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Validation of Antifeedant Properties of Certain Plants from the Kalvarayan Hills, Eastern Ghats of Tamil Nadu Used in Indigenous Traditional Preparations against Insect Pests

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The Kalvarayan / Kalrayan hills, Eastern Ghats mountains of Tamil Nadu, India, is known for its involvement in organic farming. Many farmers are following Indigenous traditional knowledge to manage insects and diseases of various crops. An array of Indigenous traditional knowledge is still existing among the farmers of the hills. Utilization of these indigenous knowledge can serve as an

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important component in development of IPM strategies against insect pests. To tap and validate the Indigenous traditional knowledge, a survey was initiated and as a result it was known that fifteen plant species took place as a component in indigenous traditional preparations used in insect pest management. Thus, all the fifteen plants were validated in the laboratory at controlled conditions by using larvae of *Spodoptera litura* (third instar) as test insect. The bio-assays of antifeedant study at 1%, 3%, and 5% concentrations revealed that *Crotalaria paniculata* has been identified as the most promising plant, displaying the higher antifeeding effect at all concentrations. The study revealed a positive correlation between extract concentration and antifeeding activity. *Holoptelea integrifolia* was also shown significant inhibition. Moderate effects were observed for *Catharanthus pusillus*, *Mucuna pruriens* and *Ipomea pandurata*. Conversely, several other plants, including *Euphorbia hirta, Polyalthia longifolia, Ocimum basilicum, Mallotus philippensis* and *Butea monosperma* showed minimal impact.

Keywords: Eastern ghats of Tamil Nadu; indigenous knowledge; antifeedant; S.litura; Crotalaria paniculata.

1. INTRODUCTION

India is rich in plant diversity, with a wide range of vegetation resulting from its varied geographical and climatic conditions. The country's ethnic diversity has allowed many indigenous cultures to preserve their traditional knowledge of native plant life [1]. India has the world's second-biggest tribal population, after Africa. Autochthonous people rely heavily on forests for their livelihood. Plants and their parts are used not just for food and medicine, but also in many tribal ceremonies that are integral to their social and religious lives [2].

Native communities living in areas rich in biodiversity have a deep understanding of the local flora and their medicinal uses. This knowledge is traditionally shared orally, passing from one generation to the next [3]. This indigenous knowledge has become a well-known strategy for discovering new sources of natural insecticides. The use of plant-based insecticides can play a crucial role in regulating insect pest populations, especially in organic farming, amidst the climate-induced changes impacting insect pest complexes across ecosystems [4]. The Kalvarayan/Kalrayan Hills are notable for their rich biodiversity, comprising numerous plants with ethnomedicinal importance recognized by indigenous communities. Previous research has recorded 64 plant species with medicinal value and 27 poisonous plant species in the area [5,6]. The purpose of this study is to investigate and validate the ethnic knowledge of natural pesticides among the Malayali and Irular tribes of the Kalvarayan hills. The goal of the research is to develop organic-based formulations to promote natural farming.

2. MATERIALS AND METHODS

2.1 Description of Study Area

The Kalvarayan Hills, part of the Southeastern Ghats, extend across the Villupuram, Salem, and Dharmapuri districts of Tamil Nadu, covering 1,145.87 square kilometers. Geographically, they are situated between latitudes 11°35'00" N to 12°05'00" N and longitudes 78°25'00" E to 79°00'00" E. The hills rise from 760 meters to 1,370 meters above sea level. They are divided into two main sections: the northern 'Chinna Kalrayan' with an average height of 823 meters and the southern 'Periya Kalrayan' with an altitude of about 1,298 meters. The study area experiences an average yearly rainfall ranging between 782.98 mm and 1787.20 mm. The temperature fluctuates from a minimum of 25ºC to a maximum of 40ºC. According to Kadavul and Parthasarathy [7], the soil in this region consists of seven different types, ranging from red loam to black clay. The survey was conducted in five hamlets of Salem (11.6643° N, 78.1460° E) (Kallathur, Karumandurai, Periyakalrayan, Puliyankurichi), three hamlets of Kallakurichi (11.7384° N, 78.9639° E) (Thalavanur,Kombur,Sitheri) districts.

