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VALIDATION OF ABORIGINAL KNOWLEDGE ON PESTICIDAL FLORA IN SOUTHERN AND HIGH PRECIPITATION ZONE OF TAMIL NADU AGAINST *Spodoptera litura* (F). (NOCTUIDAE: LEPIDOPTERA)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author ST study conception and design, data collection and interpretation of results of the manuscript. Authors ST and SA preparation draft of manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

In vitro experiments were conducted against *Spodoptera litura* to assess the antifeedant and insecticidal potential of aqueous extracts of twenty five plants collected from the southern and high precipitation zone of Tamil Nadu. It was identified that *Wrightia tinctoria* exhibited maximum potency with the concentration of 5% to give 87.24% antifeedancy accompanied by *Cipadessa baccifera* (85.51%) and *Dodonaea viscosa* (81.03%). Insecticidal activity was more pronounced in *Glorisa superba* and *Wrightia tinctoria* caused 99.99 and 99.99 % larval mortality at 2% concentration respectively.

Keywords: Artificial diet; *Spodoptera litura;* antifeedancy; insecticidal activity; aboriginal knowledge.

1. INTRODUCTION

India possesses copious vegetation with a large array of flora, because of the extreme variations in geographical and climatic conditions prevailing in the country. With the diversity of its ethnicities, many aboriginal cultures have retained their traditional knowledge on native flora Ayyanar and Ignacimuthu [1]. The largest population of tribes in the world is represented by India, which constitutes 84.51 million tribes after Africa Kala, [2]. Aboriginal people usually collect and conserve locally available wild and cultivated flora and encourage herbal medicine to medicate a variety of diseases and disorders Jeyaprakash et al. [3]. In Tamil Nadu, the Southern and High rainfall zone have rich vegetation compared to the other areas. Aboriginal people living in the biodiversity rich areas are highly familiar of the flora

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and their medicinal values and this perception is passed via oral communication from generation to generation Azaizeh [4]. This aboriginal knowledge has become a renowned tool in exploration for new sources of natural pesticides. This study aims to explore and validate the ethnic knowledge of paliyars and kaani tribes in the western ghats related to natural pesticides. The goal of research is bring organic based formulation to support natural farming.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The peak of Western Ghats covers six percent of India's landmass however have over thirty percent of the world's verdure species. The slope scopes of Western Ghats are around 1600 km long in North-South running from waterway Tapti in Gujarat to Kannyakumari in Tamil Nadu Aruna et al. [5]. The survey was conducted in five hamlets of Dindigul (Pannaikadu, Vilpatti, Vazhai giri, Ayyalur and Moolaiyur), two hamlets of Tenkasi (Courtallam and Velmalai) and three hamlets of Kanyakumari (Pechiparai hills, Perunchani hills and Keeriparai) districts. The geographical information of study areas is diversified by several ranges and hills at elevation of (1343 m, 160 m and 82 m) and mean annual rainfall of (1345 mm, 1319 mm and 2500 mm) respectively. These regions are renowned for its species wealth, variety and high endemism Ayyanar and Ignacimuthu [1] which are of high for biodiversity preservation by the International Union for Conservation of Nature.

2.2 Interviews with Aboriginal People

2.2.1 Paliyar tribes

The Aboriginal people of the study area are called Paliyar/ Paliyan/ Pazhaiyarare. They reside in the hilly regions of south Western Ghats montane rain forests of Dindigul, Madurai, Tenkasi and Tirunelveli district. They are customary nomadic hunter gathers, honey hunters and foragers. Most paliyars are illiterate and they vocalize Tamil. Ancestral herbal practitioners are the curators of the paliyans and they have an ample knowledge of medicinal plants, diseases and treatment using native flora Ignacimuthu et al. [6]. The plants suggested by these tribes are *Actiniopteris radiata, Alstonia scholaris, Alysicarpus vaginalis, Cipadessa baccifera, Dodonaea viscosa, Glorisa superba, Ipomoea obscura, Melia dubia, Ocimum basilicum, Pandanus fascicularis, Polygala arvensis, Sterculia urens* and *Wrightia tinctoria.*

