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Pesticidal activity of *Rivina humilis* L. (Phytolaccaceae) against important agricultural polyphagous field pest, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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ABSTRACT

Objective: To evaluate the pesticidal activity of antifeedant, oviposition deterrent, ovicidal and larvicidal activities of benzene, dichloromethane, diethylether, ethylacetate and methanol extracts of *Rivina humilis* at different concentrations against agricultural polyphagous pest *Spodoptera litura* (Lepidoptera: Noctuidae) (*S. litura*).

Methods: Antifeedant activities of the selected plant extract were studied as described by Isman *et al.* (1990), with slight modifications. For oviposition deterrent activity, ten pairs of (adult moths) *S. litura* were subjected in five replicates. After 48 h, the numbers of eggs masses laid on treated and control leaves were recorded and the percentage of oviposition deterrence was calculated. The ovicidal activity was determined against the eggs of *S. litura*. Twenty five early fourth instar larvae of *S. litura* were exposed to various concentrations and was assayed by using the protocol of Abbott's formula (1925); the 24 h LC₅₀ values of the *Rivina humilis* leaf extract was determined by probit analysis.

Results: All the extracts showed moderate antifeedant activity; however, significant antifeedant, ovicidal, oviposition deterrent and larvicidal activities were observed in methanol extract.

Conclusions: This study showed that the selected plant can be a potent source of natural antifeedant, oviposition deterrent, ovicidal and larvicidal activities against field pest *S. litura*.

1. Introduction

Agriculture is the backbone of the Indian economy and nearly 75% of the rural areas of Indian villagers is depending on agriculture. The amount of food production is greatly deteriorated due to the farmer's enemies (pests and diseases) that cause damage in the crop either directly by causing economic losses to the crops in the field or indirectly by causing disease and reducing the crop yield. According

to UN reports, the total world population now is about 6000 million. To feed this population we need to double the food production, which will require a threefold increase in the annual use of fertilizers and much more extensive use of pesticides[1]. *Spodoptera litura* (Lepidoptera: Noctuidae) (*S. litura*) is a major polyphagous pest, infesting more than 180 plant species[2-4]. Nowadays, synthetic insecticides have been widely used for controlling this pest on different crops, but undesirable side effects of synthetic insecticides, including the development of resistance, have necessitated a shift to more eco-friendly approaches for controlling this pest. In recent years, the use of synthetic organic insecticides in crop insect pest management programmes around the world has resulted in damage to the environment, pest resurgence, pest resistance to insecticides

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and lethal effects on non-target organisms. Due to these impacts of chemical insecticides prompted search for alternate techniques for insect pest management arises[5-8].

One possible way to reduce the high consumption of synthetic insecticides is through the application of botanical insecticides, generally considered to be environmental and medical safe. Plant derivatives are highly toxic to many insect species and more than two thousand plant species are known to possess some insecticidal properties. Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages[9]. Plant extracts and essential oils and their constituents have been reported to be an effective source of botanical pesticides[10,11]. The growing awareness of the hazards of excessive use of pesticides globally has led researchers to search for safer and more environment-friendly alternative methods for insect pest control. Therefore, extensive studies are carried out to screen plants as insect growth control agents. Over the last two to three decades, greater attention has been focused on the bioactivity of phytochemicals for their potential as pesticides against phytophagous insects[12]. Research on natural products could be alternatives to synthetic pesticides and fungicides, for example, plant extracts and essential oils, has greatly increased during recent years[13-15]. The neonate larvae initially attacking the foliage of the plants and the later stage feeds on developing seeds in the pod. The selected pest are considered as the serious pests of various economically important crops such as, cotton, groundnut, chilly, tobacco, castor, cabbage, mustard and tomato as well as some legumes, teases, etc., and also they have developed resistance in almost all commercially available chemical pesticides. Furthermore, literatures pertaining to the control of this pest using phytopesticides are scanty. Hence, the present study on the pesticidal activities of *Rivina humilis* (*R. humilis*) was evaluated against *S. litura* for its antifeedant, oviposition deterrent, ovicidal and larvicidal activity.

