

# Effect of tomatine on termites *Odontotermes wallonensis* (Wasmann) *vis-a-vis* antifeedant and repellent activity

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## ABSTRACT

A secondary alkaloid, tomatine was extracted from unripen fruits and leaves of tomato. It is a potent antifungal and insecticidal compound that interacts with sterols in the membranes of the insects and shows efficacy against insects and many species of termites. In this research, investigations were carried out to study the antifeedant and repellent activity of tomatine against the termite species *Odontotermes wallonensis* Wasmann. The result concluded that lowest consumption of food by *O. wallonensis* workers was recorded in Azadirachtin 100 ppm (0.15 g) followed by Tomatine 250 ppm (0.60 g). Soldiers and nymphs of *O. wallonensis* consumed 0.54g and 0.56g food in tomatine 250 ppm treatment whereas in treated check (Azadirachtin 100 ppm) consumed 0.14g in both castes as compared to untreated check (1.33 and 1.46 g). And also exhibited the highest repellent activity towards termites at all the doses. Tomatine 1000 ppm caused 82.13, 73.41 and 88.67 per cent repellent activity whereas Azadirachtin 100 ppm caused 88.67, 78.96 and 92.00 per cent in *O. wallonensis* workers, soldiers and nymphs, respectively.

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## INTRODUCTION

Termites are widely distributed in red, sandy loams, lateritic and red loam soils and known to damage major field crops such as wheat, maize, sugarcane, cotton, groundnut, pulses, and forest plantation trees such as Eucalyptus, Silver oak, Casuarina and all kinds of timber in buildings. Losses due to termites run to several millions

of rupees in agricultural crops alone. About 10 to 25 per cent loss is estimated in most field and forest crops (Logan *et al.*, 1990). The currently used insecticides adversely affects the soil, soil flora and fauna and this situation necessitated to evaluate the efficacy of a plant extracts against termites. Plant parts and plant extracts can be used effectively because these are less expensive

and biodegradable and hence environmentally suitable. Many farmers in Asia and Africa had been using plant extracts for controlling and repelling termites (Anonymous, 2000). Tomatine reduced the leaf consumption and altered behaviour patterns of newly emerged beetles of Colorado Potato Beetle *L. decemlineata* when fed with diet (Kowalski *et al.*, 2000). Sushell Sankhyan (1992) observed a decline in tomatine content showed strong feeding preference to the first instar larvae of *H. armigera* in tomato plants. 2-phenoxyethanol is a potent termite attractant. Nootkatone is an effective repellent and toxicant of termites either by itself or as an addition to other materials or substrates, including mulches made from vetiver grass roots or other wood products (Zhu, 2001). Vetiver oil and some of its constituents are repellent and toxic to termites (Gregg Hendreson *et al.*, 2005). These findings made the way to investigate the antifeedant and repellent activity of tomatine against *Odontotermes wallonensis*.

## MATERIAL AND METHODS

### Extraction of tomatine :

One kg unripe tomato was washed well with distilled water. The tissues were extracted by maceration with 5 per cent acetic acid (15-20 parts) and filtered using ordinary filter paper to remove the cellular debris. The filtered extract was heated at 70°C and added with NH<sub>4</sub>OH drop by drop to maintain the pH 10. The extract was centrifuged at 5,000 rpm and the supernatant was discarded. The precipitate obtained was again centrifuged at 10,000 rpm with 1 per cent NH<sub>4</sub>OH. The precipitate (tomatine) was collected, dried and weighed. Approximately 2.0 g of tomatine was obtained from 1 kg of unripe tomato. The obtained tomatine was confirmed using Wagner's reagent or Mayer's reagent.

### Preparation of stock solution :

A stock solution of tomatine was prepared by dissolving tomatine (10 mg) in ethanol (100ml). From this stock solution different concentrations of tomatine were prepared by serial dilutions and used for further laboratory experiments.

### Evaluation of antifeedant activity of tomatine :

Cardboard sheets weighing 2.5g were prepared and moistened with distilled water and shade dried for one hour. The sheets were thoroughly immersed in the

tomatine stock solution (250, 200, 150, 100 and 50 ppm) and kept aside for 12 hr in the open to evaporate the ethanol. Azadirachtin 100 ppm solution was used as the standard check and distilled water was used in the control. The treated sheet was placed in the petriplate and 50 numbers of termite workers/ soldiers/ nymphs were released, covered and kept in the BOD incubator at 25±1°C for 4 days. Once in a day the treated sheets were taken and weighed to assess the quantity of food consumed by the live workers, soldiers and nymphs. The food consumption was expressed as gm.

### Evaluation of repellent activity of tomatine :

A stock solution of tomatine was prepared by dissolving in ethanol. From this stock solution six concentrations *viz.*, 1000, 900, 800, 700 and 600 ppm were prepared by serial dilutions. Twenty four units of chambered transparent plastic containers were taken. The containers were in the size of 17.5×13×4.0 cm and each had six chamber measuring 6.5×5.75×4.0 cm. Each container was considered as one replication. The treatments were replicated thrice.