2.2.2 Kaani tribes

The tribes found in the Tenkasi and Kanyakumari district are known as Kaanikaran/ Kaani/ Kaanikars. The sound 'Kaan' meaning 'Kaatu' (forest) becomes the root of the word Kaanikars. So, this denotes that kaanikars are aboriginal people reside in forest. They are traditionally a nomadic community and vocalize malayalam mixed tamil. Kaani's have a history of customary medicine, which is communicated from generation to the next by a hierarchy of traditional curators called "Plathi and Mootukaani" Mohankumar and Velvizhi [7]. The plants suggested by these tribes are *Aglaia roxburghiana, Andrographis paniculata, Bridelia retusa, Clerodendrum trichotomum, Holoptelea integrifolia, Mallotus Philippensis, Merremia hastata, Murraya paniculata, Rauwolfia serpentina, Sapindus emarginatus, Tylophora indica* and *Tabernaemontana heyneana*. Flora especially those with ethno pharmacological uses have been the prime sources of bio pesticides.

Ethnobotanical information (local name of the plant, plant details and identifying characters, availability, medicinal and other uses) were collected through interviews and discussions among the old aged and tribal curators in and around the review region. The specimens were collected and brought to the research facility. It was identified with the help of botanists in Annamalai University and the data set on restorative plants given by the Encyclopedia of Medicinal plants. Data accumulated was cross-checked between individuals in the ensuing visits.

2.2.3 Extraction of plants

The collected plant parts were thoroughly washed with pure water and dried at room temperature (27.0 ˚C ± 2 ˚C) at Department of Entomology, Annamalai University. Then dried plants were powdered using wiley mill individually and made into 20 g thimbles using Whatman No. 40 filter paper. Then the thimbles were extracted with double distilled water at room temperature in round-bottom (500 mL) stopper flasks. After 72 h, the thimbles were removed and filtrate was used for bioassays. This was considered as 20 percent stock solution from which 2 and 5 percent concentrations were made Amer et al. [8].

2.3 Rearing of *Spodoptera litura* Fab. (Noctuidae: Lepidoptera)

The neonate and adult stages of *S. litura* are raised on an artificial diet at 27 $\pm 1^0$C, 70 ± 5% RH. The diet ingredients included wheat germ (26.0 mg), red bean flour (51.3 mg) and chickpea flour (56.0 mg) which were thoroughly mixed in a blender along with 550 mL of tepid distilled water. Separately, the agar-agar powder (16.4 g) was weighed, disintegrated in 270 mL of tepid water, boiled in a 1 L container, cooled to 50^0C and then added to the mixed ingredients. This

mixture was blended thoroughly for about 1 min at high speed. The ingredients of dried yeast powder (31.6 g), Casein (15.2 g), L-Ascorbic acid (3.2 g), Cholestrol (0.5 g), multivitamin multimineral capsules (2 nos), Castor oil (1 mL), Sorbic acid (1.3 g), methyl-ρ-hydroxybenzoate (1.8 g), Streptomycin sulphate (0.25 g) and formaldehyde solution (2 mL) were then added and the mixture stirred well. While hot, the mixture was poured into sterilized petri-dishes (15 cm diameter) and allowed to cool in refrigerator. Before using the diet, it was brought to room temperature (25– 30 ^{0}C) for 2–3 h. The solidified diet was then cut into little pieces and placed in plastic rearing vials for larval feeding Gupta et al. [9].

The diet was used for up to 10 days. Around thirty neonates (24 h-old) were raised on 8 g diet per plastic container (10 X 6.5 cm), covered by gada cloth and secured with elastic band. After five days, these larvae were reared individually in multi-cavity trays (30 X 22 cm) of 24 cells until pupation to avoid cannibalism. Pieces of fresh diet were provided once in two days after cleaning the container. Pupation took place in the sand layer provided. Collected pupae were washed with 0.02% formaldehyde solution, sexed and transferred into oviposition cages (1'×1'×1') at the rate of ten pairs per cage. During egg laying and hatching, the adults were fed with 10% sucrose solution fortified with multivitamin drops (2 mL) for efficient and uniform egg production.