2. Materials and methods

2.1. Plants collection and solvent extraction

The plant *R. humilis* L. (Phytolaccaceae) was collected from in and around Yercaud hill station (11.77940 N, 78.20340 E) Salem Districts of the Tamilnadu, India. The leaves were collected during June 2013-January 2014 and brought to the laboratory where they were washed thoroughly with tap water and kept in sunlight for 45 min for the complete evaporation of water and then shade dried on blotting paper spread at room temperature [(28±2)°C]. The dried plant material was powdered using electric blender and extracted with selected solvent using Soxhlet apparatus. The solvent from the crude extract was evaporated using rotary evaporator at 45°C until the complete evaporation of solvent from the crude and the crude extract was stored in an aseptic amber bottle vials at 4°C in the refrigerator.

2.2. Insect rearing

The armyworm, *S. litura* (Noctuide: Lepidoptera) was cultured and maintained in the laboratory on castor leaves (*Ricinus communis* L.). Rearing conditions were a 12 h photo regime at (28±2)°C and (75±5)% relative humidity. An insect culture was continuously refreshed with wild moths captures by a light trap in the vicinity of the agricultural farm of Koothur Village, Sirkali Taluk, Nagapattinam District. Generally, hale, healthy and uniform sized fourth instar larvae, fresh eggs and newly emerged adult moths of cultured species were used for the experiments.

2.3. Antifeedant activity

Antifeedant activities of the selected plants extract were studied using leaf disc no-choice method as described by Isman *et al.*[16] with slight modifications. Fresh castor leaf discs (for *S. litura*) of 3 cm diameter were used for the experiments. Selected plant extracts of different concentrations viz., 100-500 mg/L were treated individually on the fresh leaf discs. One treatment with acetone alone was used as positive control and one treatment without solvent was considered as negative control (0 mg/L). In each Petri discs (1.5 cm×9 cm) wet filler paper was placed to avoid early drying of the leaf disc single fourth instar larva of *S. litura* was introduced individually. Five replicates were maintained for each concentration and the progressive consumption of leaf area by the larvae after 24 h was recorded in control and treated discs using leaf area.

2.4. Oviposition deterrent activity

For oviposition deterrent activity 100-500 mg/L concentration of individual extract was sprayed on respective fresh host plant and similar controls as mentioned above were also used here. The petioles of the treated leaves were inserted into a conical flask (cotton plugged) containing dechlorinated water to avoid early drying and placed inside the cage (60 cm×45 cm×45 cm). Ten pairs of (adult moths) *S. litura* were introduced in individual cages. Ten percent (w/v) sucrose solution with multivitamin drops was provided for adult feeding to increasing fecundity, five replicates were maintained for control and treatments. After 48 h, the numbers of eggs masses (*S. litura*) laid on treated and control leaves were recorded and the percentage of oviposition deterrence was calculated[17].

2.5. Ovicidal activity

For ovicidal activity, scales from the egg masses of *S. litura* were carefully removed using fine camel brush. Five hundred eggs from the selected lepidopterans were separated into five lots and each having 100 eggs and dipped in 100-500 mg/L concentrations of plant extracts and controls as mentioned above. Number of eggs hatched in control and treatments were recorded and the percentage of ovicidal activity was calculated using Abbott's formula[18].

2.6. Larvicidal activity

For the evaluation of larvicidal activity, 125-1 000 mg/L concentrations were used against *S. litura* larvae. Petioles of the leaves were tied with wet cotton plug to avoid early drying and placed in plastic trough (29 cm×8 cm) 20 pre starved (4 h) fourth instar larvae of test organisms were introduced individually and covered with muslin cloth. Five replicates were maintained and the number of dead larvae after 48 h was recorded and the percentage of larval mortality was calculated using Abbott's formula [18].

2.7. Statistical analysis

The average mortality data were subjected to probit analysis for calculating LC_{50} , LC_{90} and other statistics *Chi*-square, slope and regression values were calculated by using the software using statistical package of social science (SPSS) version 18.0 for windows, significance level was set at $P<0.05$.

3. Results

3.1. Feeding deterrent activity of different solvent extracts of *R. humilis*

Methanol extract of *R. humilis* showed (22.55±1.52)% feeding deterrence against the fourth instar larvae of *S. litura* at 100 mg/L concentration, whereas, (36.49±1.68)% and (71.92±2.67)% of antifeedant activity was recorded in methanol extract of *R. humilis* at 200 and 300 mg/L, respectively. Similarly, at higher concentrations such as 400 and 500 mg/L, (86.57±3.80)% and (98.54±3.24)% antifeedant activity was recorded respectively against the above said concentrations (Table 1). In general, the antifeedant activity is directly proportional to the increase in the concentration and also among the various extracts tested, methanol extract was found to have significant activity than the other solvent extracts.

