Research Article

Methanolic Leaf Extracts of Certain Plants as Larvicides against Teak skeletonizer, *Eutectona machaeralis*

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ABSTRACT

Aim: The coveted Indian hardwood teak (Tectona grandis) grows well in particular tropical temperatures and soil types. With 8.9 million hectares, India's teak forests are essential to the luxury market. Indian teak trees are seriously threatened by the teak skeletonizer (*Eutectona machaeralis* Walker), which causes extensive defoliation. As a result, less timber is produced and trees grow slower. **Materials and Methods**: In this work, methanolic leaf extracts of *Wrightia tinctoria*, *Murraya koenigii* and *Prosopis juliflora* were tested for their larvicidal efficacy against the fourth instar larvae of *E. machaeralis*. **Results**: All extracts had high levels of alkaloids, flavonoids and polyphenols, according to phytochemical analyses. The findings showed that mortality rates were concentration-dependent. *M. koenigii* had the maximum effectiveness, with mortality rates of 61.43±1.76% at 200 ppm and 92.86±2.40% at 400 ppm. **Conclusion:** These results highlight the potential of these plant extracts as efficient, environmentally beneficial teak pest management options.

Keywords: *Tectona grandis, Wrightia tinctoria, Murraya koenigii, Prosopis juliflora*, Teak leaf skeletonizer, *Eutectona machaeralis*, Larvicidal activity.

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INTRODUCTION

Teak, scientifically known as *Tectona grandis*, is a tree species that has a broad but fragmented distribution in India. It grows well in humid, warm, tropical regions and does well on deep fluvial soil that has a pH of roughly 6.5. It grows below 24°N latitude, in southern as well as certain eastern and western regions of India.[1] The teak-bearing forests in India cover nearly 8.9 million hectares; falling within a precipitation range of 800 to 2500 mm. Teak is a highly sought-after hardwood species with a growing demand in luxury markets. As a result, teak plantations have expanded rapidly outside of their natural habitats to countries in Asia, Latin America, Africa and Oceania. Currently, teak is grown in approximately 70 tropical countries, with Myanmar, India and Indonesia accounting for most of the plantations.[2] Teak plantations have a significant presence in India, with the first one established in 1846 at Nilambur, Kerala. According to,[3] India has teak plantations on over 1.5 million hectares, with 50,000 hectares being added yearly.

In India, the teak skeletonizer (*Eutectona machaeralis* Walker) is a serious pest of teak trees. It is regarded as one of the most damaging teak pests, causing extensive defoliation every year in nurseries, plantations and wild forests throughout teak-growing regions.[4-6] The larvae of this insect have a distinct feeding behavior, consuming only the fleshy leaf tissues while excluding the veins. This feeding pattern has detrimental effects on the overall growth and vitality of the teak tree. Furthermore, it may cause several anomalies that ultimately result in a decline in the amount and quality of timber production. Over the years, numerous

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researchers have conducted in-depth studies and compiled significant documentation on the effects of the teak skeletonizer on teak trees.[7,8]

Since the start of the Green Revolution, synthetic insecticides have become a prevalent tool for controlling agricultural pests. However, these insecticides, while aiding in increasing agricultural productivity, also showed lethal consequences on non-target organisms. [9] The surge in pest populations is a direct consequence of these insects' developing resistance to insecticides, a situation aggravated by the overuse of these synthetic pesticides. The existence of hazardous residues in various environmental compartments is a result of indiscriminate pesticide application and has a major negative influence on human well-being.[10] In light of these concerns, there is a growing recognition of the significance of botanical pesticides, or biopesticides, as viable alternatives to synthetic insecticides. These organic plant extracts hold the advantage of being biodegradable and pose fewer hazards to beneficial organisms in comparison to synthetic pesticides.

Several studies were conducted to test the efficacy of botanicals against the larvae of *E. machaeralis*. In one study[11] the extracts from thirteen different medicinal plants were tested at concentrations of 5% and 10%. Among these, *Azadirachta indica, Nerium oleander, Strychnos nux-vomica* and *Tylophora indica* exhibited remarkable effectiveness, resulting in significant inhibition of the pest. Conversely, the extract from *Mirabilis jalapa* showed the least impact. In another study[12] 5% concentration of crude extract from fresh leaves of 32 distinct medicinal plants was evaluated for antifeedant and insecticidal properties against the third instar larvae of *E. machaeralis*. *Calotropis procera* emerged as the most effective, demonstrating potent biopesticidal properties. Following closely were extracts from *Datura metel* and *A. indica*. In this study, methanolic extracts were prepared from the leaves of *W. tinctoria, M. koenigii* and *P. juliflora* and their larvicidal efficacy was evaluated against the fourth instar larvae of *E. machaeralis*.