2.4 Antifeedant and Insecticidal Assays

The 4.5 cm diameter of castor leaf disc was cut out and 200 µl of each concentration (2 & 5 percent) was smeared on both adaxial and abaxial surfaces of the leaf using a blunt glass rod separately. Treated leaf discs were air dried and placed in a plastic container. Three newly shed, 3 h pre starved, third instar larvae were introduced into the container and covered with muslin material held set up by an elastic band, after 12 h of treatment, uneaten leaf discs were collected from treatment and control and then the larvae were provided with untreated fresh castor leaves and reared upto adult emergence. There were 27 treatments including absolute and positive controls and replicated 3 times. The graphical method was used to measure the area of leaves collected from each treatment, and per cent leaf area protection over control was computed and the antifeedancy was rated Rani and Arivudainambi [10].

$$\text{Percent leaf area protection over control} = \frac{\%\text{ leaf area protection in treatment} - \%\text{ leaf area protection in control}}{100 - \%\text{ leaf area protection in control}} \text{ X } 100$$

The antifeedancy was rated as per the scale given below.

Chart 1. Rating scale

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

Sreedevi et al. [11]; Chitra and Ramakoteeswara [12]

The larvae transferred to fresh leaves were observed daily upto adult emergence and percent larval mortality, pupal malformation and adult emergence were worked out. If the larva suffers mortality after feeding on the treated leaves (usually within 24 h), the extract was classified as insecticidal. If it avoided feeding the treated leaves after initial nibbling, the respective extract was considered to possess antifeedant properties and if the larva moves away from the treated discs without feeding, the extract was considered as repellent and if any malformation observed, it was noted as insect growth regulator Michael villiani and Fred Gould [13].

2.5 Statistical Analysis

The data obtained from laboratory experiment were analyzed in a Completely Randomized Block Design by "F" test for significance. Standard Error of difference (S.E (d)) and Critical difference values were calculated at 5 per cent probability level and the treatment means values of the experiments were compared using Duncan's Multiple Range Test (DMRT) Steel and Torrie [14].

Table 1. Antifeedancy of certain botanicals against *S. litura*

Treatments (5% concentration)	Per cent leaf area protection over control	Antifeedant grading
Actiniopteris radiata	8.63	(+)
Aglaia roxburghiana	30.00	(++)
Alstonia scholaris	14.14	(+)
Alysicarpus vaginalis	47.59	(++)
Andrographis paniculata	61.73	(+++)
Bridelia retusa	30.00	(++)
Cipadessa baccifera	**85.51**	**(++++)**
Clerodendrum trichotomum	31.04	(++)
Dodonaea viscosa	**81.03**	**(++++)**
Glorisa superba	64.49	(+++)
Holoptelea integrifolia	44.83	(++)
Ipomoea obscura	70.00	(+++)
Mallotus Philippensis	63.80	(+++)
Melia dubia	50.69	(+++)
Merremia hastata	23.11	(++)
Murraya paniculata	33.45	(++)
Ocimum basilicum	47.94	(++)
Pandanus fascicularis	30.00	(++)
Polygala arvensis	56.90	(+++)
Rauwolfia serpentia	12.07	(+)
Sapindus emarginatus	64.49	(+++)
Sterculia urens	67.25	(+++)
Tabernaemontana heyneana	57.59	(+++)
Tylophora indica	53.45	(+++)
Wrightia tinctoria	**87.24**	**(++++)**
Positive control (Azadirachtin 1500 ppm)	70.69	(+++)
Control	0.00	-

