FEEDING DETERRENCE INDEX OF *Momordica cymbalaria* FENZL EX HOOK AGAINST COMMON CUT WORM, *Spodoptera litura* FABRICIUS

ABSTRACT

Plant secondary metabolites exhibit a wide range of biological activity and may act as feeding and oviposition deterrents, repellents, fumigants, and behavior modifiers. Fruits and tubers of *Momordica cymbalaria* Hook (Fenzl.) possess anti-ovulatory and abortifacient properties that could be exploited in the management of insect pests. Hence, the feeding deterrent activity of *M. cymbalaria* leaf, peel (fruit skin), seed and tuber solvent extract against *Spodoptera litura* Fabr. was studied. Different concentrations of acetone extract of leaf from 0.3% to 0.8%, performed better in deterring *S. litura* larval feeding. Peel acetone extract showed a strong feeding deterrence (94.83% leaf area protection over the absolute control) at 0.9% concentration followed by medium deterrence at 0.8, 0.7, and 0.6% concentrations (85.62, 77.90, and 67.08 % protection respectively). The hexane seed extract was comparatively oilier and had a distinct rancid smell noticeable in the higher concentrations (0.8, 0.9, and 1%). Tubers did not exhibit any significant leaf area protection below 0.2% concentration. However, 1% tuber acetone extract showed 99.36 percent protection. The effective concentrations of leaf acetone extract for 50 and 95% deterrence were 0.27and 0.84 percent respectively. The effective concentrations of other plant parts were ranked as follows; Seeds>Peels>Tubers.

Keywords: Feeding deterrence; *Momordica cymbalaria* Fenzl ex hook; leaf; peel; seed; tuber.

1. INTRODUCTION

Plant secondary metabolites *viz.*, alkaloids, terpenoids, flavonoids, phenols, amino acids, sugars, etc. play a crucial role in protecting crops from the attack of insect pests. They exhibit a wide range of biological activity and may act as feeding and oviposition deterrents, repellents, fumigants and behavior modifiers [1,2,3]. Development of resistance and resurgence and residues due to indiscriminate use of synthetic pesticides have renewed interest in natural pesticides. Their easy degradability, wide spectrum of action, lack of resistance development, and ease of sourcing makes them an attractive choice. Thousands of plant species are found to possess insecticidal properties, indicating that the plant kingdom is a vast storehouse of potentially useful chemicals for pest control [4].

Momordica cymbalaria Fenzl ex Hook (Syn.: *Luffa tuberosa* (Roxb.); *Momordica tuberosa* (Roxb.)), a vine found in India, in the states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, and Tamil Nadu has been used in various Asian traditional medicine systems for a long time. In addition, fruits and tubers possess anti-ovulatory and abortifacient properties which could be exploited in the management of insect pests.

Spodoptera litura Fab. (Beet armyworm; Tobacco cutworm), a devasting polyphagous pest known to cause cent percent yield loss under outbreak conditions, is regularly managed using chemical insecticides and developed multi-pronged resistance [5,6,7]. Hence, the feeding deterrent activity of *M. cymbalaria* leaf, peel (fruit skin), seed and tuber solvent extracts against *S. litura*, was evaluated.

2. MATERIALS AND METHODS

The present study was carried out during 2019 to 2022 in the Phyto-insecticide Laboratory, Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu, India.

2.1 Mass Culturing of *S. litura*

The initial culture (egg mass) was purchased from the National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, India, and the accession number was NBAII-MP-NOC-02. Continuous culture was maintained in the Department of Entomology insectary throughout the study period. The rearing trays and other materials used for the rearing purpose were disinfected with 0.1percent formaldehyde solution.

The neonate larvae were allowed to feed on fresh disease-free, castor leaves. The fresh castor leaves were washed with water, shade dried, and fed to the larvae. The first three instars were reared in groups (fifty larvae per container) using small plastic containers (16 cm x16 cm x10cm). From the fourth instar onwards, they were transferred to larger round plastic containers (60 cm dia. x 25cm height) @ 30 larvae per container. The fully grown larvae were allowed to pupate in sterilized sawdust. Three days after pupation they were taken out carefully, sexed, and placed inside an adult emergence cum oviposition cage (45 cm x 45cm x 45 cm) in a male: female ratio of 10:15. The emerged adults were provided with 10% sugar solution mixed with five drops of vitamin E (by dipping a cotton wig at the top of the 15ml glass vial) as adult feed.

