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A Novel Approach for Studying Antitermite Efficacy of Different Leaf Extracts of *Thevetia peruviana* from Polluted and Non-polluted Sites

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Authors' contributions

The work was carried out in collaboration between all the authors. Author NS designed the study and wrote the protocol. Author JK carried out the experiments, performed the statistical analyses and wrote the first draft of the manuscript. Author GS managed the literature searches and the analyses of the study. All the authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

A novel method for evaluating antitermite activity was standardized whereby filter papers treated with 50% methanolic, 90% methanolic and aqueous extracts of *Thevetia peruviana* leaves from polluted and non-polluted sites against *Odontotermes obesus. In vivo* investigation was carried out and observations were recorded without disturbing the termitorium. 90% methanolic extract was found to be the most effective control for termites, followed by 50% methanolic and aqueous extracts. However, antitermite efficacy was found to be higher in aqueous extracts of samples collected from polluted sites than non-polluted sites. Results were found statistically significant, with the value of Pearson's Correlation Coefficient 'r'=-.693. proving the negative correlation between average weight loss and antitermite efficacy.

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1. INTRODUCTION

Termites are soft-bodied arthropods described as social insects. They are classified under the order Isoptera. Termites are important because their activities have positive as well as negative impact on environment. Termites contribute significantly to maintain most of the world's ecosystems. They help breakdown and recycle wood and other plant materials producing organic matter. Their tunneling activities help to aerate soils. Termite activities result in improvement of soil composition and fertility. Compact soils do not support plant life in general but as soon as termites tunnel through such soils, they help in reclaiming them. They also contribute significantly to atmospheric gases [1]. Certain termite species in tropical countries grow fungus within their nests which develop into large mushrooms. These mushrooms (cultured and cultivated by termites) are eaten in some communities of Africa. Moreover, children and women widely consume termite-infected soil for nutritional or other benefits encouraged by indigenous belief systems [2]. Termites constitute a supplementary source of protein for man and mot birds, lizards, frogs and anteaters.

Termites also have negative impact on the economy by causing damage to physical structures such as buildings, bridges, dams, railway sleepers, furniture and even roads. They are also a threat to agriculture as they damage crops and forest trees causing significant loss to annual and perennial crops. Termites pose a great threat to furniture, paper and clothing especially in the semi-arid and sub-humid tropics. In case of severe infestations, their activities result in the loss of structural strength of buildings. The damage caused by termites alone is reported to be more than the combined annual destruction caused by fires, tornadoes and earthquakes [3-4].

Termites inhabit approximately 70% of the world. There are now over 2700 species of termites described from 282 genera but these can be grouped in four major categories according to their nesting habitats and association with moisture. These are damp wood, dry wood, subterranean and arboreal termites [5].

Subterranean termites are the most economically important family of termites with 80% considered to be among economically important pests in the world [6]. There are several genera of subterranean termites found in the literature. They include *Coptotermes, Odontotermes, Microtermes, Reticulotermes* and *Hetrotermes*. Their cryptic and subterranean natures make them more difficult to control. Adedeji et al. 2017 studied the effect of bark, stem and leaf extracts of *Lawsonia inermis* against both termite and fungal decay and found it best to control termites [7].

Odontotermes obesus (Rambur) is a subterranean termite and it belongs to family Termitidae of Order Isoptera. It is one of the most wood-destroying termites causing about 33% damage to timber in India [8]. The present investigation was an attempt to test the antitermite efficacy of aqueous and methanolic leaf extracts of *Thevetia peruviana* against *Odontotermes obesus* (Rambur) from polluted as well as non-polluted sites.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant and Termite

Thevetia peruviana was identified at the Department of Botany, Kurukshetra University, Kurukshetra. The accredition number provided was KUK/BOT/IPS-19.

Fig. 1. *Odontotermes obesus*

Three different native sites with maximum infestation of termite were selected. Mixed sampling of 15-20 soldiers and workers termites were collected from the native sites. Collected samples were identified and authenticated (Fig. 1). The termite species was identified to be

soldiers and workers of *Odontotermes obesus* using identification keys provided by O.B. Chhotani, 1997 in Fauna of India, Isoptera Termites Vol. 22.

2.2 Collection, Processing and Extraction of Plant Material

Leaves of *Thevetia peruviana* from both polluted as well as non-polluted sites were collected and washed thoroughly with running tap water and then with double distilled water. Leaves were first air dried and then oven dried at 60°C for 12 hours. Dried tissues were powdered in a grinder and kept in an air tight polythene bags. To minimize the error and to calculate the extraction efficiency of the plant material, moisture content was determined using moisture balance (AND, MX-50). 50% methanol, 90% methanol and aqueous extracts of leaves were prepared.