2.2 Interview with Indigenous People

2.2.1 Malayali tribes

The high topography and surrounding plains of the Southern Eastern Ghats are solidly populated, and the tribes in this region are known as "Malayalees" [8]. According to Thurston [9], the term Malayali is derived from the words "malai" (hill) and "al" (person), thus referring to hill people [2]. Their survival primarily relies on resources from agriculture and forests [10].

Plants recommended by the malayali tribes are *Crotalaria paniculata, Dioscorea bulbifera, Euphorbia hirta, Euphorbia neriifolia, Holoptelea integrifolia, Ipomea pandurata, Mallotus philippensis, Mucuna pruriens, Murraya paniculata.*

2.2.2 Irular tribes

The local Irulars are well known for their unique cultural practices, extensive knowledge of herbal medicine, and proficiency in agriculture. Farming, hunting, and collecting honey are among their traditional pursuits, and they have a thorough understanding of the area flora and animals. Natural medicine practitioners and elderly people involved in farming activities from both tribes had ample amount of knowledge pertaining to the flora. Plants suggested by Irular tribes are *Butea monosperma, Catharanthus pusillus, Clerodendrum trichotomum, Ocimum basilicum, Rauvolfia tetraphylla, Polyalthia longifolia*.

Ethnobotanical information (local plant name, plant characteristics and distinguishing traits, availability, medicinal and other uses) were gathered through interviews and conversations with elders and tribe curators in and around the review region. The samples were collected and sent to the research center. Botanists at Annamalai University assisted in identifying the plant samples, as did the Encyclopedia of Medicinal herbs on restorative herbs. During successive visits, data was cross-checked between individuals [11].

2.2.3 Extraction of plants

The plant samples intended for harvest were collected and placed in A3 paper bags, each labelled with a sticker indicating the plant's common or vernacular name. After being washed, wiped, and shade-dried for 15 to 20 days, the dried plant materials were ground into powder using an electric blender. The sealed bags were then stored in a deep freezer at -20°C in the Phyto-insecticides laboratory, Department of Entomology for further analysis. The powdered plant samples were individually formed into 50g thimbles using Whatman No. 40 filter paper and extracted with distilled water at room temperature for 72 hours. After extraction, the thimbles were carefully removed, and the extracts were collected. To create a 50% stock solution, 100 ml of distilled water was added to each 50g thimble. Various concentrations such as 5%, 3% and 1% were then prepared from this stock solution and used in preliminary bioassays [12].

2.3 Mass Culturing of *Spodoptera litura* Fabricius

The mass rearing of *S. litura* began with the collection of egg masses from infested blackgram, cotton fields in Vallampadugai village (11.3471^0 N, 79.7091^0 E), Cuddalore District. Collected egg masses were treated with 0.02% of sodium hypochlorite solution and placed in the tray which consisted of tender castor leaves and allowed to hatch. Newly hatched neonates were transferred to fresh castor leaves using a thin hairbrush. The laboratory conditions were maintained at 25 ± 2°C, with 70 ± 5% relative humidity and a photoperiod of 16 hours light and 8 hours dark. Fresh castor leaves replaced older ones regularly, and maintenance was consistently performed. Sterilized moist sandy soil was provided for the fifth instar larvae for pupation. Pupae that were three days old were treated with a 0.02% formaldehyde solution to prevent infection and took about a week to emerge. These pupae were then placed in oviposition cages (1' × 1' × 1'). After the adults emerged, they were sexed and fed with cotton lumps dipped in 5% honey water and provided with *Nerium oleander* for oviposition. The plants in the oviposition cages were monitored regularly, and the newly laid eggs were treated with a 0.05% sodium hypochlorite solution. The F1 generation neonates were transferred to castor leaves, and this culture was continuously maintained, with third instars being used for further experiments [13].

2.4 Screening of Plant Extracts for Antifeedant Activity against *S.litura*

Antifeedant properties of selected plant extracts were evaluated using a no-choice leaf disc bioassay method. Leaf discs, each with a diameter of 4.5 cm, were incised using a cork borer. Concentrations such as 5%,3%,1% (200 μl) of each extract was applied to both the adaxial and abaxial surfaces of the leaf discs with a blunt glass rod. After allowing the discs to air dry, they were placed in petri dishes, and third instar larvae of *S.litura* that had been pre-starved for 3 hours were introduced separately. The experiment included 15 treatments, with positive control using 2% neem oil and an absolute control with water. The feeding activity of the larvae was monitored over a 24-hour period, and the leaf area consumed was measured using a

List 1. Rating scale

Per cent leaf area protection	Grade	
> 80	Strong Inhibition	(++++)
50-79	Medium Inhibition	(+++)
20-49	Weak Inhibition	(++)
< 19	Insignificant inhibition	(+)

[14]

leaf area meter for both the control and treated discs. The protected leaf area, relative to the control, was calculated, and antifeedant activity was assessed using the specified grading scale.