Values are mean (%) of the three replicates ± standard error

3. RESULTS AND DISCUSSION

3.1 Antifeedant Activity

The result obtained from antifeedant assay at 5 per cent concentration against third instar of *S. litura* showed that among twenty five plants, immature pod extracts of *W. tinctoria* exhibited strong inhibition of 87.24% leaf area protection over control followed by leaf extract of *C. baccifera* (85.51%) and *D. viscosa* (81.03%). Insects exposed to treatment showed low respiration rates, reduced locomotor activity and avoidance responses to treated leaves. The more pronounced antifeedant activity in *W. tinctoria* may be due to the presence of wrightial, a triterpenoid phytochemical along with cycloartenone, cycloeucalenol, β amyrin and β sitosterol Ramachandra et al. [15]. Li et al. [16] reported antifeedant properties of *C. baccifera* due to presence of limonoids and three tetranortriterpenoids. The pure compound kaempferol is isolated from *D. viscosa* (leaf) showed as termiticide on tunneling and midgut enzyme activities of *Odontotermes obesus* Nisar et al. [17] (Table 1) (Fig. 1).

About 11 treatments showed moderate inhibition with the range from 50.69 to 70.00%. Among these, medium percent leaf area protection over control was observed in *M. dubia* (50.69%) at 5% whereas maximum leaf area protection over control in moderate inhibition was observed in *I. obscura* (70%). Our findings coincide with the findings of Koul et al. [18] who indicated the extract of *M. dubia* possess antifeedant activity against *S. litura* and *Helicoverpa armigera* and had growth inhibitory and antifeedant bio-activities increase with increasing polarity of extracting solvents. Sahayaraj et al. [19] revealed that choloroform extracts of *I. carnea* showed pronounced antifeedant and haemotoxic effect on *Achaea janata*. Nine treatments showed weak antifeedancy and ranges from 23.11 to 47.94%. *O. basilicum* leaf extracts showed 47.94% antifeedancy and least percent leaf area protection over control was observed in *M. hastata* (23.11%). This results in accordance with the statement made by Wilson et al. [20] 10% aqueous extracts of *O. basilicum* caused at least 50% mortality of *Spodoptera frugiperda* larvae. The antifeedancy rating showed insignificant inhibition in *A. radiata* (8.63%),