A small opening (0.5 cm diameter) was made at the bottom of the two inner walls, connecting the three chambers. A filter paper was provided to each of the two lateral side chambers of all containers. One lateral side was marked treated and filter paper in this side was treated with 125 µl of tomatine solutions. Filter papers in the opposite side were left untreated. The control was similarly prepared in which both the sides of filter paper received only distilled water. The containers were left uncovered for 4 hrs at ambient condition for solvent evaporation. Then 250 µl of double distilled water was added to each filter paper. A total number of fifty workers and fifty soldiers were released in the middle chamber. Containers were kept at laboratory conditions covered with their lids and an opaque black sheet to eliminate the effect of light.

### Observations :

Number of workers in each lateral chamber taken at 2, 4, 6, 8, 10 hours and containers were rotated after each observation. The following formula was used to calculate the per cent repellent activity.

$$\text{Per cent repellency} = \frac{\text{No. of termites repelled from single chamber}}{\text{Total number of termites in the container}} \times 100$$

For laboratory studies, the per cent mortality was

corrected using Abbot's (Abbott, 1925) formula. The data on percentage values and number were transformed into arcsine and square root values, respectively before subject to statistical analysis. Analysis of variance was done in AGRESS and AGDATA Packages. Duncan Multiple Range Test was applied for comparing the treatment means.

## RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

### Effect of tomatine on the consumption of food by termites :

The data pertaining to the antifeedant and repellent activity of tomatine was presented in the Tables 1 and 2. Tomatine 250 ppm effectively reduced the food consumption in workers (0.60g), soldiers (0.54 g) and larvae (0.56 g) of *O. wallonensis*. The food consumption in untreated check was higher (1.50 g , 1.33 g and 1.46 g) by workers, soldiers and larvae, respectively (Table

1). The present finding is in accordance with the findings of Grace and Yates (1992) who observed *C. formosanus* workers fed less on paper containing azadirachtin at 100ppm concentration and with the findings of Lu and Chu (1992) who reported that application of 0.1- 0.4 per cent tomatine solution on cabbage significantly reduced the DBM feeding from 1/6 to 1/41 of that of the control.

The results of the experiment conducted by Hirano *et al.* (1994) supports well with this concept. They stated the strong antifeeding activity of tomatine on *Thrips palmi* Karny. Gunther *et al.* (1997) examined lethal concentration of tomatidine and solanidine reduced the feeding and acted as deterrent to Potato aphid (*Microsiphum euphorbiae* Thomas). Tomatine reduced the leaf consumption and altered behaviour patterns of newly emerged beetles of Colorado Potato Beetle *L. decemlineata* when fed with diet (Kowalski *et al.*, 2000). Sushell Sankhyan (1992) observed a decline in tomatine content showed strong feeding preference to the first instar larvae of *H.armigera* in tomato plants. The suggestions of Pathak *et al.* (2000) was in consonance with these results. They conducted an experiment on

**Table 1 : Evaluation of antifeedant activity of tomatine against *Odontotermes wallonensis***

Sr. No.	Treatments	Dose (ppm)	Mean weight of food consumed (gm)		
			Workers	Soldiers	Nymphs
1.	Tomatine	250	0.60 <sup>b</sup> (0.74)	0.54 <sup>b</sup> (0.69)	0.56 <sup>b</sup> (0.71)
2.	Tomatine	200	0.63 <sup>c</sup> (0.71)	0.56 <sup>c</sup> (0.72)	0.59 <sup>c</sup> (0.74)
3.	Tomatine	150	0.74 <sup>d</sup> (0.84)	0.67 <sup>d</sup> (0.80)	0.70 <sup>d</sup> (0.81)
4.	Tomatine	100	0.84 <sup>e</sup> (0.90)	0.78 <sup>e</sup> (0.87)	0.80 <sup>e</sup> (0.88)
5.	Tomatine	50	0.96 <sup>f</sup> (0.96)	0.87 <sup>f</sup> (0.92)	0.92 <sup>a</sup> (0.94)
6.	Azadirachtin	100	0.15 <sup>a</sup> (0.36)	0.14 <sup>a</sup> (0.35)	0.14 <sup>f</sup> (0.35)
7.	Untreated check	-	1.50 <sup>g</sup> (1.21)	1.33 <sup>g</sup> (1.14)	1.46 <sup>g</sup> (1.20)
	S.E.±		0.74	0.97	0.009
	C.D. (P=0.05)		1.47	2.09	0.018