Percent leaf area protection over control=

$$\frac{\%\ \text{leaf area protection in treatment} - \%\ \text{leaf area protection in control}}{100 - \%\ \text{leaf area protection in control}} \times 100$$

2.5 Statistical Analysis

The study data were analyzed using analysis of variance (ANOVA) under a completely randomized design (CRD) following the methods outlined by Gomez and Gomez [15]. Prior to analysis, necessary data transformations were performed. The calculations were carried out using the ICAR-WASP software package.

3. RESULTS AND DISCUSSION

Among the tested plant species at 1%, *C paniculata* displayed the highest antifeedant activity with 76.11% leaf area protection over control, indicating medium inhibition. It was followed by *Holoptelea integrifolia* (69.42%), *Catharanthus pusillus* (57.39%), *Mucuna pruriens* (50.34%) against third instar larvae of *S.litura*. Positive control with treatment of 0.15% Azadirachtin exhibited medium inhibition with 66.66% leaf area protection over control. Weak antifeedancy recorded in *Ipomea pandurata* (43.18%), *Rauvolfia tetraphylla* (30.83%), *Murraya paniculata* (23.78%) and *Euphorbia neriifolia* (19.65). Insignificant feeding inhibition showed in *Dioscorea bulbifera* (15.46%), *Clerodendrum trichotomum* (14.19%), *Mallotus philippensis* (11.38%), *Polyalthia longifolia* (8.62%), *Ocimum bacilicum* (7.91%), *Butea monosperma* (3.88%) (Table 1 and Fig. 1).

At 3% concentration, the results revealed that *C.paniculata* ranked first among 15 plant species recording strong antifeedancy with 82.29% of leaf area protection over control. It was followed by *H.integrifolia* exhibiting 80.18% of leaf area protection over control. Medium feeding inhibition was observed in *C.pusillus* (61.98%), *M. pruriens* (57.32%), *Ipomea pandurata* (50.66%). The positive control followed with azadirachtin 0.15% recorded 66.66% leaf area protection over control. Weak feeding inhibition noted in *R. tetraphylla* (34.22%), *M. paniculata* (30.43%), *E.neriifolia* (28.87%), *Dioscorea bulbifera* (25.28%), *C. trichotomum* (23.66%), *M. philippensis* (22.82%) and *P.longifolia* (20.77%). Insignificant inhibition exhibited in *O. bacilicum* (18.32%), *E. hirta* (12.82%) and *B. monosperma* (8.37%) (Table 2 and Fig. 2).

At 5% concentration, *C.paniculata* and *H.integrifolia* resulted in strong antifeedancy, recording 87.73%, 83.97% of leaf area protection over control. Medium feeding inhibition is observed in *C.pusillus* (75.22%), *M.pruriens* (65.80%),*I.pandurata* (59.67%). Weak antifeedancy noticed in *R.tetraphylla* (43.04%), *M.paniculata* (41.49%), *E.neriifolia* (39.88%), *D.bulbifera* (36.33%), *C.trichotomum* (35.72%), *P.longifolia* (32.14%), *O.basilicum* (26.84%) and *E.hirta* (19.65). *B.monosperma* resulted in insignificant feeding inhibition with 12.66% of leaf area protection over control (Table 3 and Fig. 3).