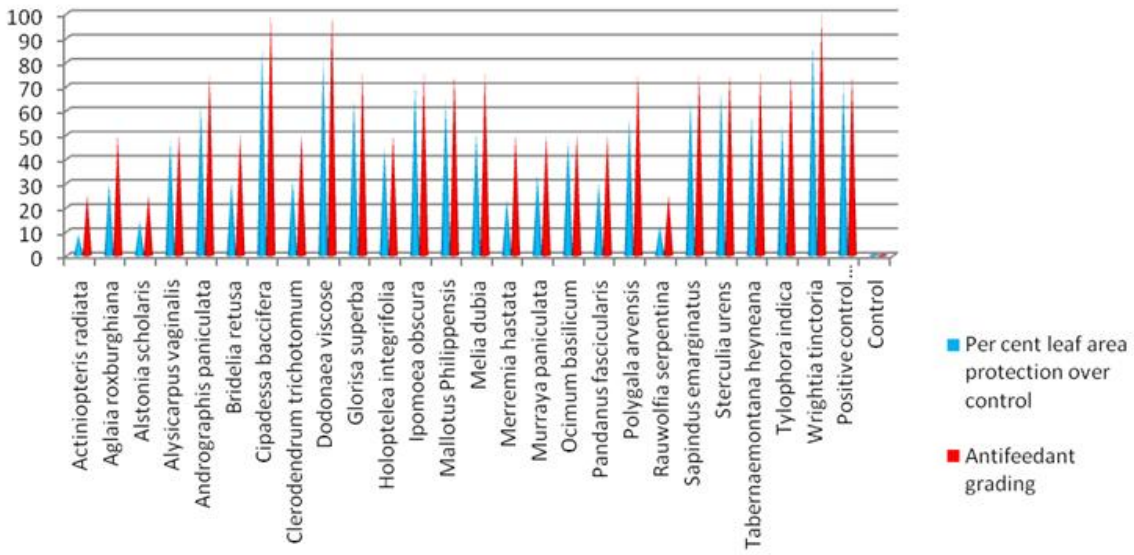

Fig. 1. Antifeedancy of certain botanicals against *S. litura*

A. scholaris (14.14%) and *R. serpentina* (12.07%) (Table 1) (Fig .1).

3.2 Insecticidal Assay

The highest larval mortality was found in *W. tinctoria* (33.33%) and *M. dubia* (33.33%) at 2% concentration which was followed by *A. radiata*, *A. vaginalis*, *A. paniculata*, *C. baccifera*, *D. viscosa*, *T. heyneanaa* and *T. indica* caused 16.66% larval mortality. Sakthivadivel et al. [21] reported aqueous fruit extract of *W. tinctoria* against *Culex quinquefasciatus* exhibited highest larvicidal activity followed by aqueous leaf extract with LC50 value of 0.17 and 0.09%, 0.21 and 0.11% after 24 and 48 h respectively. The highest pupal deformities was observed in *W. tinctoria* (66.67%) followed by *M. dubia* (50%). The precocious pupation may be due to the presence of phytoecdysone compounds. Sahayaraj et al. [19] treated pupa showed incompletely and deformed head capsule and reminants of larval thoracic appendages and morphologically dead. This is due to retarded food consumption and digestive enzyme activity. The highest adult malformation was noted in *G. superba* (83.33%) due to the presence of colchicine, colchicoside and 2 demethyl colchicines caused growth inhibitory activity against *S. litura* Nebapure et al. [22] followed by *A. scholaris* recorded 33.33% adult molformation (Table 2) (Fig 2). The overall insecticidal activity was more pronounced in *G. superba* (99.99%) followed by *W. tinctoria* (99.99%) and *M. dubia* (83.33%). Moderate activity was observed in *A. paniculata*, *C. baccifera*, *T. heyneana* and positive control caused 66.65%. Absence of insecticidal activity was recorded in *C. trichotomum*, *I. obscura*, *M. hastate*, *P. fascicularis*, *S. emarginatus* and control (0.00%) (Table 2) (Fig 2).

Table 2. Insecticidal activity of certain botanicals against *S. litura*

Treatments (2% concentration)	Larval Mortality	Pupal Deformation	Adult Malformation
Actiniopteris radiata	16.66 (24.03)^{b}	16.66 (24.03)^{d}	16.66 (24.03)^{c}
Aglaia roxburghiana	0.00 (35.25)^{a}	16.66 (24.03)^{d}	16.66 (24.03)^{c}
Alstonia scholaris	0.00 (2.02)^{c}	16.66 (24.03)^{d}	33.33 (35.25)^{b}
Alysicarpus vaginalis	16.66 (24.03)^{b}	16.66 (24.03)^{d}	0.00 (2.02)^{d}
Andrographis paniculata	16.66 (24.03)^{b}	33.33 (35.25)^{c}	16.66 (24.03)^{c}
Bridelia retusa	0.00 (2.02)^{c}	16.66 (24.03)^{d}	16.66 (24.03)^{c}
Cipadessa baccifera	16.66 (24.03)^{b}	33.33 (35.25)^{c}	16.66 (24.03)^{c}
Clerodendrum trichotomum	0.00 (2.02)^{c}	0.00 (2.02)^{e}	0.00 (2.02)^{d}
Dodonaea viscose	16.66 (24.03)^{b}	33.33 (35.25)^{c}	0.00 (2.02)^{d}
Glorisa superba	**0.00 (2.02)^{c}**	**16.66 (24.03)^{d}**	**83.33 (65.96)^{a}**