2.3 Experimental Design

Blotting paper sheet (rich in cellulose) was cut to uniform size i.e. 5 cm x 5 cm. Experimental sets were prepared by dipping the blotting paper in respective extract (Fig. 2). Single dipping was given to each paper. Then these were air dried for 2 hrs. Weight after dipping and drying was noted down.

In total, 7 experimental sets were prepared according to Table 1. Each set was kept in replicates of 3. Untreated blotting paper was kept as a control. The experimental sets were then incubated to the selected sites and kept as such for 15 weeks. The experiment was observed at regular intervals of 2 weeks and photography was also done. Fig. 3 is showing native termite infested sites selected for the experiment. After 15 weeks, blotting papers were removed from the respective sites and weighed. Average weight loss and change % of weight loss was calculated.

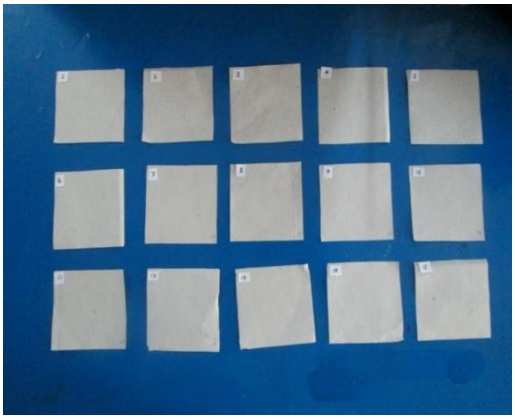

Fig. 2. Exprimental set

Fig. 3. Native termite-infested sites

Table 1. Showing experimental sets for antitermite efficacy

S. no.	Coding	Type of site	Type of extract
1	A_1,A_2,A_3	Polluted	Aqueous
2	B_1,B_2,B_3	Polluted	50% methanolic
3	C_1,C_2,C_3	Polluted	90% methanolic
4	D_1,D_2,D_3	Non Polluted	Aqueous
5	E_1,E_2,E_3	Non Polluted	50% methanolic
6	F_1,F_2,F_3	Non Polluted	90% methanolic
7	G_1,G_2,G_3	Control	Control

2.4 Statistical Analysis

Data was statistically analyzed by applying Pearson's correlation test which proved a definite relationship between the type of solvent and antitermite efficacy. SPSS 11.5 for windows was used to analyze the data statistically.

3. RESULTS AND DISCUSSION

7 experimental sets were prepared to check antitermite efficacy of different leaf extracts of *Thevetia peruviana*. Experimental sets were prepared by dipping the blotting paper in respective extract. After 15 weeks, blotting papers were removed from the respective sites and weighed. Average weight loss was calculated. It was observed that the maximum antitermite activity was exhibited by 90% methanolic leaf extract followed by 50% methanolic leaf extract and aqueous leaf extract. Maximum weight loss was observed in Control. In case of leaf extracts, aqueous extract showed maximum weight loss, 50% methanolic extract showed minimal weight loss whereas 90% methanolic extract does not show any weight loss. Antitermite efficacy was found to be higher in aqueous extracts of polluted sites than non polluted sites as indicated in Table 2 in which extracts of polluted site suffered lesser weight loss than extracts of non polluted sites. A weight loss of 2.97% was observed in case of aqueous leaf extract from polluted site whereas 21% weight loss has been observed in case of aqueous leaf extract from non-polluted site. Fig. 4 shows the termitorium built by termite over control samples. Control samples showed the maximum signs of damage after the termination of experiment i.e. 15 weeks. Aqueous extracts from non-polluted sites showed more damage than those of polluted sites (Fig. 5). 50% methanolic extracts from polluted sites as well as non-polluted sites showed minimal damage. 90% methanolic extracts from polluted sites as well as non-polluted sites showed minimal damage (Figs. 6-11). Pearsons Correlation was applied to the dataset and a strong negative correlation was found significant with r = -.693 (Table 3). It can be interpreted that the antitermite efficacy of leaf extract of *Thevetia peruviana* depends upon the type of solvent. Aqueous solvent showed higher weight loss percentage and when the concentration of methanol was increased, weight loss decreased which means antitermite efficacy was increased.

Termites are a huge menace to crops, buildings and other commercial products. They are the most problematic pest causing a threatening loss to urban environment. They cause significant loss to wooden components especially in semi-arid and sub humid tropics [9].

