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Larvicidal activity of *Rorippa indica* L. against *Spodoptera litura* Fab.

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ABSTRACT

Larvicidal activity of seed and root extracts of Rorippa indica L. at different concentrations (1.0, 2.0, 3.0 4.0 and 5ppm) was evaluated at room temperature against Spodoptera litura Fab. Among the different extracts tested (petroleum ether, chloroform, ethanol, methanol and aqueous) the methanolic root extract without elicitor treatment (Jasmonic acid) depicted the highest mortality rate at 5ppm (73 %) and elicitor treated methanolic root extract, a high mortality rate was observed at 5 ppm (100 %). The methanolic elicitor treated root extracts of R.indica showed highest morality rate at 5 ppm. Interestingly no morality was observed in control. Hence, this plant could be used to isolate active principle and develop a new botanical formulation in pest management programme.

Key words: *Rorippa indica*, Plant extracts, *Spodoptera litura*, Elicitor, Jasmonic acid.

INTRODUCTION

The insect pests have developed resistance to a variety of insecticides due to the indiscriminate use of chemical pesticides. Insecticides affect the non-target organisms and human beings, directly or indirectly. Plant materials are effective against a variety of agricultural insect pests; they are easily degradable. This is beneficial for both the environment and agriculture product consumers [1]. Plant extracts or pure compounds manifest their effect on insects in several ways including toxicity, mortality, antifeedancy, growth inhibition, suppression of reproductive behavior and reduction of fecundity and fertility [2]. Feeding deterrency is caused due to the action of botanicals[3], which prevent the motility of the gut [4]. Some compounds, either separately or synergistically, makes up a chemical defense barrier in the plant against certain pests [5]. Many workers have highlighted the importance of developing botanical insecticides from plants. It has been suggested that tremendous interest has been generated in recent years about the use of pesticidal plants, particularly those that can be harvested, formulated and used easily. This created a world-wide interest in the development of alternative strategies, including the search for new types of insecticides used as traditional botanical pest control agents. Plant crude extracts often consist of complex mixtures of compounds which may act synergistically [6]. Botanical pesticides are highly effective, safe and ecologically acceptable [7]. *Spodoptera litura* infests a wide range of cultivated food crops numbering around 112, belonging to 44 families [8]. Eight larvae of*S. Litura* on a *Dysdercus koenigii* can reduce the yields up to 50% [9] *S.litura* is one of the most economically important insect pests in many countries including India, Japan, China and other countries of South East Asia [10]. This pest may become serious during the seedling stage causing extensive loss of

agricultural production all over the world [11]. In the present investigation *Rorippa indica* were evaluated for their biological activities against the economically important polyphagous pest *S.litura* Fab.

MATERIALS AND METHODS

Plant Collection

Seeds and root of *Rorippa indica* L. were collected from Kollidam river bank, Tiruchirappalli District, Tamil Nadu, India, during the month of February and March.

Extract preparation

Regenerated plants of *Rorippa indica*(seeds and roots) were air-dried and ground into fine powder in blender**.** Powdered seed and root of *Rorippa indica* was weighed and was wrapped in Whatmann No. 1 filter paper. 40 grams of the enveloped seed and root powder was introduced into the soxhlet chamber at one time for the extraction. In the round bottom flask 350 ml of Petroleum ether was taken as solvent. The apparatus was run till the solvent in the soxhlet chamber became transparent. Then the extract along with the solvent was removed and solvent mixture was subjected to vacuum drying and the paste was stored at 4°C untill the bioassay was performed. Paste was treated as the pure extract and for bioassay, different concentrations of the seed and root extracts were prepared. Similar procedure was repeated with chloroform, methanol, ethanol and aqueous as solvent.

Insect Culture

Collection and rearing of pest

Egg masses and larvae of *Spodoptera litura Fab* were collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Collected leaves with egg masses were transferred on to the filter paper and kept in petridishes under laboratory conditions (27°C + 10° C temperature; 65 - 70%RH, 11 L : 13 D). Newly hatched first instar larvae were reared in plastic trough(28 x 21 x 9 cm) on castor leaves. Third instar larvae of *Spodoptera litura* alone were used for this experiment.