Table 1

Antifeedant activity of *R. humilis* extracts against the fourth instar larvae of *S. litura*.

Solvent tested	Antifeedant activity (%)				
	100 mg/L	200 mg/L	300 mg/L	400 mg/L	500 mg/L
Benzene	8.83±0.84 ^b	21.52±1.82 ^b	56.78±2.66 ^b	72.53±3.68 ^b	83.54±2.66 ^b
Dichloromethane	17.38±1.66 ^d	30.80±1.56 ^d	64.90±2.93 ^d	81.85±2.35 ^d	92.55±2.48 ^d
Diethyl ether	9.69±0.78 ^c	25.78±2.87 ^c	58.46±2.78 ^c	75.43±3.74 ^c	86.12±2.88 ^c
Ethyl acetate	19.82±0.94 ^c	33.55±2.72 ^c	67.44±2.52 ^c	83.26±2.44 ^c	95.55±3.42 ^c
Methanol	22.55±1.52 ^f	36.49±1.68 ^f	71.92±2.67 ^f	86.57±3.80 ^f	98.54±3.24 ^f
Control	1.45±0.38 ^a	1.45±0.38 ^a	1.45±0.38 ^a	1.45±0.38 ^a	1.45±0.38 ^a

Values represent mean±SD of five replications. Different alphabets in the column are statistically significant at $P<0.05$. (MANOVA; LSD-Tukey's test). Control groups were fed with tender host leaf disc with no phytochemicals.

3.2. Oviposition deterrent activity of different solvent extracts of *R. humilis*

Methanol extract of *R. humilis* showed (23.42±1.69)% oviposition deterrence against the gravid moths of *S. litura* at 100 mg/L

concentration, whereas, (33.52±1.83)% and (68.49±2.67)% of oviposition deterrence was recorded in methanol extract of *R. humilis* at 200 and 300 mg/L, respectively. Similarly, at higher concentrations such as 400 and 500 mg/L (80.66±2.24)% and (97.18±2.22)% oviposition deterrence was recorded respectively against the above said concentrations (Table 2). In general, the oviposition deterrence is directly proportional to the increase in the concentration and also among the various extracts tested, methanol extract was found to have significant activity than the other solvent extracts.

Table 2

Oviposition deterrent activity of *R. humilis* extracts against the gravid moths of *S. litura*.

Solvent tested	Oviposition deterrent activity (%)				
	100 mg/L	200 mg/L	300 mg/L	400 mg/L	500 mg/L
Benzene	9.49±0.73 ^b	22.21±1.57 ^b	46.65±2.56 ^b	62.54±2.56 ^b	85.42±2.33 ^b
Dichloromethane	17.46±1.84 ^d	28.76±1.73 ^d	57.34±2.63 ^d	73.38±2.82 ^d	91.64±2.44 ^d
Diethyl ether	15.79±1.57 ^c	24.58±1.36 ^c	55.17±2.49 ^c	65.37±2.76 ^c	89.39±2.89 ^c
Ethyl acetate	21.14±1.46 ^c	31.85±2.94 ^c	66.54±2.34 ^c	76.58±2.95 ^c	95.75±2.42 ^c
Methanol	23.42±1.69 ^f	33.52±1.83 ^f	68.49±2.67 ^f	80.66±2.24 ^f	97.18±2.22 ^f
Control	1.85±0.84 ^a	1.85±0.84 ^a	1.85±0.84 ^a	1.85±0.84 ^a	1.85±0.84 ^a

Values represent mean±SD of five replications. Different alphabets in the column are statistically significant at $P<0.05$ (MANOVA; LSD-Tukey's test). Control groups were allowed to lay eggs on host plant sprayed with no phytochemicals.

3.3. Ovicidal activity of different solvent extracts of *R. humilis*

Methanol extract of *R. humilis* showed (21.49±1.89)% ovicidal activity against the eggs (0-6 h) of the *S. litura* at 100 mg/L concentration, whereas, (38.53±1.24)% and (74.78±3.32)% of ovicidal activity was recorded in methanol extract of *R. humilis* at 200 and 300 mg/L respectively. Similarly, at higher concentrations such as 400 and 500 mg/L (87.29±2.48)% and (98.25±2.36)% ovicidal activity was recorded respectively against the above said concentrations (Table 3). In general, the ovicidal activity is directly proportional to the increase in the concentration and also among the various extracts tested, methanol extract was found to have significant activity than the other solvent extracts.