MATERIALS AND METHODS

Plant materials

W. tinctoria, M. koenigii and *P. juliflora* leaves were obtained from adjacent villages in Sangareddy town, Telangana, India. They were then shade-dried and powdered using an electric grinder before being stored separately in airtight containers

Preparation of leaf extracts

In the Soxhlet apparatus, 100 grams of plant powders were steeped in 200 mL of methanol for 6 hours at 60ºC. The methanol was later evaporated using a rotary evaporator to get semisolid extracts of the test plants. These semisolid extracts were then preserved in clean bottles at 4ºC until use.

Phytochemical Analysis

The prepared plant extracts were subjected to qualitative phytochemical analyses to find out the presence of various secondary metabolites. Mayor's reagent test (Alkaloids), Alkaline reagent test (Flavonoids), Salkowski test (Terpenoids), Froth test (Saponins), Keller-Killiani Test (Glycosides) and NaOH test (Phenols) were employed to confirm the presence of secondary metabolites

Preparation of test solutions

To make the 1000 ppm stock solutions of the test plant extracts, 1 g of the extract was thoroughly mixed in 10 mL of methanol, later 990 mL of distilled water was added to it. Using the serial dilution procedure, test solutions of 50, 100, 200 and 400 ppm were produced from the stock solutions. The control solution was made with identical proportions of methanol and distilled water as the test solution but without the extracts.

Collection of larvae

Early instar larvae of *E. machaeralis* were recovered from the teak plants on the Osmania University campus. They were raised by providing fresh teak leaves. The fourth instar larvae from the reared population were employed in the larvicidal bioassays.

Larvicidal activity

Topical application and leaf spray method were followed in the larvicidal bioassay. 10 larvae were taken in each test batch. They and the fresh teak leaves were sprayed with test solutions, once the leaves were air-dried, they were placed in separate Plastic jars. The number of dead larvae was counted and noted. The experiment continued until all the larvae were dead or they metamorphosed into the next instar. The same experiment was replicated five times. Mortality results were corrected by using the[13] formula.

Corrected mortality=(% test mortality-% control mortality)/(100-control mortality)x100

RESULTS

The results of the phytochemical analyses of the test plant extracts (Table 1) indicated that all three extracts are rich in Alkaloids, Flavonoids and Polyphenols content. Terpenoids were abundantly present in *P. juliflora* and *W. tinctoria* extracts, while they are moderately present in

M. koenigii extracts. Saponins were moderately present in *W. tinctoria* and *M. koenigii* extracts and absent in *P. juliflora* extracts. Glycosides were moderately observed in *M. koenigii* extracts; however, they are absent in both, *P. juliflora* and *W. tinctoria* extracts.

Table 1: Identified secondary metabolites through phytochemical analyses. Absence and presence are denoted by '-' and '+', respectively. The number of '+' is proportional to the relative abundance of the secondary metabolites.

Extracts	Alkaloids	Flavonoids	Saponins	Terpenoids	Polyphenols	Glycosides
M. koenigii	+++	+++	++	++	+++	++
P. juliflora	+++	+++	-	+++	+++	-
W. tinctoria	+++	+++	++	+++	+++	-

The larvicidal bioassays results of the present study are given in Table 2 and Figure 1. At 50 ppm, which is the lowest concentration tested, *M. koenigii* extracts caused a mortality rate of 21.43%±2.40, while *P. juliflora* and *W. tinctoria* recorded mortality rates of 17.86%±2.67 and 21.43%±2.40, respectively. Increasing the concentration to 100 ppm resulted in higher mortality rates: *M. koenigii* showed 39.29%±2.11, *P. juliflora* recorded 32.14%±1.76 and *W. tinctoria* had a mortality rate of 32.14%±3.67. At 200 ppm, *M. koenigii* demonstrated an even more substantial effect, with a mortality rate of 61.43%±1.76. *P. juliflora* and *W. tinctoria* exhibited mortality rates of 46.43%±2.0 and 46.43%±2.83, respectively. The highest concentration tested, 400 ppm, led to *M. koenigii* achieving the highest mortality rate of 92.86%±2.40. *P. juliflora* and *W. tinctoria* still demonstrated substantial efficacy, with mortality rates of 78.57%±2.83 and 71.43%±2.11, respectively.

Table 2: Larvicidal efficacy of different test extracts against the 4th instar larva of *E. machaeralis*.