Well-cleaned fresh *Nerium oleander* leaves with their petiole immersed in a 250ml conical flask containing water served as ovipositional substrate. Egg masses laid in groups were carefully removed on alternate days, scales removed, dipped in 0.1 percent sodium hypochlorite solution for 2 minutes, shade dried, and stored in a plastic container covered with a cotton cloth. Upon hatching, the neonates were reared on castor leaves and the cycle continued.

2.2 Collection of Plant Samples and Preparation of Extracts

Leaf, peel (Fruit skin), seed, and tuber of *M. cymbalaria*were collected from Sangarankoil of Tenkasi district, Virudhunagar, Sattur, and Aruppukottai of Virudhunagar district, and T. Kallupatti, Saptur, and Tirumangalam of Madurai district. Identified by Dr. M. Murugan, Sri Kaliswari College (Autonomous), Sivakasi. Specimens were submitted at Department of Botany, Annamalai University. The vine along with the entire plant was collected during the rainy and winter season (October – January). The succulent tubers were collected during the pre-monsoon season once they started germinating (September – October). The collected plant parts were washed with distilled water and shade dried until the moisture content was completely removed. The dried samples were ground into a fine powder with a traditional stone grinder, sieved to uniform particle size using a 30 mesh sieve, and stored in air-tight plastic containers (500 ml capacity) separately.

2.3 Extraction Method

The powdered plant samples were extracted using three different analytical grade solvents *viz.*, hexane (Non-polar, BP – 69°C), acetone (Polar, BP – 56°C), and methanol (Most polar, BP – 65°C) sequentially at room temperature. 30 g of each powdered plant

sample was soaked in 300 ml of the respective solvent sequentially (from non-polar to most polar) for seven days each in a 500 ml conical flask, covered with aluminum foil and shaken intermittently in a magnetic stirrer (Remi 2-MLH) [8]. Later, each solvent extract was concentrated using a rotary evaporator, the extractive was collected separately, and stored in glass vials (30 ml Capacity) kept in a refrigerator until further use.

2.4 Preliminary Evaluation of the Toxicity of Solvent Extracts against *S. litura*

Oral toxicity of all the extractives was evaluated through poison food bioassay. The extractive was diluted using 0.1 percent emulsified water (0.1 ml of triton X-100 in 100 ml of water) to prepare desired concentrations [9,10]. The concentrations tested were 1, 10, 20, 30, 50, 70, 90 and 100 percent.0.3ml of each concentration of each extractive was smeared on three cm diameter castor leaf discs (both abaxial and adaxial surface) using a camel hair brush, air dried for 10 minutes, and placed inside Petri dishes (15 cm dia.) lined with moist filter paper. 10 four-hour pre-starved third instar *S. litura* larvae were released into each Petri dish containing three treated leaf discs. 0.5 percent neem oil-treated leaf discs acted as the positive control. Solvent control and absolute control were also maintained. The experiment was replicated three times and carried out as a Completely Randomized Design. All the bioassays were performed at 25 ± 3° C and 80 % Relative Humidity. After six hours, the leaf area fed was measured using a leaf area meter (Systronics India Ltd., Model. No: 211), and percent leaf area protection over absolute control was computed and feeding deterrent activity was calculated using the formula:

$$\text{Deterrency Index (DI)} = \frac{C - T}{C + T} \times 100$$

Where C = Per cent leaf area consumed in control discs

T = Per cent leaf area consumed in treated discs.

2.5 Confirmatory Evaluation of the Toxicity of Solvent Extracts against *S. litura*

A confirmatory bioassay was performed with selected effective extracts at lower concentrations *viz.*, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 per cent as detailed earlier. The desired concentrations were obtained by dissolving 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6. 0.7, 0.8, 0.9 and 1 g of respective effective extract in 100ml of emulsified water. The experiment was replicated three times and percent leaf area protection over absolute control was computed.