Table 3

Ovicidal activity of *R. humilis* extracts against the eggs (0-6 hrs) of the *S. litura*.

Solvent tested	Ovicidal activity (%)				
	100 mg/L	200 mg/L	300 mg/L	400 mg/L	500 mg/L
Benzene	7.96±1.29 ^b	18.28±1.53 ^b	56.94±1.54 ^b	69.58±2.54 ^b	85.55±2.84 ^b
Dichloromethane	15.47±1.34 ^d	27.55±1.32 ^d	68.65±1.42 ^d	82.49±2.59 ^d	92.55±2.36 ^d
Diethyl ether	10.95±1.64 ^c	21.38±1.27 ^c	59.30±1.68 ^c	78.25±2.37 ^c	89.49±2.57 ^c
Ethyl acetate	18.72±1.56 ^c	34.67±1.94 ^c	70.92±1.89 ^c	85.75±2.56 ^c	94.44±2.86 ^c
Methanol	21.49±1.89 ^f	38.53±1.24 ^f	74.78±3.32 ^f	87.29±2.48 ^f	98.25±2.36 ^f
Control	2.57±0.86 ^a	2.57±0.86 ^a	2.57±0.86 ^a	2.57±0.86 ^a	2.57±0.86 ^a

Values represent mean±SD of five replications. Different alphabets in the column are statistically significant at $P<0.05$. (MANOVA; LSD-Tukey's Test). Eggs in control groups were sprayed with no phytochemicals.

3.4. Larvicidal activity of different solvent extracts of *R. humilis*

Maximum larvicidal activity was observed in the methanol extract of *R. humilis* followed by ethylacetate, dichloromethane,

diethylether and benzene. Among five solvents tested the methanol extract was found to be most significant solvent which brings the significant larvicidal activity against the fourth instar larvae of *S. litura* which is represented in Figure 1. It is noteworthy to observe that as the concentration increased the larval mortality is also increased. The maximum larvicidal activity was recorded from the highest concentration of methanol extract at 1000 mg/L and the least larvicidal activity was recorded from the 125 mg/L concentration of benzene extract. Furthermore, the larval mortality observed from the 250, 500, 750 and 1000 mg/L concentrations extracts showed (34.20±1.94)%, (62.80±2.35)%, (76.60±2.64)% and (98.60±2.22)%, respectively with methanol extract. These results obtained from the present experiment have been proved significant statistically, and they are all on par with the control groups. In nut shell, the experimental larvae subjected to 1000 mg/L concentrations were found more susceptible to the plant extracts tested since the lethality of the larvae were found to be maximum among the test concentrations.

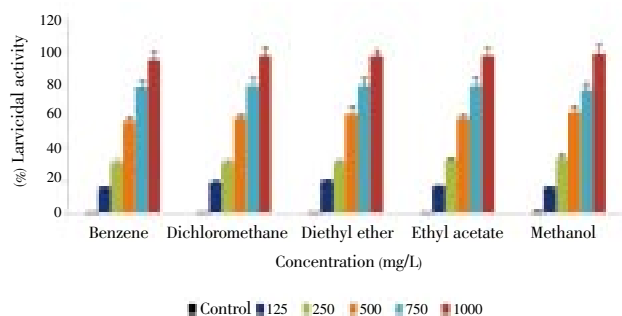


Figure 1. Larvicidal activity of *R. humilis* extracts against freshly moulted fourth instar larvae of *S. litura*.

3.5. Lethal activity of different solvent extracts of *R. humilis*

Lethal concentration observed against fourth instar larvae of *S. litura* with various solvent extracts are shown in Figure 2. The LC₅₀ value of benzene, diethylether, dichloromethane, ethyl acetate and methanol extract of *R. humilis* were 469.20, 446.96, 445.13, 440.42 and 440.30 mg/L respectively. The Chi-square values are significant at $P \leq 0.05$ level. The Chi-square values in the bioassays indicated probably the heterogeneity of the test population. Among five solvent extracts, the methanol extract was responsible for strong lethal activity observed against selected pest species.