Bioassay

The oral toxicity of the crude extracts against *Spodoptera litura* larvae was investigated through no choice method[12]. Bioassays using leaf discs (3 to 4 cm in diameter)was prepared from Chinese cabbage leaves cut by a cork borer. Each leaf disc was impregnated with the extract solution (1, 2, 3, 4 and 5 ppm). All discs were left at room temperature to air-dry, where after the discs were placed in plastic petri dish (10 cm diameter, top was covered with fine cloth) and padded with moist filter paper marked on one side. Ten 3rdinstar of *S. litura* larvae were then introduced in each petri dish containing a leaf disc. The mortality rate of the larvae was observed at 24, 48 and 72 hrs. In addition, the effects of continuous exposure of the plant extracts on larval development and survival during the 15 days treatment were also recorded.

Statistical analysis

Data for the bioassay was corrected for mortality in the control using Abbott's formula [13]. The experiment was designed in three completely randomized replicates. The data obtained was statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT).

RESULTS AND DISSCUSION

The *Spodoptera litura* infests a wide range of cultivated crops. Botanical insecticides provide an alternative to synthetic insecticides because they are generally considered safe, biodegradable and can often be obtained from local sources [14]. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive and are readily available throughout world. Many studies have drawn attention of the toxic effects of plant extracts on related Diptera[15]. However, the present work is a further mile stone in the same line in the present investigations, *R.indica* showed biopesticidal activity against *S. litura.* The larvicidal effect of resinous exudates from the tender leaves of *Azadirachta indica*. Crude extract of leaves of *Solanumnigrum* in water showed larvicidal activity against *A. culcifacies, C.quinque fasciatus* and *A. aegypti*. *Albizzia amara* and *Ocimum basilicum* considerably affect the mosquito survival and pronounced high repellent potential *[16], [17]*. Anti-acdysteroid activity of neem seed kernel extract in *Aedes aegypti*, resulting in growth inhibition and prolonged developmental period. [18].

Table 1: Mortality Rate of *Spodoptera litura* F. After Feeding on Host Plants Treated with *Rorippa indica* L. Seed Extracts

Solvent	Plant Extract (ppm)	Mortality rate (%)		
		24	48	72
	Control	00	00	00
Petroleum Ether	1.00	10^{d}	10^{e}	12^{d}
	2.00	13^{c}	14^{d}	16^{c}
	3.00	23^{b}	25^{c}	27^{b}
	4.00	32^{ab}	36^{b}	40^{ab}
	5.00	43^{a}	45^{a}	47^{a}
Chloroform	1.00	10^{e}	16^{d}	23^{d}
	2.00	23^{d}	30^{c}	36^{c}
	3.00	36^{c}	46^{b}	48^{b}
	4.00	46^{ab}	56^{ab}	59^{ab}
	5.00	56^{a}	63^{a}	65^{a}
Methanol	1.00	13^{e}	18^{e}	26^{e}
	2.00	26^{d}	32^{d}	38^{d}
	3.00	36^{c}	42^{c}	53^{c}
	4.00	45^{b}	56^{b}	58^{b}
	5.00	5.2^{a}	62^{a}	68^{a}
Ethanol	1.00	0.0^{d}	10^{e}	10^{e}
	2.00	10^{cd}	20^{d}	23^{d}
	3.00	16^{c}	26^{c}	30^{c}
	4.00	30^{b}	43^{ab}	45^{b}
	5.00	43^{a}	53^{a}	58^{a}
Aqueous	1.00	03^{e}	06^{d}	10^{e}
	2.00	13^{d}	20^{c}	23^{d}
	3.00	23^{c}	30^{b}	33^{c}
	4.00	36^{b}	40^{b}	46^{ab}
	5.00	46^{a}	52^{a}	56^{a}

Data was based on 3rd instars larvae, 10 larvae/ replication of 3 replications, Mean in column with the same plant extract followed by the same common letters are not significantly different at the 5% level as determined by DMRT ($P < 0.05$).