The most effective extracts imparting medium feeding deterrence (50 to 80%) at 0.5 percent concentration were selected and their effective concentrations deterring 50 and 95percent (EC_{50} and EC_{95}) of *S. litura* larvae, was deduced by linear regression analysis.

3. RESULTS AND DISCUSSION

Preliminary bioassay conducted with different concentrations *viz.* 1, 10, 20, 30, 50, 70, 90, and 100 per cent revealed supremacy of all the solvent extracts in imparting feeding deterrence. All the solvent extracts exhibited 100% feeding deterrence from 10% concentration onwards. At the lowest concentration tested (1%), the feeding deterrent activity got reduced and ranged from 85% to 99% across the solvent extracts tested.

From the results of the confirmatory bioassay, based on observations of unfed leaf area measured using leaf area meter, it was evident that the acetone extracts *M. cymbalaria* leaf, peel, seed and tuber imparted medium feeding deterrence from 0.5% concentration onwards. This was followed by methanol extract of all the plant parts tested. Among the plant parts tested, the acetone leaves extract imparted highest feeding deterrence followed by seed, peel and tuber extracts.

At the lowest concentration tested (0.05 %), all the leaf solvent extracts imparted insignificant activity which was found to improve drastically in leaf acetone extract along increasing concentrations. At 0.3% concentration itself, leaf acetone extract was found to impart medium feeding deterrent action while the hexane and methanol extracts were showing weak feeding deterrence. The leaf acetone extracts imparted strong feeding deterrence from 0.6% onwards whereas the other two solvent extracts reached that level only at 0.8% concentration.

The seed acetone extract showed almost a similar trend as that of the acetone leaf extract. However, the leaf acetone extract performed better in deterring the feeding of *S. litura* larvae till 0.8% concentration. With regard to hexane and methanol seed extracts a similar trend was noticed till 0.7% concentration. Nevertheless, hexane seed extract at higher concentrations (0.8, 0.9 and 1%) performed better than the methanol seed extract. The non-polar nature of hexane might have played a role in extracting out more oily active ingredients which favoured a more pronounced feeding deterrent action. This was evidenced by the fact that the hexane seed extract was comparatively more oily and a distinct rancid smell noticeable in the above mentioned higher concentrations (0.8, 0.9 and 1%). Peel acetone extract

was also found superior in deterring the *S. litura* larvae. It showed a strong feeding deterrence (94.83% leaf area protection over the absolute control) at 0.9% concentration followed by a medium action of 85.62%, 77.90% and 67.08%protection at 0.8%, 0.7% and 0.6% concentrations respectively. Peel hexane and methanol extracts were found to be strong deterrents at 1%, medium deterrents at 0.7% and 0.8% and weak deterrents at 0.4% and 0.3 % concentrations. Below 0.3% both the extracts were found to impart insignificant feeding deterrence. A similar trend as noticed in all the other plant parts was found in tuber hexane, acetone and methanol extracts. However, tubers did not exhibit any significant leaf area protection below 0.2% concentration while compared with solvent extracts of other plant parts.

3.1 Effective Concentrations of Effective Extract (DI_{50} and DI_{95})

Acetone extract of leaf, peel, seed and tuber which imparted medium to strong feeding deterrence were selected as most effective extract and their effective concentrations to deter 50% and 95 % feeding in *S. litura* third instar larvae was deduced. The leaf acetone extract imparted 50% and 95% deterrence at lowest concentrations among the plant parts tested. The effective concentrations were 0.44 (DI_{50}) and 0.89 (DI_{95}). The effective concentrations of the other plant parts were ranked as follows; Seeds>Peels>Tubers.

Feeding deterrence was noticed in many plant extracts as noticed in *M. cymbalaria* acetone extract. Feeding deterrence of *Azadirachta indica* A. Juss. acetone extract was reported by [11]. The supremacy of acetone extract in other botanicals over other solvent extracts were reported earlier by [12,10,13,14]. The oily nature of hexane seed extract as found in the present study was corroborated by many researchers in various botanicals like plant oils [15] *Syzygium lineare* leaf [16] and *C. fistula* flower [17].