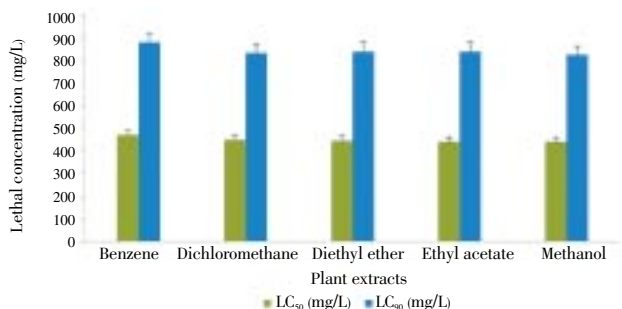


Figure 2. Lethal concentrations of *R. humilis* extracts against freshly moulted fourth instar larvae of *S. litura*.

4. Discussion

The results showed that Indian medicinal plant *R. humilis* have significant antifeedant activity, oviposition deterrent activity, ovicidal activity and larvicidal activity against selected important agricultural lepidopteran field pest *S. litura*. The results are comparable with an earlier report by Pavela^[19] who reported that twenty essential oils applied by fumigation were highly toxic to the third instar of *Spodoptera littoralis* larvae. Two essential oils *Nepeta cataria* and *Thuja occidentalis* were highly toxic with LC₅₀ 10.0 mL/m³ (5.5 and 6.5 mL/m³, respectively). Recently, Duraipandiyar *et al.*^[20] have reported that larvicidal activities of rhein isolated from *Cassia fistula* flower against lepidopteron pests *S. litura* and *H. armigera* and the LC₅₀ values was 606.50 mg/L for *H. armigera* and 1192.55 mg/L for *S. litura*. The survived larvae produced malformed adults. Elumalai *et al.*^[21] have reported that maximum ovicidal activity was found in *Ocimum basilicum* and *Zingiber officinale*. *S. litura* eggs were 100% of mortality (No hatchability) recorded in 300 mg/L, respectively. Krishnappa *et al.*^[22] have reported that *Tagetes patula* volatile oil contained 10 compounds and they were tested against the fourth instar larvae of *S. litura* for their antifeedant activity by leaf disc bioassay. Among the compounds tested, terpinolene was the most effective feeding deterrent agent against *S. litura*. Jeyasankar *et al.*^[23] observed that high larval mortality indicates potential insecticidal properties present in the *Syzygium lineare* plant extract and the isolated crystal compound. Jeyasankar *et al.*^[24] reported that a new crystal compound 2,5-diacetoxy-2benzyl-4,4,6,6-tetramethyl-1,3-cyclohexanedione was isolated from the leaves of *S. lineare*. The insecticidal activity of the compound was assessed against fourth instar larvae of *S. litura*. Its activity was better than the positive control azadirachtin. The compound was responsible for growth inhibition on *S. litura*. It induced larval, pupal and adult deformities even at low concentration. Anandan *et al.*^[25] have reported that crude extracts of *Hyptis suaveolens* and *Melochia chorchorifolia* against *S. litura*, four fractions obtained from *H. suaveolens*, fraction III was found to inhibit the feeding ratio of the *S. litura* and it is apparent from the table. While in *Melochia chorchorifolia* only three fractions have been obtained, among them fraction II was found to induced more feeding deterrent activity at 2000 mg/L concentration.

Krishnappa *et al.*^[26] reported that the *Clausena dentate* leaves essential oil against Armyworm, *S. litura* it produce significant larvicidal activity, with 24 h LC₅₀ 111.54 mg/L and LC₉₀ 205.38 mg/L, respectively. The major chemical compositions larvicidal activities were also tested. The LC₅₀ and LC₉₀ values of sabinene were LC₅₀ 21.42 mg/L and LC₉₀ 40.39 mg/L, respectively. This was closely followed by biofloratriene LC₅₀ 23.31 mg/L and LC₉₀ 43.62 mg/L. Baskar *et al.*^[27] have observed pupicidal activity in different crude extract of *Atalantia monophylla* against *H. armigera*. Malarvannan *et al.*^[28] observed that *Argemone mexicana* extracts reduced adult emergence and increased pupal mortality of *S. litura*. Baskar *et al.*^[29] have reported that bioefficacy of leaf and root extracts of *Aristolochia tagala* against *S. litura*. Effects on feeding,