C.paniculata resulted in higher antifeedant activity when compared with other plant species in all 1%, 3% and 5% concentrations, Whereas *B.monosperma* recorded insignificant antifeedancy in all concentrations.*C.paniculata* ranked first among all the fifteen plants in exhibiting antifeedant properties against *S.litura* in all three antifeedant bioassays. The study results were supported by [16], demonstrated the efficacy of aqueous seed extracts of *C.stipularia* against *Tribolium castaneum.* Results revealed that 10% seed extracts of *C.stipularia* exerted 100% mortality and moderate deterrent activity, resulting in a 70 % reduction in food consumption compared to the normal food intake of *T.castaneum.* Arivoli and Tennyson [17] investigated the antifeedant properties of various plant extracts against *S.litura.* Hexane extracts of seeds of *Abrus Precatorious* Linn which belongs to same family Fabaceae as *Crotalaria* *paniculata* exhibited 78.61% of antifeedancy against *S.litura* which are in line with our study results. Also, our results were in accordance with Arivoli and Tennyson [17], where the hexane extracts of *Cassia fistula* from fabaceae resulted in 76.48% antifeedancy against *S.litura.* Genus *Crotalaria* comprises more than 700 species and the insecticidal properties of *Crotalaria spp.* is due to the presence of pyrrolizidine alkaloids. Lopez et al. [18] reported the presence of iminosugar group alkaloid 1β,2β-epoxy-1α-methoxymethyl-8α-pyrrolizidine in abundance in *C. longistrata* which exerted an 73.2-100% mortality on the nymphs of *Bactericera cockerelli*

Table 1. Antifeedant assay of selected plant species at 1% concentration against third instar larvae of *S. litura*

S.No	Aqueous extract (1% concentration)	Per cent Leaf area fed	Per cent Leaf area protection over control	Antifeedant rating
1	*Butea monosperma*	96.12 (79.20)[b]	3.88	+
2	*Catharanthus pusillus*	42.61 (40.74)[j]	57.39	+++
3	*Clerodendrum trichotomum*	85.11 (67.50)[ef]	14.89	+
4	*Crotalaria paniculata*	23.89 (29.25)[l]	76.11	+++
5	*Dioscorea bulbifera*	84.54 (66.86)[ef]	15.46	+
6	*Euphorbia hirta*	94.68 (76.88)[bc]	5.32	+
7	*Euphorbia neriifolia*	80.35 (63.78)[fg]	19.65	++
8	*Holoptelea integrifolia*	30.58 (33.57)[k]	69.42	+++
9	*Ipomea pandurata*	56.82 (48.92)[i]	43.18	++
10	*Mallotus philippensis*	88.62 (70.35)[de]	11.38	+
11	*Mucuna pruriens*	49.66 (44.80)[ij]	50.34	+++
12	*Murraya paniculata*	76.22 (60.88)[g]	23.78	++
13	*Ocimum basilicum*	92.09 (74.02)[cd]	7.91	+
14	*Rauvolfia tetraphylla*	69.17 (56.27)[h]	30.83	++
15	*Polyalthia longifolia*	91.38 (73.02)[cd]	8.62	+
16	Positive control (0.15% azadiractin)	33.34 (35.26)[k]	66.66	+++
17	Absolute Control	100.00 (87.97)[a]	-	
	CD (P=0.05)	4.12		

Values are mean of three replications
Values in parentheses are arc sine transformed
Values with various alphabets differ significantly

Table 2. Antifeedant assay of selected plant species at 3% concentration against third instar larvae of *S. litura*

S.No	Aqueous extract (3% concentration)	Per cent Leaf area fed	Per cent Leaf area protection over control	Antifeedant rating
1	*Butea monosperma*	91.63 (73.30)[b]	8.37	+
2	*Catharanthus pusillus*	38.02 (38.06)[kl]	61.98	+++
3	*Clerodendrum trichotomum*	76.34 (60.96)[ef]	23.66	++
4	*Crotalaria paniculata*	17.71 (24.88)[m]	82.29	++++
5	*Dioscorea bulbifera*	74.72 (59.82)[fg]	25.28	++
6	*Euphorbia hirta*	87.18 (69.06)[c]	12.82	+
7	*Euphorbia neriifolia*	71.13 (57.53)[gh]	28.87	++
8	*Holoptelea integrifolia*	19.82 (26.43)[m]	80.18	++++
9	*Ipomea pandurata*	49.34 (44.62)[j]	50.66	+++
10	*Mallotus philippensis*	77.18 (61.48)[ef]	22.82	++
11	*Mucuna pruriens*	42.68 (40.79)[k]	57.32	+++
12	*Murraya paniculata*	69.57 (56.55)[hi]	30.43	++
13	*Ocimum basilicum*	81.68 (64.73)[d]	18.32	+
14	*Rauvolfia tetraphylla*	65.78 (54.20)[i]	34.22	++
15	*Polyalthia longifolia*	79.23 (62.90)[de]	20.77	++
16	Positive control (0.15% azadiractin)	33.34 (35.26)[l]	66.66	+++
17	Absolute Control	100.00 (87.97)[a]	-	-
	CD (P=0.05)	2.88		