Odontotermes obesus cause great loss in agriculture [10]. It has been reported that termites become economic pests when they

Table 2. Antitermite efficacy of different plant extracts of *Thevetia peruviana*

S.No.	Plant extract	Initial wt (g)*	Final wt (g)*	Weight loss (g)	Weight loss (change %)
1	TP (P) Aqueous	0.202	0.196	0.006	2.97
2	TP (P) 50% methanolic	0.215	0.215	0.000	0.00
3	TP (P) 90% methanolic	0.207	0.207	0.000	0.00
4	TP (NP) Aqueous	0.219	0.173	0.046	21.00
5	TP (NP)50% methanolic	0.215	0.214	0.001	0.46
6	TP (NP)90% methanolic	0.205	0.205	0.000	0.00
7	Control	0.224	0.142	0.082	36.60

**Mean of three replicates*

Table 3. Showing correlation analysis for antitermite activity

		WLOSS	SOLVENT
W LOSS	Pearson correlation	1	-.693
	Sig. (2-tailed)	.	.084
	N	7	7
SOLVENT	Pearson correlation	-.693	1
	Sig. (2-tailed)	.084	.
	N	7	7

start destroying wooden products of human homes and other commercial products. Termites cause billions of dollar damage throughout the world annually [11].

The most common prevention measure to control the infestation of termites is the use of synthetic termiticides viz. Disodium Octaborate Tetrahydrate (DOT), Chlorpyriphos, etc. Excessive use of these chemicals is harmful for environment and the target insects develop resistance from the prolonged use of termiticides. Therefore, it is necessary to search for alternative means of termite control [12].

Fig. 4. Termite building termitorium on control sample

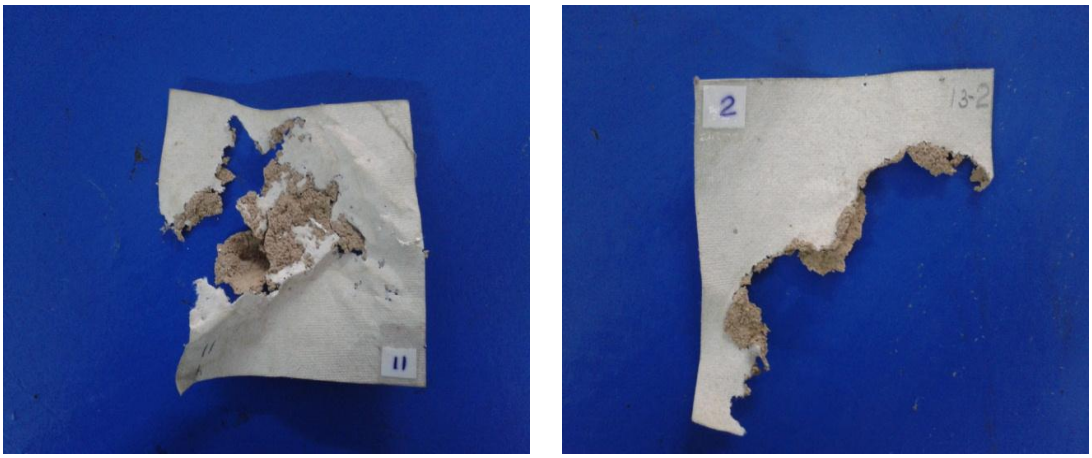

Fig. 5. Control sample eaten by termite

Fig. 6. Non-polluted aqueous extract

Fig. 7. Polluted aqueous extract

Fig. 8. 50% methanolic_non-polluted

Fig. 9. 50% methanolic_polluted

Fig. 10. 90% methanolic_non-polluted

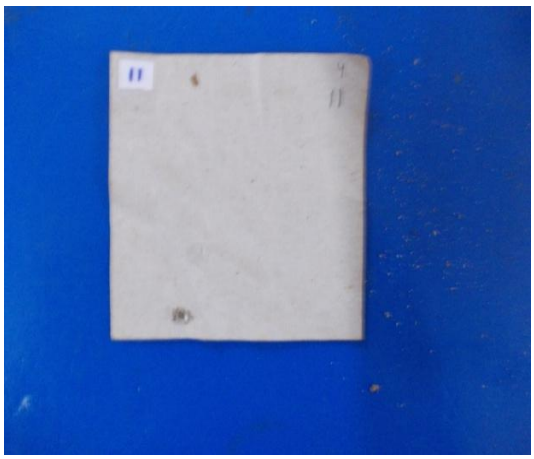

Fig. 11. 90% methanolic_polluted

Note: all the photographs were collected after 15 weeks of induction of experiment.