Table 1. Percent feeding deterrence in *M. cymbalaria* leaf extract treated discs fed by third instar *S. litura* larvae

Conc. (in per cent)	Deterrency Index*		
	Hexane extract	**Acetone extract**	**Methanol extract**
0.05	6.59 (14.87) [k]	9.93 (18.37) [k]	7.78 (16.20) [k]
0.1	8.94 (17.40) [j]	15.22 (22.96) [j]	10.80 (19.19) [j]
0.2	10.77 (19.16) [i]	27.43 (31.58) [i]	15.52 (23.20) [i]
0.3	20.65 (27.03) [h]	39.14 (38.73) [h]	23.32 (28.88) [h]
0.4	27.71 (31.76) [g]	46.00 (42.71) [g]	34.13 (35.75) [g]
0.5	34.58 (36.02) [f]	56.59(48.79) [f]	44.87(42.06) [f]
0.6	47.75(43.71) [e]	67.08(54.99) [e]	54.28(47.46) [e]
0.7	60.15(50.86) [d]	77.90(61.96) [d]	66.08(54.38) [d]
0.8	68.55(55.89) [c]	85.62(67.72) [c]	74.42(59.62) [c]
0.9	82.72(65.44) [b]	94.83(76.86) [b]	87.69(69.46) [b]
1	93.95(75.76) [a]	99.35(85.38) [a]	97.37(80.67) [a]
Positive Control (Neem oil 0.5%)	93.85(75.64) [a]	92.55(74.16) [b]	94.05(75.88) [ab]
Solvent Control (Acetone)	0.11(1.90) [l]	0.09(1.72) [l]	0.07(1.52) [l]
Absolute Control	0.22	0.23	0.25
S. Ed	0.52	0.53	0.69
CD (0.05)	1.06	1.08	1.40

**Mean of three observations; Values within parentheses are arcsine transformed; Means with different alphabets within a column differ significantly*

Table 2. Percent feeding deterrence in *M. cymbalaria* seed extract treated discs fed by third instar *S. litura* larvae

Conc. (in per cent)	Deterrency Index*		
	Hexane extract	**Acetone extract**	**Methanol extract**
0.05	0.83(5.23) [k]	4.57(12.34) [k]	8.51(16.96) [k]
0.1	3.62(10.97) [j]	7.34(15.72) [j]	15.73(23.37) [j]
0.2	8.47(16.92) [i]	10.91(19.29) [i]	21.49(27.62) [i]
0.3	12.03(20.29) [h]	17.67(24.86) [h]	25.89(30.59) [h]
0.4	17.21(24.51) [g]	23.57(29.04) [g]	39.27(38.80) [g]
0.5	25.50(30.33) [f]	31.88(34.38) [f]	45.11(42.19) [f]

Conc. (in per cent)	Deterrency Index*		
	Hexane extract	**Acetone extract**	**Methanol extract**
0.6	33.66(35.46)^{e}	42.33(40.59)^{e}	58.99(50.18)^{e}
0.7	45.06(42.16)^{d}	59.73(50.61)^{d}	75.05(60.03)^{d}
0.8	60.47(51.04)^{c}	71.88(57.98)^{c}	83.42(65.97)^{c}
0.9	77.62(61.77)^{b}	92.10(73.68)^{b}	94.94(77.00)^{b}
1	90.52(72.07)^{a}	99.43(85.67)^{a}	94.86(76.90)^{a}
Positive Control (Neem oil 0.5%)	90.0(71.57) ^{a}	91.55(73.10) ^{b}	94.05(75.88)^{ab}
Solvent Control (Acetone)	0.06(1.40) ^{l}	0.05(1.28) ^{l}	0.06(1.40) ^{l}
Absolute Control	0.30	0.19	0.23
S. Ed	0.47	0.61	0.45
CD (0.05)	0.95	1.23	0.91

**Mean of three observations; Values within parentheses are arcsine transformed; Means with different alphabets within a column differ significantly*

Table 3. Percent feeding deterrence in *M. cymbalaria* peel extracts treated discs fed by third instar *S. litura* larvae