Values are mean of three replications
Values in parentheses are arc sine transformed
Values with various alphabets differ significantly

Table 3. Antifeedant assay of selected plant species at 5% concentration against third instar larvae of *S. litura*

S.No	Aqueous extract (5% concentration)	Per cent Leaf area fed	Per cent Leaf area protection over control	Antifeedant rating
1	*Butea monosperma*	87.34 (69.21)[b]	12.66	+
2	*Catharanthus pusillus*	24.78 (29.85)[k]	75.22	+++
3	*Clerodendrum trichotomum*	64.28 (53.31)[ef]	35.72	++
4	*Crotalaria paniculata*	12.27 (20.50)[m]	87.73	++++
5	*Dioscorea bulbifera*	63.67 (52.93)[fg]	36.33	++
6	*Euphorbia hirta*	80.35 (63.70)[c]	19.65	++
7	*Euphorbia neriifolia*	60.12 (50.84)[gh]	39.88	++
8	*Holoptelea integrifolia*	16.03 (23.59)[l]	83.97	++++
9	*Ipomea pandurata*	40.33 (39.42)[i]	59.67	+++
10	*Mallotus philippensis*	64.56 (53.46)[ef]	35.44	++
11	*Mucuna pruriens*	34.20 (35.79)[j]	65.80	+++
12	*Murraya paniculata*	58.51 (49.90)[h]	41.49	++
13	*Ocimum basilicum*	73.16 (58.82)[d]	26.84	++
14	*Rauvolfia tetraphylla*	56.96 (49.00)[h]	43.04	++
15	*Polyalthia longifolia*	67.86 (55.46)[e]	32.14	++
16	Positive control (0.15% azadiractin)	33.34 (35.26)[j]	66.66	+++
17	Absolute Control	100.00 (87.97)[a]	-	-
	CD (P=0.05)	2.25		

Values are mean of three replications
Values in parentheses are arc sine transformed
Values with various alphabets differ significant

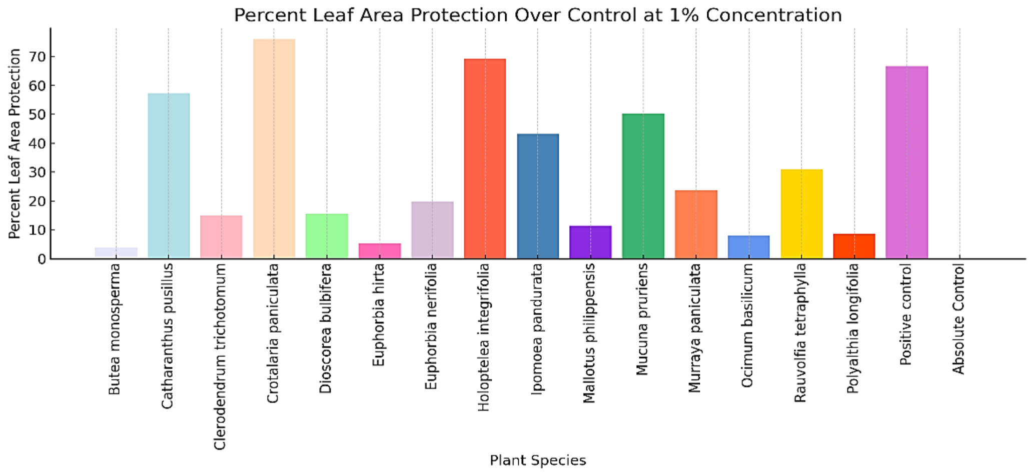

Fig. 1. Anifeedancy of selected botanicals against *S.litura* at 1 % concentration