Many workers have reported the effect of plant essential oils on termite control. Zhu et al. 2001 reported that essential oil of vetiver grass was found to be effective against subterranean termites [13]. Sharma et al. in 1999 and Tellez et al. in 2001 reported the antitermite activity of aerial parts of *Tagetes erecta* against *Odontotermes obesus* [14-15].

Antitermite efficacy of different plant parts of *Thevetia peruviana* has also been studied by various workers. The antitermite properties of petroleum ether, ethanol and aqueous root extracts of *Thevetia peruviana* have been studied and it was reported that ethanolic extract offered significant protection against termites [16]. Most of the researchers have limited their studies to *in vitro* model i.e. by disturbing the termite colony and bringing the termite species to the laboratory. This may lead to the automated death of termite due to the variation in environment and also the change in innate behavior pattern of termite. So, the termicidal reports may prove doubtful. Abbas et al. 2013 evaluated the antitermitic activity of ethanolic seed extracts of 15 medicinal plants by *in vitro* monitoring of mortality of *Odontotermes obesus* [17]. The activity of ethanolic seed extracts of various medicinal plants was studied against *Coptotermes heimi* [18]. Indrayani et al. in 2006 studied the antitermite effect of 0.1% eugenol-based concentration of *Melaleuca leucadendra* on *Coptotermes formosanus [*19]. Ahmed et al. 2011 studied the effect of methanolic and aqueous leaf extracts of *Thevetia peruviana* on the mortality of *Microtermes obesi in vitro [*20]. Edori and Dibofori-Orji in 2016 reported the mortal effect of aqueous seed extracts of *Raphiafarinifera* on termites *in vitro [*21]. The repellency effect of *Tagetes erecta* and *Citrus sinensis* seed oil was investigated against *Odontotermes obesus* [22].

The present investigation was carried out *in vivo* where the treated blotting papers were kept as such at the termite-infested sites infected with soldier and worker population of *Odontotermes obesus* and observed periodically without disturbing the termitorium. In such conditions, termites are more active as they remain in the same vicinity with respect to their social and confined behavior within the termitoria. The duration of *in vitro* experiments was 2-3 weeks or even lesser than that [17-18,21]. But in present investigation, duration of experiment was 15 weeks. This gave long term authentic information on antitermite bioassay. Weight loss percentage was caluculated which clearly indicated the antitermite efficacy of different plant extracts. Weight loss is inversely proportional to the antitermite efficacy i.e. lesser the weight loss, greater the antitermite activity. It is clear from Table 2, that 90% methanolic leaf extract of *Thevetia peruviana* proved to be the most efficacious extract for antitermite activity followed by 50% and aqueous. Aqueous extract had least effect on termites. It may be due to the fact that the secondary metabolite content was very less to be effective against termites [23]. Also methanol and the secondary metabolites present in the leaf of *Thevetia peruviana* had a synergistic effect on controlling the growth of *Odontotermes obesus.* The results were statistically analyzed and a significant negative correlation (r=-.693) was observed which clearly indicated that the antitermite efficacy was negatively correlated with the concentration of solvent. As the concentration of methanol was increased, the weight loss percentage was decreased i.e. antitermite efficacy was increased (Table 3).

4. CONCLUSION

The effect of pollution on the synthesis of secondary metabolites was studied by Kaur et al. in 2015 and found that extraction efficiency of 90% methanolic leaf extracts was more than 50% and aqueous leaf extracts [24]. Keeping this view in mind, the antitermite efficacy of leaf extracts from non-polluted sites was compared to that of leaf extracts of polluted sites and it was observed that aqueous leaf extracts from polluted sites had better antitermite activity than those of non-polluted sites as indicated by the lesser weight loss percentage of 2.97% than non-polluted site where the weight loss percentage was 21% (Table 2). This may be due to the fact that some non-polar phytoconstituent has been oversynthesized in aqueous extract which might be responsibe for antitermite efficacy and methanol was unable to extract that phytoconstituent. This approach of assaying antitermite efficacy is beneficial in two ways: firstly, it is reclaiming the polluted soils and secondly it is leading towards scale-up of phytoconstituents responsible for antitermite efficacy. *Thevetia peruviana* has been extensively planted on National Highway Dividers where pollution stress is on the pace. Its plantation will reclamate the soil as well as pollution stress with over synthesize important phytoconstituents responsible for various bioactivities. It is concluded from the results that

the leaf extracts of *Thevetia peruviana* may provide a renewable source of safe natural antitermitic agent. List of numerous medicinal uses of *Thevetia peruviana* can now be extended to include natural antitermitics. These extracts should be used alone or in combination with the chemical pesticides to lower their harmful effect. The results obtained have opened the possibility of further investigations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval is not applicable for our manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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