Extracts	*M. koenigii*	*P. juliflora*	*W. tinctoria*
Control	0±1.76	0±1.76	0±1.76
50	21.43±2.40	17.86±2.67	21.43±2.40
100	39.29±2.11	32.14±1.76	32.14±3.67
200	61.43±1.76	46.43±2.0	46.43±2.83
400	92.86±2.40	78.57±2.83	71.43±2.11
Regression equation	y=0.2218x +9.7335	y=0.1857x +7.1435	y=0.1643 x+9.6429
R2	0.9545	0.9699	0.9396

The regression equations were derived through regression analysis (Figures 2-4) to model the relationship between the concentration of the plant extract (x) and the corresponding mortality rate (y). For *M. koenigii*, the equation is y=0.2218x+9.7335, for *P. juliflora* it is y=0.1857x+7.1435 and for *W. tinctoria* it is y=0.1643x+9.6429. These equations allow for the estimation of mortality rates at concentrations not directly tested in the experiment. Furthermore, the high R-squared (R2) values indicate a strong fit of the regression lines to the actual data points. For *M. koenigii,* R2=0.9545, for *P. juliflora*, R2=0.9699 and for *W. tinctoria*, R2=0.9396. This underscores the reliability of the regression equations in predicting mortality rates based on the concentration of the plant extract

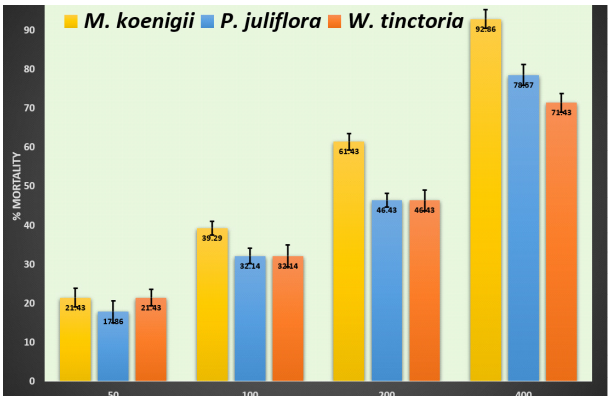

Figure 1: Larvicidal bioassay results of the methanolic leaf extracts of *M. koenigii, P. juliflora* and *W. tinctoria* against the 4th instar larvae of *E. machaeralis.*

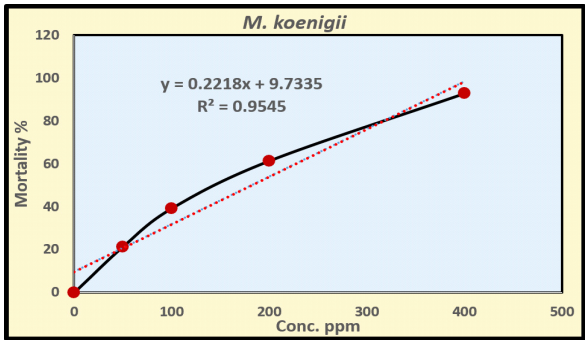

Figure 2: Regression analysis of the *M. koenigii's* methanolic leaf extracts bioassays against the 4th instar larvae of *E. machaeralis.*

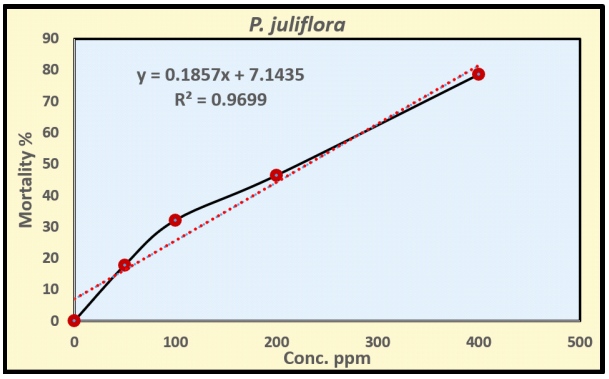

Figure 3: Regression analysis of the *P. julifora's* methanolic leaf extracts bioassays against the 4th instar larvae of

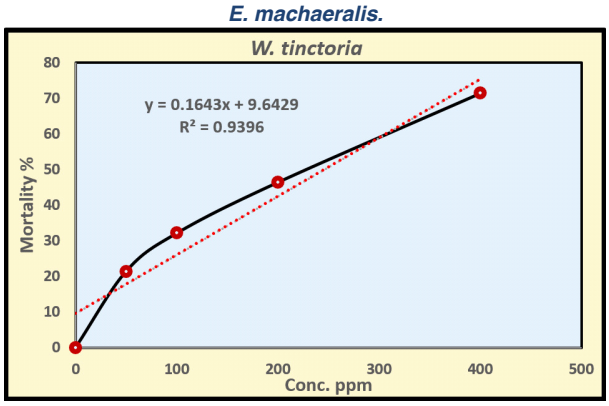

Figure 4: Regression analysis of the *W. tinctoria's* methanolic leaf extracts bioassays against the 4th instar larvae of *E. machaeralis*.