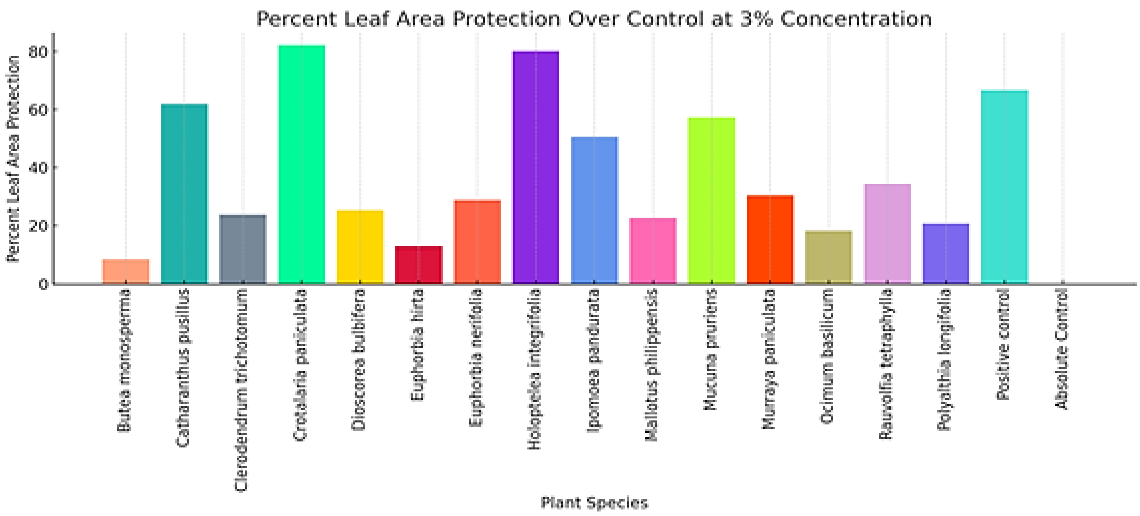

Fig. 2. Anifeedancy of selected botanicals against *S.litura* at 3 % concentration

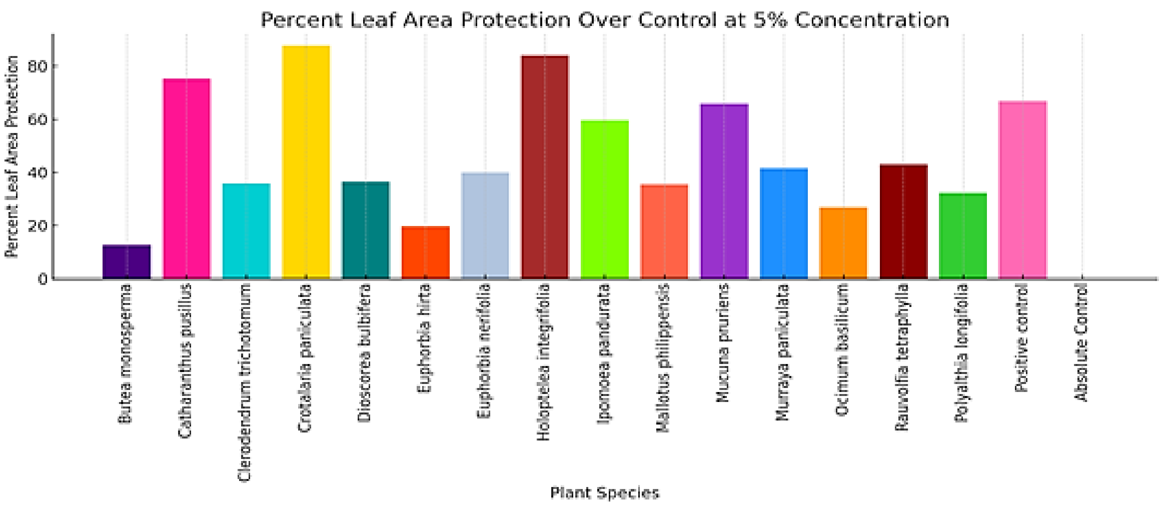

Fig. 3. Anifeedancy of selected botanicals against *S.litura* at 5 % concentration

4. CONCLUSION

Thus, a systematic search for pesticidal plants in the Kalvarayan hills of Tamil Nadu's Eastern Ghats indicated the presence of plants with pesticidal properties. Following preliminary screening, the flora from the families Fabaceae, Ulmaceae, and Amaranthaceae have strong pesticidal properties. Further extraction, characterization and isolation may result in the development of better, more effective, and environmentally friendly pest management phyto compounds.Evaluating plant extracts for their antifeedant properties is a important method in the search of new plant based insecticides,as secondary metabolites can prevent insects from feeding.Plant based insecticides can play a major role in developing Integrated pest management (IPM) strategies against *S.litura*. In addition to having better insecticidal activity than some chemical pesticides, phytoinsecticides offer the advantage of being less hazardous to non-target organisms besides the target pests.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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