Treatments (2% concentration)	Larval Mortality	Pupal Deformation	Adult Malformation
Holoptelea integrifolia	0.00 (2.02) [c]	33.33 (35.25) [c]	16.66 (24.03) [c]
Ipomoea obscura	0.00 (2.02) [c]	0.00 (2.02) [e]	0.00 (2.02) [d]
Mallotus Philippensis	0.00 (2.02) [c]	16.00 (24.03) [d]	16.00 (24.03) [c]
Melia dubia	**33.33 (35.25) [a]**	**50.00 (45.00) [b]**	**0.00 (2.02) [d]**
Merremia hastate	0.00 (2.02) [c]	0.00 (2.02) [e]	0.00 (2.02) [d]
Murraya paniculata	0.00 (2.02) [c]	33.33 (35.25) [c]	16.66 (24.03) [c]
Ocimum basilicum	0.00 (2.02) [c]	16.00 (24.03) [d]	16.66 (24.03) [c]
Pandanus fascicularis	0.00 (2.02) [c]	0.00 (2.02) [e]	0.00 (2.02) [d]
Polygala arvensis	0.00 (2.02) [c]	16.66 (24.03) [d]	16.66 (24.03) [c]
Rauwolfia serpentia	0.00 (2.02) [c]	0.00 (2.02) [e]	16.66 (24.03) [c]
Sapindus emarginatus	0.00 (2.02) [c]	0.00 (2.02) [e]	0.00 (2.02) [d]
Sterculia urens	0.00 (2.02) [c]	16.66 (24.03) [d]	0.00 (2.02) [d]
Tabernaemontana heyneana	16.66 (24.03) [b]	16.66 (24.03) [d]	16.66 (24.03) [c]
Tylophora indica	16.66 (24.03) [b]	16.66 (24.03) [d]	0.00 (2.02) [d]
Wrightia tinctoria	**33.33 (35.25) [a]**	**66.67 (54.75) [a]**	**0.00 (2.02) [d]**
Positive control	0.00 (2.02) [c]	33.33 (35.25) [c]	16.66 (24.03) [c]
Control	0.00 (2.02) [c]	0.00 (2.02) [e]	0.00 (2.02) [d]
SE(d)	1.063	1.471	1.319
C.D.	2.136	2.955	2.649

Means of three replications
Values in parenthesis are arc sine transformed values.
The values followed by same letters do not differ significantly at p=0.05

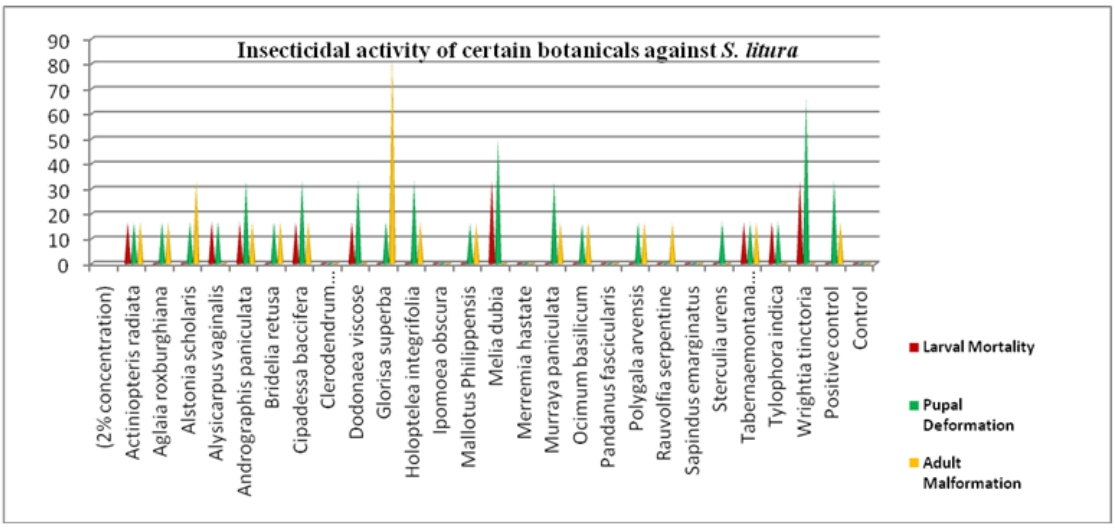

Fig. 2. Insecticidal activity of certain botanicals against *S. litura*

4. CONCLUSION

Thus the detailed survey for pesticidal plants of southern and high rainfall zone of Tamil Nadu revealed the availability of wild poisonous plant. After preliminary screening, the flora belonging to the family Apocynaceae, Meliaceae and Colchicaceae have pronounced pesticidal value. Further isolation and characterization may end with newer, more effectual and eco-friendly compounds in pest management.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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