Table 2: Mortality Rate of *Spodoptera litura* After Feeding on Host Plants Treated with *Rorippa indica* L. Root Extracts

Solvent	Plant Extract (ppm)	Mortality rate (%)		
		24	48	72
	Control	00	00	00
Petroleum Ether	1.00	20^{e}	03^{e}	06^{e}
	2.00	13^{d}	06^{d}	20^{d}
	3.00	26^{c}	20^{c}	30^{c}
	4.00	36^{b}	33^{b}	46^{b}
	5.00	46^{a}	53^{a}	60^{a}
Chloroform	1.00	03^{e}	05^{e}	08^{e}
	2.00	13^{d}	10^{d}	13^{d}
	3.00	23^{c}	22^{c}	23^{c}
	4.00	30^{b}	36^{b}	43^{b}
	5.00	50^{a}	53^{a}	56^{a}
Methanol	1.00	03^{e}	10^{e}	16^{e}
	2.00	20^{d}	23^{d}	33^{d}
	3.00	33^{c}	40^{c}	46^{c}
	4.00	46^{b}	53^{b}	60^{b}
	5.00	63^{a}	66^{a}	73^{a}
Ethanol	1.00	00^{d}	03^{e}	06^{e}
	2.00	06^{cd}	13^{d}	20^{d}
	3.00	16^{c}	23^{c}	30^{c}
	4.00	36^{b}	40^{b}	43^{b}
	5.00	46^{a}	50^{a}	53^{a}
Aqueous	1.00	00^{e}	03^{e}	13^{e}
	2.00	16^{d}	20^{d}	26^{d}
	3.00	30^{c}	33^{c}	43^{c}
	4.00	43^{b}	46^{b}	53^{b}
	5.00	56^{a}	60^{a}	66^{a}

The activity of crude plant extracts is often attributed to the complex mixture of active compounds. The study of mortality is one of the methods of potential larvicidal activity of plants popularly used for this purpose. Larvicidal activity of seed and root of *R.indica* were analysed against *S.litura* in the present study (Table 1 and 2). Petroleum ether, chloroform, methanol, ethanol and aqueous seed and root extracts were tested for their larvicidal effect on

*S.litura.*1st and 2nd instar larvae were highly sensitive when compared with 3rd and 4th instar [19].Therefore the age of the insects should be considered when testing larvicidal activity of any compound or extracts.The insect bioassay was carried out using a series of five different concentrations of extracts (1.0, 2.0 3.0, 4.0 and 5.0 ppm) on each 3rd instar of *S. litura* and observation were made at 24, 48 and 72 hrs. .The mortality rate of seed and root extracts of different solvents at 5 ppm was 40, 65, 68, 58, 56 % and 60, 56, 73, 53, 66 % respectively. (Table 1 and 2). After 72 hours of without elicitor seed extracts exposure the rate of mortality in *S.litura* was (68 %)and in rest of the tested plants comparatively lesser effects were found and in control no activity found. The rate of mortality was found to increase with higher concentrations of plant extract which indicates direct relationship between the dose and rate of mortality.

Without elicitor methanolic root extract showed maximum larvicidal effect (73%) at 5 ppm followed by aqueous (66 %), petroleum ether (60 %), chloroform (56 %) and ethanol (53 %) extract suggesting that solvent plays an important role in dissolving the plant constituents and is therefore of great significance. The mortality was also found to be co-related with dose concentration highest being at 5 ppm. Plants are great source of secondary plant metabolites which control herbivores and pathogens. The results of this investigation clearly indicate that by increasing concentrations, the rate of mortality increases. Similar observations were recorded in all extracts of *R.indica*.

have demonstrated the larvicidal effects of *Terminalia catappa* seed extracts against agricultural pests, *Spodoptera litura* (F.) and *Achaea janata* (Lepidoptera: Noctuidae) and their role in controlling the pests [20]. Similar observation was reported in extract of *Myrtillocactus geometrizans* against *Spodoptera frugiperda*[21], *Yucca periculosa Atalantia monophylla* against *Helicover paarmigera*[22], [23]. Insect growth regulation properties of plant extracts are very interesting and unique in nature. For example, the enzyme ecdysone plays a major role in the phenomenon ecdysis or moulting i.e. shedding of old skin. When the active plant compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying [24].

