricultural plants such as wheat (Larran *et al.*, 2002a), bananas (Pocasangre *et al.*, 2000; Cao *et al.*, 2002), soybeans (Larran *et al.*, 2002b), and tomatoes (Larran *et al.*, 2001) have been isolated. Role of endophytic fungi in plant protection such as protection from insects (Breen, 1994; Clement *et al.*, 2005), plant parasitic nematodes (Elmi *et al.*, 2000), and plant pathogens (Dingle and McGee, 2003; Wicklow *et al.*, 2005). In the present study, pesticidal activity of endophytic fungi isolated from healthy leaves of common weed *Clerodendron inerme* was studied.

MATERIALS AND METHODS

Isolation of endophytic fungi

Endophytic fungi was isolated from surface sterilized leaflets of *Clerodendron inerme* by the modified method of Vijay Verma *et al* (2010) .In this method, fresh and healthy leaves of *Clerodendron inerme* collected from the University campus in sterile polythene bag and brought to the laboratory immediately. The collected leaves were cut into 1mm square surface using sterile blade and rinsed in sterile distilled water. The cut sections were washed successively in sterile distilled water containing 0.1%HgCl2, washed leaflets was blotted on sterile tissue paper and transferred to the Potato Dextrose Agar medium(PDA). The plates were incubated at 28ºC for 10 days. Daily observation was made to record the fungal growth. The respective fungal growths from the leaves were transferred to PDA plates for colony morphology and further identification. Identification was carried out based on standard methods Identified fungi were maintained on PDA slant

Crude extraction of bioactive compounds

The fungi grown in PDA slants were flooded with sterile distilled water containing 0.1%Tween 80 and scrapped with surface sterilized glass rod and slurry was filtered through cheese cloth to remove mycelial debris. 0.1 ml of spore suspension of the culture was added to 100 ml of sterile Potato Dextrose Broth and kept in a shaker in room temperature at 150 rpm for 7 to 10 days. After the incubation period, the media was filtered and the collected filtrate was extracted with the double the volume of ethyl acetate, the crude extract was vacuum dried at 60ºC and the concentrated extract was used for further studies.

Laboratory pesticidal assay on *S. litura* larva

The egg masses and larval instars of *S. litura* were collected from the groundnut field in an area around Kanchipuram and Thiruvallur district, Tamil Nadu, India. Collected larvae were maintained on groundnut leaves (TMV-7 variety). Twenty larvae in each instars separately were sprayed with different concentration of metabolites (10, 25, 50, 75 and 100 µg using hand sprayer. The treated larvae were introduced into the plastic container (34 mm x 21 mm) provided with moist cotton swap 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. For control category, another 20 larvae of each instar treated with distilled water only. The containers were incubated at room temperature 28 ± 0.5^0 C in an incubator (Remi BOD incubator, Mumbai, India). Daily observation on larval mortality was recorded for a period of 4 days. After 96 hours of the conidial treatment, all the surviving larvae from each treatment were transferred to another container of the same size for further development. The total larval and pupal durations, adult longevity and the adult emergence were recorded. The LT_{50} of the dose of fungi to kill the different larval instars was assessed in hours following Blever and Hostetter (1971).

$$LT_{50} = a + e\frac{(c - b)}{D}$$

Where, a = the number 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 reached; c = 50% of the total number tested; D = the number of larvae dying in 24 hours period during which the 50% mortality was reached and e = the number of hours between mortality counts. The dose mortality data were subjected to profit analysis (Finney, 1962) for LC_{50}.

Pupa and adult

About 300 gram of finely sieved soil was taken in 500 ml capacity bottle and autoclaved at 15 psi pressure for 30 minutes. After the sterilization this soil was transferred in to a clean surface sterilized 500 ml capacity plastic container (65 x 32 mm) and the soil moisture was maintained by adding 5 ml of sterile-distilled water. Respective concentrations of metabolite was added separately. Pupa of *S. litura* was placed individually. Each treatment was replicated 10 times. Another set was maintained by adding only distilled water as control. Observations on the pupal mortality and adult emergence were recorded.

RESULTS AND DISCUSSION

Endophytic microorganisms are those that inhabit the interior of plants, especially leaves, branches and stems, showing no apparently harm to the hosts