DISCUSSION

Secondary metabolites, formerly thought to be useless, but now seen as vital plant components that provide selection and adaptation benefits. Plants have evolved secondary metabolites to resist herbivorous animals and insect pest attacks and adapt to their environment, preventing them from feeding.[14,15] Countless studies revealed the pesticidal properties of secondary metabolites in various plant extracts. Secondary metabolites like terpenes, phenolics, flavonoids, saponins and glycosides have various pesticidal properties and can be used in plant protection.[16]

In a previous study[17] ethanolic leaf extracts of *M. koenigii* were analyzed qualitatively revealing the presence of saponins, tannins, alkaloids, glycosides, terpenoids and phenols. In their study, Flavonoids were not detected. However, we discovered the presence of flavonoids also including the other secondary metabolites. In our study, we used methanol as a solvent in acquiring leaf extracts of *M. koenigii*. This could be the reason for flavonoids being not identified in the analysis made by.[17] Furthermore, the results of the present study are supported by other studies where, *M. koenigii* leaf extracts were reported to be rich in Alkaloids,[18] Terpenoids,[19,20] Polyphenols[21] and Flavonoids.[22] In the study made by,[23] methanolic leaf extracts of *W. tinctoria* identified all secondary metabolites except glycosides are in line with the results of the present study. Ethanolic leaf extracts of *P. juliflora* revealed the presence of all other phytochemicals except, Saponins and Terpenoids.[24] In our study, saponins and glycosides were not detected in methanolic extracts of *P. juliflora* leaves, suggesting the role of solvent in acquiring the secondary metabolites.

Extracts of various plants were reported to possess good pesticidal properties against the larvae of *E. machaeralis*. In a study by,[25] five-leaf extracts (*Adhatoda vasica, Vitex negundo, Azadiracta indica, Ricinus communis and Pongamia glabra*), *A. indica seed* kernel extract and A. indica seed oil were investigated for their larvicidal properties against *E. machaeralis* larvae to find five-leaf extracts at 6% showed better larvicidal efficacy than neem oil and neem seed kernel extract. However, the commercial formulation Grub kill and Bacillus thuringiensis, demonstrated marginally superior larvicidal activity. In another study[26] Panchagavya with crude extracts of Seaweed, Sargassum wightii, was tested against *E. machaeralis* and reported the highest larvicidal efficacy of 71% at 5000 ppm concentration after 72 hr of the application. However, in the present study, all three tested plant extracts showed better larvicidal efficacy at much lower concentrations suggesting their potential in the control of *E. machaeralis*. It is evident from the results of the larvicidal bioassays that *M. koenigii, P. juliflora* and *W. tinctoria* extracts demonstrated varying degrees of larvicidal activity. Notably, at higher concentrations, the mortality rates substantially increased, suggesting a concentration-dependent effect. The regression equations and the high R-squared (R^2) values indicate a strong fit of the regression lines to the actual data, affirming the reliability of the results.

It is a well-established fact that Alkaloids, Polyphenols, Flavonoids, Terpenoids, Saponins and Glycosides possess pesticidal properties[27] and other biologically important activities. The larvicidal bioassay results of the present study, combined with the phytochemical analysis, suggest that secondary metabolites in the tested plant extracts contribute to the observed larvicidal activity against the larvae of *E. machaeralis*. Comparatively, *M. koenigii* extracts showed higher larvicidal efficiency than the remaining two plant extracts. The synergistic effect of all six secondary metabolites-Alkaloids, Polyphenols, Flavonoids, Terpenoids, Saponins and Glycosides in the *M. koenigii* leaf extracts might be the reason for its superior larvicidal efficacy over the other two extracts.

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AUTHORS CONTRIBUTIONS

Madhavi Maddala: Conceived the idea, conducted experiments and edited the manuscript.

Mahesh Lingakari: Conducted experiment and prepared the first draft.

Srikanth Bandi: Conducted experiment and analyzed results.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

pH: Potential of Hydrogen; **N:** North; **mm:** Millimetre; **°C:** degree Celsius; **NaOH:** Sodium Hydroxide; **ppm:** Parts Per Million; **ml:** Millilitre.

SUMMARY

The study evaluated the larvicidal efficacy of *W. tinctoria*, *P. juliflora* and *M. koenigii* methanolic extracts against *E. machaeralis*, a teak tree pest. Alkaloids, flavonoids and polyphenols were discovered by phytochemical examination of the extracts in this Telangana, India-based study. Larvicidal bioassays showed effects that varied with concentration; at 400 ppm, *M. koenigii* extracts showed the maximum effectiveness, with 92.86% mortality. Significant effectiveness was also demonstrated by *W. tinctoria* and *P. juliflora*, which exhibited 71.43% and 78.57%, respectively, at the greatest concentration. The results of the study demonstrated the possibility of these extracts as environmentally acceptable substitutes for teak leaf skeletonizer management; the greater performance of *M. koenigii* was related to its synergistic metabolite actions. Further research is needed to determine the secondary metabolites responsible for the observed results in the present study.

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