Chander and Ahmed (1982) earlier evaluated petroleum ether extracts of leaves of *Cestrum nocturnum* and roots of *Withania somnifera* against *Callosobruchus chinensis* and recorded the effectiveness up to 90 days which was in conformation with the present findings. Maximum mortality with the extracts of seeds of *Solanumdulcamara* were compared to other plant [25].Alcohol extract of fruits (4 %) of *Solanum indicum* was most effective against aphid *Macrosiphum rosae*[26].The fruits of *Capsicum frutescens* showed contact toxicity and also had an effect on development of *Callosobruchus chinensis*[27].The toxicity of ethanol extracts of *Datura metel*, *Datura stramonium* and *Solanum nigrum* against storage beetles and reported that *Solanum nigrum* was most toxic [28].The study to the effectiveness of *Peganum harmala* against *Callosobruchus chinensis* showed that ether extract at high concentration (10 %) caused adult mortality [29]. The present findings therefore suggest that the plant *R.indica* possesses certain chemicals, especially in the root, which results in the mortality of the pest.

Elicitor (Jasmonic acid) treated plant seed and root extracts of five different solvents (Petroleum ether, Chloroform, Methanol, Ethanol and Aqueous) were analysed for the larvicidal bioassay on *Spodoptera litura.* The results of larvicidal activity of seed extracts are presented (Table 3). All plant extracts showed larvicidal activity in 24, 42 and 72 h exposure tests. In the present investigation, it was observed that the methanolic elicitor treated seed extracts exhibited maximum larvicidal activity at 5 ppm(71, 75,80, 72 and 70 %). The highest mortality rate observed in methonolic extracts (80 %). Similar observation was reported by extract of *Myrtillocactus geometrizans* on fall army worm, *Spodoptera frugiperda*[29]and *Yucca periculosa[30]*.

The larvicidal activity of the crude extracts of *R.indica* at different concentrations (1.0, 2.0, 3.0, 4.0 and 5ppm) against the 3rd instar larvae of *S. litura*is given in(Table 4). The jasmonic acid treated methanolic root extract of *Rorippa indica* displayed the highest larvicidal activities with at 5 ppm concentration. The result revealed that the *R.indica* extract mixed with leaf disc enter into the alimentary canal while feeding and caused mortality. Statistically significant larvicidal activity (100 %) was observed in methanol extract of *R.indica* at 5 ppm concentration followed byethanol, petroleum ether, aqueous and chloroform extracts. The results of this investigation clearly indicate that by increasing concentrations, the rate of mortality increases as earlier report on *P. interpunctella* adults by various essential oils [31].The extracts from *Rorippa indica* showed minimum activity aqueous extracts 5ppm concentration (65 %).

Table 3: Mortality Rate of *Spodoptera litura* After Feeding on Plants Treated with Seed Extracts Obtained from Jasmonic Acid Treated *Rorippa indica* L.

Solvent	Plant Extract (ppm)	Mortality rate (%)		
		24	48	72
	Control	00	00	00
Petroleum ether	1.00	04^{e}	13^{d}	23^{e}
	2.00	20^{d}	30^{c}	40^{d}
	3.00	32^{c}	46^{b}	53^{c}
	4.00	52^{b}	60^{ab}	63^{b}
	5.00	62^{a}	65^{a}	71^{a}
Chloroform	1.00	03^{de}	03^{e}	13^{d}
	2.00	13^{c}	23^{d}	23^{c}
	3.00	26^{bc}	36^{c}	43^{b}
	4.00	43^{b}	50^{b}	60^{ab}
	5.00	56^{a}	63^{a}	75^{a}
Methanol	1.00	10^{e}	13^{d}	20^{d}
	2.00	23^{d}	23^{cd}	33^{c}
	3.00	37^{cd}	43^{b}	53^{bc}
	4.00	53^{b}	63^{ab}	70^{b}
	5.00	70^{a}	77^{a}	80^{a}
Ethanol	1.00	03^{e}	10^{d}	16^{e}
	2.00	20^{d}	22^{c}	30^{d}
	3.00	36^{c}	46^{bc}	46^{c}
	4.00	50^{b}	60^{b}	63^{b}
	5.00	66^{a}	70^{a}	72^{a}
Aqueous	1.00	02^{e}	04^{e}	10^{e}
	2.00	12^{d}	21^{d}	30^{d}
	3.00	30^{c}	40^{c}	50^{c}
	4.00	46^{b}	53^{b}	60^{b}
	5.00	63^{a}	66^{a}	70^{a}

Data was based on 3rd instars larvae, 10 larvae/ replication of 3 replications, Mean in column with the same plant extract followed by the same common letters are not significantly different at the 5% level as determined by DMRT (P < 0.05).