(Azevedo, 1998). The capability of colonizing internal host tissues has made endophytes valuable as a tool to improve crop performance in agriculture (Zhang *et al.,* 2011). Endophytic microorganisms have received considerable attention in the last 20 years because of their capacity to protect hosts against insects pests and pathogens. Toxic metabolites produced by endophytic microorganisms in many plants can greatly reduce the populations of associated insects. The extracts of foliar fungal endophytes isolated from Picea rubens Sarg. (red spruce) needles were toxic to the forest pest *Choristoneura fumiferana* Clem. (eastern spruce budworm) in dietary bioassays (Miller *et al.,* 2008; Sumarah *et al.,* 2010). Toxic metabolites produced by endophytic fungi (*Epichloëand Neotyphodium* species) in fescue grasses greatly reduce the populations of associated herbivorous insects. These fungi produce various alkaloids that affect herbivore growth (Clay and Schardl, 2002). Thyagarajan and Namasivayam (2011) isolated seven genera of endophytic fungi residing in *Vitex negundo* and mycelium extract of *Penicillium* sp and *Fusarium* sp revealed pesticidal activity. Various agro active compounds such as herbicidal and pesticidal compounds have been isolated from endophytic fungi (Kusari *et al.,* 2012). In the present study, a total of 75 isolates belong to five genera belong to *Alternaria sp, Cladosporium sp, Paecilomyces sp, Rhizopus sp, Tricoderma sp* were isolated from the leaves of Clerodendron *inerme* (Table 1).Among the different fungal genera, *Cladosporium sp* was found to be dominant (50%) followed by *Paecilomyces sp* (20%), *Rhizopus sp* (15%) and *Tricoderma sp* (10%), *Alternaria* sp (5%). All the fungal isolates were identified based on cultural and morphological characteristics. Among the fungal isolates, ethyl acetate extract showed pesticidal activity. Pesticidal activity revealed all the life stages of *Spodoptera litura* susceptible as dose dependent manner. Concentration dependent variation on mortality was observed in all the life stages (Table 1). In general, the mortality is a concentration dependent factor (Bai. and Kandasamy, 1981, Sahayaraj and Sekar, 1996). Such a dose dependent mortality with the fungal spores was observed in *Sitophilus zeamais* (Adane *et al.,* 1996, *H. armigera* (Wadyalkar *et al.,* 2002) *S. litura* (Manjula *et al.,* 2004) *Plutella xylostella* (Kennedy *et al.,* 2001) *Hemosepilachna vigintioctopunctata* (Hafez *et al.,* 1994) *Phthorimaea operculella* (Devaprasad *et al.,* 1990). In the case of larval development the total larval period was statistically significant at 5% level in higher concentrations when compared to the control. Larval period was not recorded in 100, 75, 50 µg/ml. Distinct reduction of larval period was reported in 25 and 10 µg/ml which showed 12.0 and 13.5 days. Pupal and adult emergence was not recorded in all the tested concentration of fungal metabolites except 10 µg/ml. The percentage of pupal and adult emergence was 15.0 and 10.0% respectively and the same concentration revealed 5.0 hours of adult longevity.

Distinct effect on LT_{50} and LC_{50} was recorded in metabolites treatment LT_{50} increased as the larvae grew older as well as with the increase in the concentration of the metabolites used. As the instar advanced, a decrease in mortality and an increase in time for initial mortality were recorded. Furthermore, the first and second, third instars larvae were highly susceptible to the fungus with 100, 75/and 50 µg which recorded the total larval mortality of 100% followed by fourth (90.4 %), fifth and sixth instar larvae (80.2 and 71.3 %) LT_{50} value of the 100 µg for the fourth, fifth and sixth instars was found to be 0.12,0.91 and 1.54 days (Table 2).

Table 3. Effect of fungal metabolites on LC_{50} Parameters of *S. litura.*

Instars	Regression equation (Y = a + bx)	LC_{50} µg / ml	Variance	Chi-square value	95% Confidence Limit	
					Lower Limit	Upper Limit
I	–0.44 +	0.2	0.45	1.59	1.35	1.76
II	– 3.48 +	1.5	0.50	1.61	1.55	1.98
III	– 0.52 +	1.7	0.38	2.92	1.87	2.10
IV	0.16 +	2.0	1.53	4.93	2.22	2.95
V	0.26 +	2.5	2.10	5.13	3.23	3.21
VI	0.31 +	3.0	3.01	6.13	4.42	6.01

A gradual increase in LT 50 was recorded in concentration dose and stage of the instar. Similar observation was also recorded in remaining concentration. The results of LC_{50} values determined through probit analysis were presented in Table 4.

Among the various estimate of the regression based probit analyses, 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 *S. litura* in response to varying concentration showed

an increased trend in the LC_{50} value when the age of the larva advanced. The present study clearly reveals that metabolites was found to infecting life stages of *S. litura* and distinct effect could be observed. Further purification, identification and field evaluation of the fungal metabolites is now progress.

Table 2. Effect of crude metabolites on *developmental* period of *S.litura*

Concentration	Developmental parameters			
	LP	PE	AE	AL
10	13.5	15.0 [a]	10.0 [a]	5.0 [a]
25	12.0[a]	-	-	-
50	-	-	-	-
75	-	-	-	-
100	-	-	-	-
Control	18.2	100.0	100.0	36.2

LP-larval period (days), PE-pupal emergence (%) AE-Adult emergence (%) AL -adult longevity (hours) In column, the mean carries the same letter is statistically significant at 5% level by DMRT

Table 1. Effect of fungal metabolites on LT 50 (days) and mortality (%) on *S. litura*

S.No	Instars	LT 50 (Days)					Mortality (%)				
		10	25	50	75	100	10	25	50	75	100
1	I	-	-	-	-	-	100.0	100.0	100.0	100.0	100.0
2	II	-	-	-	-	-	100.0	100.0	100.0	100.0	100.0
3	III	-	-	-	-	-	100.0	100.0	100.0	100.0	100.0
4	IV	4.24	3.0	2.0	0.71	0.12	45.0	61.0	73.2	83.0	90.4
5	V	5.31	4.21	3.31	1.0	0.91	34.0	51.0	62.4	72.0	80.2
6	VI	6.23	4.31	3.61	2.71	1.54	03.0	14.0	30.0	50.1	70.3

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