**Table 2 : Evaluation of repellent activity of tomatine against *Odontotermes wallonensis***

Sr. No.	Treatments	Dose (ppm)	Mean per cent repellent action		
			Workers	Soldiers	Nymphs
1.	Tomatine	1000	82.13 <sup>a</sup> (68.72)	73.41 <sup>b</sup> (57.73)	88.67 <sup>b</sup> (72.54)
2.	Tomatine	900	75.20 <sup>b</sup> (63.58)	66.34 <sup>c</sup> (54.73)	83.33 <sup>c</sup> (66.68)
3.	Tomatine	800	62.53 <sup>c</sup> (52.70)	59.00 <sup>d</sup> (50.30)	78.34 <sup>d</sup> (63.13)
4.	Tomatine	700	49.73 <sup>c</sup> (44.73)	53.00 <sup>e</sup> (46.73)	67.33 <sup>e</sup> (55.55)
5.	Tomatine	600	37.06 <sup>d</sup> (37.56)	43.06 <sup>f</sup> (40.87)	63.33 <sup>e</sup> (53.05)
6.	Azadirachtin	100	88.67 <sup>e</sup> (72.13)	78.96 <sup>a</sup> (65.91)	92.00 <sup>a</sup> (78.20)
7.	Untreated check	-	0.57 <sup>f</sup> (5.05)	2.84 <sup>g</sup> (8.80)	3.51 <sup>f</sup> (9.02)
	S.E.±		0.008	0.007	1.47
	C.D.(P=0.05)		0.015	0.014	2.95

termites by using murrya leaves. The wood pieces of mango were treated with murrya leaves and the treated wood were less consumed by the termites and recorded only 20 per cent damage than control in which the wood pieces were completely damaged. Sand treated with vetiver oil or its alkaloid nootkatone at 100µg/g of substrate effectively reduced the food consumption and survival of *C. formosanus* termites (Lara Maistrello *et al.*, 2001).

Schmutterer (1990) suggested that azadirachtin acted as a antifeedant and has the sublethal and chronic effects on *Eurygaster* sp. The present findings are in agreement with findings of Kumar *et al.* (2003) who evaluated the biological efficacy of eight commercial neem formulations (CNFs) against second instar larvae of diamondback moth (DBM), *Plutella xylostella*. Similar results were recorded by Isman (1996) who indicated that secondary alkaloids are behaviour modifying substance that deters feeding through a direct action on peripheral sensilla (taste organs) in insects. Amy (2006) suggested that azadirachtin's interference with phagostimulants (play a role in feeding behaviour of insects) and involvement in synthesis of Prothoracicotropic hormone (PTTH) was the reason for antifeedant and insecticidal activity, respectively.

From the results of current study it was concluded that tomatine at different concentrations effectively shows the antifeedant activity on all castes of termites. But while comparing the antifeedant activity of tomatine it was maximum in soldiers, because less amount of food consumption by soldiers (0.54 g) while workers and nymphs consumed high amount of food. (0.60g and 0.56g). This may be due to dissimilarity in feeding behaviour and food preference by termite castes. Workers and nymphs have the highest preference to food source and workers consume large quantity of food than soldiers which consume low amount of food.

#### Studies on repellent activity of tomatine against termites :

Tomatine at different concentrations effectively repelled the termites. Tomatine 1000 ppm had the highest repellent activity on workers (82.13%), soldiers (73.41%) and larvae (88.67 %) of *Odontotermes wallonensis*. It had the highest repellent activity in workers compared to soldiers because soldiers can withstand its concentration and larvae were effectively repelled by

tomatine (Table 2). These findings were supported by Hirano *et al.* (1994) who suggested that tomatine can be used as repellent for *Thrips palmi* Karny. Plant derivatives of nootkatone, one of the extracts vetiver oil was found to be an effective repellent against the termites either by itself or with addition of other materials including mulches from vetiver grass root or wood products (Zhu, 2001). Findings of Verna *et al.* (2003) were in agreement with present findings who conducted a short term bioassay on the subterranean termites, isoborneol (a bicyclic monoterpenoid derived from *Nicotiana tabaccum*) repelled workers of *Reticulitermes santonensis* after adding the substance to the substrate. Gregg Hendreson *et al.* (2005) revealed that Naphthalene derivatives from *Wallastonia biflora* effectively repelled the workers and soldiers of *C. formosamus* termites. Accordance with these results, Grace and Yates (2007) stated that termites avoided long term contact with the various concentration of azadirachtin treated sand. In the current study it revealed that tomatine showed higher repellency on nymphs (88.67 %) and workers (82.13 %), than soldiers (73.41%). When the nymphs were exposed to tomatine, they were repelled suddenly from the source. This might be due to the sensitiveness of the nymphs to tomatine. From the present study it was concluded that tomatine caused mortality effectively in all castes of *Odontotermes wallonensis*. Further studies are needed to use tomatine as alternate termiticide in the management of subterranean termites.

#### Conclusion :

Finally it concluded that tomatine effectively showed the antifeedant and repellent activity on all castes of termites. Future research may focus on effect of tomatine on other insects.

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