Conc. (in per cent)	Deterrency Index*		
	Hexane extract	**Acetone extract**	**Methanol extract**
0.1	2.17(8.47)^{k}	9.10(17.56)^{j}	4.38(12.08)^{k}
0.2	6.58(14.86)^{j}	14.29(22.21)^{i}	9.13(17.59)^{j}
0.3	10.76(19.15)^{i}	20.98(27.26)^{h}	13.12(21.24)^{i}
0.4	14.47(22.36)^{h}	27.07(31.35)^{g}	17.81(24.96)^{h}
0.5	22.39(28.24)^{g}	32.85(34.97)^{f}	25.64(30.42)^{g}
0.6	28.25(32.11)^{f}	41.15(39.90)^{e}	33.27(35.23)^{f}
0.7	38.10(38.12)^{e}	49.52(44.72)^{d}	43.34(41.17)^{e}
0.8	47.07(43.32)^{d}	63.37(52.75)^{c}	55.64(48.24)^{d}
0.9	56.18(48.55)^{c}	77.83(6.91)^{b}	67.77(55.41)^{c}
1	74.01(59.35)^{b}	91.36(72.91)^{a}	81.69(64.67)^{b}
Positive Control (Neem oil 0.5%)	92.00(73.57) ^{a}	90.25(73.62)^{a}	90.05(71.61) ^{a}
Solvent Control (Acetone)	0.07(1.52) ^{l}	0.09(1.72)^{k}	0.06(1.40) ^{l}
Absolute Control	0.29	0.21	0.20
S. Ed	0.57	0.99	0.42
CD (0.05)	1.15	2.01	0.85

**Mean of three observations; Values within parentheses are arcsine transformed; Means with different alphabets within a column differ significantly*

Table 4. Percent feeding deterrence in *M. cymbalaria* tuber extracts treated discs fed by third instar *S. litura* larvae

Conc. (in per cent)	Deterrency Index*		
	Hexane extract	**Acetone extract**	**Methanol extract**
0.2	0.51(4.10)^{j}	6.81(15.13)^{i}	5.25(13.25)^{j}
0.3	3.62(10.97)^{i}	12.39(20.61)^{h}	9.77(18.21)^{i}
0.4	7.56(15.96)^{h}	21.56(27.67)^{g}	15.73(23.37)^{h}
0.5	12.55(20.75)^{g}	28.78(32.44)^{f}	21.78(27.82)^{g}
0.6	18.70(25.62)^{f}	38.64(38.43)^{e}	28.91(32.53)^{f}
0.7	24.87(29.91)^{e}	46.11(42.77)^{d}	35.99(36.86)^{e}
0.8	32.10(34.51)^{d}	55.05(47.90)^{c}	44.86(42.05)^{d}
0.9	55.75(48.30)^{c}	69.71(56.61)^{b}	61.19(51.47)^{c}
1	81.49(64.52)^{b}	98.73(83.53)^{a}	84.45(66.78)^{b}
Positive Control (Neem oil 0.5%)	90.15(71.71) ^{a}	94.55(76.50) ^{b}	93.05(74.71) ^{a}
Solvent Control (Acetone)	0.12(1.99) ^{k}	0.13(2.07) ^{j}	0.09(1.72) ^{k}
Absolute Control	0.17	0.23	0.22
S. Ed	0.56	1.00	0.66
CD (0.05)	1.15	2.05	1.35

**Mean of three observations; Values within parentheses are arcsine transformed; Means with different alphabets within a column differ significantly*

Table 5. Effective concentrations (DI_{50}& DI_{95}) of *M. cymbalaria* effective extracts imparting medium to strong feeding deterrence against *S. litura*

Sample	DI_{50}	DI_{95}	Correlation Coefficient	R^2	Intercept (a)	Slope (b)
Leaf acetone extract	0.44	0.89	0.99	0.99	6.5	99.82
Seed acetone extract	0.59	1.05	0.97	0.94	-8.43	98.08
Peel acetone extract	0.65	1.20	0.98	0.96	-3.76	82.22
Tuber acetone extract	0.72	1.24	0.99	0.98	-13.38	87.74

4. CONCLUSION

It is evident that *M. cymbalaria* possess very effective feeding deterrence activity against the common cut worm *S. litura.* The effective concentrations of leaf acetone extract for 50 and 95% deterrence were 0.27and 0.84 percent respectively. The effective concentrations of other plant parts were ranked as follows; Seeds>Peels>Tubers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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