Table 4: Mortality rate of *Spodoptera litura* After Feeding on Plants Treated with Root Extracts Obtained from Jasmonic Acid Treated *Rorippa indica* L.

Solvents	Plant Extract (ppm)	Mortality rate (%)		
		24	48	72
	Control	00	00	00
Petroleum ether	1.00	06^{e}	10^{e}	23^{de}
	2.00	23^{d}	26^{d}	40^{d}
	3.00	36^{c}	43^{c}	53^{c}
	4.00	56^{b}	60^{b}	70^{b}
	5.00	60^{a}	62^{a}	76^{a}
Chloroform	1.00	13^{d}	16^{d}	23^{d}
	2.00	26^{c}	33^{c}	36^{c}
	3.00	43^{bc}	53^{bc}	56^{b}
	4.00	60^{b}	56^{b}	66^{ab}
	5.00	66^{a}	73^{a}	76^{a}
Methanol	1.00	16^{e}	20^{e}	26^{e}
	2.00	33^{d}	40^{d}	43^{d}
	3.00	46^{c}	56^{c}	63^{bc}
	4.00	63^{b}	73^{b}	73^{b}
	5.00	80^{a}	90^{a}	100^{a}
Ethanol	1.00	04^{e}	11^{e}	16^{e}
	2.00	22^{d}	23^{d}	33^{d}
	3.00	38^{c}	43^{c}	56^{c}
	4.00	50^{b}	60^{ab}	66^{b}
	5.00	63^{a}	70^{a}	76^{a}
Aqueous	1.00	03^{e}	06^{e}	10^{e}
	2.00	16^{d}	20^{d}	23^{d}
	3.00	33^{c}	33^{c}	42^{c}
	4.00	46^{b}	50^{b}	56^{b}
	5.00	56^{a}	63^{a}	65^{a}

Data was based on 3rd instars larvae, 10 larvae/ replication of 3 replications, Mean in column with the same plant extract followed by the same common letters are not significantly different at the 5% level as determined by DMRT (P < 0.05).

In response to wounding or insect feeding, linolenic acid is released from membrane lipids and then converted enzymatically into Jasmonic Acid (JA). It in turn causes the transcriptional activation of genes encoding ProtienaseInhibitors (PIs) and of enzymes involved in the production of volatile compounds or of secondary compounds such as nicotine, numerous phenolics and other defence related compounds. Naturally occurring protease inhibitors have been explored since they interact and block the active center of the digestive enzymes, both proteases and amylases in the gut system. Commonly used inhibitors include crystalline soybean trypsin inhibitor of Kunitz, Bownman - Birk soybean trypsin inhibitor, lima bean inhibitor, chickpea trypsin inhibitor, chymotrypsin inhibitor and ovomucoid[32]. These proteins bind tightly to the active site of the enzyme in the midgut preventing access to normal substrates. Failure by the pest insect to overcome this inhibition of digestion results in death by starvation.The aqueous plant extracts of *Acorus calamus*, *Xanthium strumarium*, *Polygonum hydropiper* and *Clerodendronin fortunatum* showed 50 % mortality of red spider mite at higher concentrations (10 %) under laboratory conditions [33]. This difference observed in mortality due to extracts from different plant parts might have variation in the concentration of the chemical constituents. It could be suggested that root in this case consists of more toxic compounds as compared to other plant parts (seeds).

All plant extracts showed moderate toxic effect on larvae after 24 h of exposure; however, the highest mortality was found in methanol root extracts. This has been observed earlier the potential of larvicidal activity of *Matricaria chamomile* flowers' extract was studied against engorged *Rhipicephalus annulatus* mortality rate from 6.67% to 26.7%, whereas no mortality was recorded for non-treated control group [34].

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