larvicidal and pupicidal activities and larval-pupal duration were studied. Higher antifeedant activity (56.06%), lethal concentration for feeding inhibition (3.69%), larvicidal (40.66%), pupicidal (28%), total mortality (68.66%) and prolonged larval-pupal duration (12.04–13.08 d) were observed in ethyl acetate leaf extract at 5.0% concentration. bioassay. Earlier, Anandan *et al.*[30] reported that efficacy of ethyl acetate, methanol and aqueous extracts of *Acrocalamus*, *Corchorus aestuauus*, *Cammelina bengalinsis*, *Embllica ficus religiosa* and *Lantena Camera* were tested at 1000 mg/L for their antifeedant activity against fourth instar larvae of *H. armigera* using leaf disc (no-choice) method. The aqueous extract of *C. collinus* was found to have maximum antifeedant activity followed by *E. fisheri* *F. religiosa* and *C. aestuauus*. Jayasankar *et al.*[31] reported that *Mentha* oil showed minimum ovicidal activity at 0.25% concentration 18.33±3.15 and maximum ovicidal activity at highest concentration tested (2.0%-28.99±7.11). Ovicidal activity recorded from 0.5 and 1.0% were less significant (23.25±4.66 and 24.74±5.47, respectively).

Earlier, Elumalai *et al.*[32] reported that the maximum larval mortality was found in the essential oil of *Zingiber officinales*, *Citrus limonum*, *Acorus calamus*, *Rosmarinus officinalis*, *Ocimum basilicum*, *Cuminum cyminum* and *Coriandrum sativum* tested against armyworm, *S. litura* agricultural important lepidopteron pest with the LC₅₀ values were 15.00, 34.55, 36.13, 38.2, 57.55, 63.99 and 65.07 mg/L, respectively. Baskar *et al.*[33] reported that the ethyl acetate extract of *Couroupita guianensis* exhibited 69.7% against *H. armigera* at 5% concentration. The antifeedant activity was due to the presence of steroids, phenols, flavonoids and alkaloids in the ethyl acetate extract of leaf. Gokulakrishnan *et al.*[34] reported that the line of experiment was attempted with plant oil formulation, showed maximum percentage of ovipositional repellent activity against the gravid moths of *H. armigera* followed by *S. litura* and *E. vitella* were 84.75, 79.90 and 76.55, respectively. Gokulakrishnan *et al.*[3] reported that the most significant antifeedant activity was observed in *Achaea janata* at 1000 mg/L concentration of *Salvia officinalis* (85.56), *S. litura*, *Mentha spicata* (82.85), *H. armigera* and *M. spicata* (90.55) *Achaea janata*. Thirugnanasampandan *et al.*[35] reported these phytochemical compounds do not cause any harmful effects on human or environment since these phytochemicals have shown effective antioxidant and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging potential. It may be possible to produce botanical insecticides by phytopharming through genetic engineering of an existing field crop to produce high value natural products. This research found that even relatively short-term exposure of larvae, pupae and eggs to plant extracts doses can markedly increase their mortality over time, and thus reduce the total number of viable larvae, pupae and eggs leading to a possible significant reduction in the total population dynamics of pests. Moreover, in insects, as determined by other authors, short-term exposure of larvae, pupae, eggs and adults to essential oils may cause a significant reduction in fecundity and fertility[36–38]. The present investigation suggests that the biological activity of botanicals is plant species-specific and

the exposure concentrations have a direct impact on the efficacy of test species. Thus, the subsequent processes of isolation of active principle responsible for above activities are under processing. Conclusively, the broad-spectrum activities of phytochemicals, an extractable molecule imparts unique qualities to the plant and its potential needs to be further exploited for insect control programme.

An attempt has been made to evaluate the role of different extracts of *R. humilis* for their antifeedant activity, oviposition deterrent activity, ovicidal activity and larvicidal activities against various life stages of selected lepidopteran field pest *S. litura*. The results reported in this study opened the possibility for further investigations of the efficacy of antifeedant activity, oviposition deterrent activity, ovicidal activity and larvicidal activities of natural product extracts as a potential agent for combating important agricultural field pest *S. litura*.

Conflict of interest statement

We declare that we have no conflict of interest.

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