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Effect of tomatine on *Termitomyces* fungus in termitaria of subterranean termites *Odontotermes wallonensis* Wasmann

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ABSTRACT

Tomatine is a glycoalkaloid found in the stems and leaves of tomato plants, which has fungicidal properties. This laboratory studies were carried out to find out the efficacy of tomatine on the *Termitomyces* fungus found in the termitaria of *O. wallonensis*, it was applied through Difco media for fungal growth. The overall period of observations (15 days) revealed that tomatine 1000 ppm effectively reduced the mean diameter growth of fungus (1.41 cm) followed by tomatine 900 ppm (1.52 cm). The fungal growth of fungus in the standard check carbendazim 500 ppm was 1.32 cm whereas it was 8.17 cm in untreated check. The highest suppression of fungus was noticed in carbendazim 500 ppm (84.59 %) which was on par with tomatine 1000 ppm (83.44 %). Tomatine 900 ppm was also suppressed the fungus effectively at 82.08 per cent. The least mean per cent suppression (68.37 %) was noticed in the lower concentration of tomatine 600 ppm. The per cent suppression of fungal growth was decreased from 5 th day to 15 th day of observation. This study was supported by many earlier contributions. Finally the result was concluded that tomatine could be used as a termiticide to control fungus growing termites.

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INTRODUCTION

Termite is a diverse group of insect with respect to nutritional ecology. They are divided into four groups according to diets. Grass-harvesters, which are physiologically more advanced, consist of Hodotermitidae and some species of "higher termite" Termitidae (Wood, 1978), Wood-feeders (living tree, dead wood and litter) occupy the majority of "lower termites" (Mastotertmitidae, Kalotermitidae, Termopsidae, Hodotermitidae, Rhinotermitidae and Serritermitidae) (Waller and La fage, 1987) which are physiologically primitive except Hodotermitidae. Fungus-growers are members of Mastotermitinae (Termitidae). They culture a fungus, belonging Genus *Temitomyces* (Basidiomycete) in their nest, and fungus-comb is eaten by young colony individuals. The fourth group is soil-feeders. Approximately 60 per cent species of Termitidae consume soils which contain minerals, carbohydrates, soil micro-organism, and polyphenolic compounds (Collins, 1983). Thus, the preference of termites on foods naturally contributes to diversity of nutritional physiology. The fungus helps the termites to degrade the plant-derived material (e.g., wood, dry grass, and leaf litter) on which they live (Johnson *et al*., 1981). The fungus comb is maintained by the termites through continuous addition of predigested plant substrate while the older comb material is consumed. (Rouland-Lefevre, 2000). With reference to fungicidal activity, tomatine also has antimicrobial properties against certain classes of microbes although some microbes produce an enzyme called tomatinase which can degrade tomatine, rendering it ineffective as an antimicrobial. Many investigations showed the efficacy of tomatine against many species of termites in different ways (Nisha *et al*., 2013). The present study was undertaken to evaluate the antifungal properties of a tomato alkaloid, tomatine against *Termitomyces*.

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 70^0C and added with NH_4OH 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_4OH. 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 antifungal activity of tomatine on *Termitomyces* fungus :

The *Termitomyces* fungus was collected from fungal garden in the mound of *Odontotermes wallonensis* located on the coconut field bund at Agriculture College and Research Institute, Madurai. The fungus was cultured in to the Difco 4 media mixed with different concentrations of tomatine extract (1000, 900, 800, 700 and 600 ppm) and in medium with out the tomatine extract. The standard check used was Carbendazim 500 ppm. The following observations *viz.*, mean diameter growth of *Termitomyces* (cm) and mean per cent suppression were observed.

Composition of difco media :

NH_2 NO_3 - 1 g, KH_2PO_4 - 0.90 g, $MgSO_4$ - 0.70g, Glucose- 20g, Difco yeast extract powder-0.3 g, Distilled water- 1 liter, pH - 6.4, Agar - 2 g

For laboratory studies, the per cent mortality was corrected using Abbot's (Abbot, 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 mean per cent suppression of *Termitomyces* fungal growth was high in carbendazim 500 ppm (84.59 %) which was at par with tomatine1000 ppm (83.44%). During all the periods of observation the per cent suppression of fungal growth decreased from 5th day to 15th day. On 5th Day of observation the mean per cent suppression of fungal growth ranged from 91.55 per cent to 68.37 per cent. The least mean per cent suppression (68.37 %) was noticed in the lower concentration of tomatine 600 ppm.

Similar trend of suppression was noticed up to 15th Day of observation. In 10th day and in 15th day the fungal suppression was 82.96, 81.11 and 83.70 per cent and 77.41, 75.93 and 78.52 per cent, respectively in tomatine 1000, 900ppm and carbendazim 500ppm (Table 1).While comparing the overall period of observation tomatine 1000 ppm effectively reduced the growth of *Termitomyces* fungus (1.41cm). The fungal growth in carbendazim 500ppm was 1.32 cm whereas it was 8.17 cm in untreated check. During the initial period of observation (5th Day) tomatine 1000 ppm effectively reduced the fungal growth (0.65cm), 900 ppm (0.70 cm), 800 ppm (0.82 cm) and they were on par with the standard check carbendazin 500 ppm (0.55 cm). This was followed by 700 ppm (1.27 cm) and 600 ppm (2.07cm). The untreated check recorded the mean fungal growth of 6.5 cm. The mean fungal growth was minimum in tomatine 1000 ppm (2.03 cm) and tomatine 900 ppm (1.70 cm) and they were on par with standard check carbendazim 500ppm (1.93 cm) at 15th day. Next in the order the efficacy was tomatine 800 ppm (2.8 cm) (Table 1).

The antifungal activity of tomatine was already reported by Irving *et al.* (1946). According to their study, tomatine exhibited marked fungistatic activity towards two strains of *Aspergillus clavatus.* On the tomatine treated *A. clavatus* plates after 19 hrs of incubation the inhibition zones were perfectly clean and devoid of all growth. This is supported by the view of Dow and Callow (1978) who discussed that tomatine was both fungistatic and fungicidal and caused an irreversible leakage of electrolytes from the hyphae, which resulted in inhibition of hyphal elongation of the fungi *Cladosporium fulvum.* Same result was observed by Costa and Gaugler (1989) who showed in their results that colony formation and growth of the fungus *Beauveria bassiana* were inhibited by tomtine at 100 mg/lit. The present findings are in accordance with Glazener and Wouters (1981) and Quidde *et al*. (1998) who suggested that α- tomatine affected the fungal formation and confirming the resistance towards *Botrytis cinerea.*

Among the different tomatine concentrations, tomatine 1000ppm recorded minimum growth (1.41cm) and highest suppression of fungus (83.44%). The findings of present studies were in corroboration with findings of Datchina Murthy *et al*. (2010) who evaluated antifungal properties of tomatine against *Termitomyces* sp found in termitaria of *O.wallonensis*. They found that tomatine 1000 ppm recorded 2.64 cm growth and 69.27 per cent of suppression of fungus. Earlier reports were also supportive to these findings. Osbourn (1996) evaluated the antifungal activities of tomatine against pathogenic fungi *Fusarium oxysporum* that cause tomato wilt, and other *Fusarium* species that cause wilt in peas and cabbage. According to Muller Jurgen (1998) another alkaloid from tomato plant, Solanine also showed fungicidal activity. Effect of tomatine on virus was found by Balashova *et al*. (1984) who explained tomatine at 0.005 per cent inhibited the growth of the tobacco mosaic virus in infected tomato plants under *in vitro* condition.

Table 1 : Effect of tomatine against *Termitomyces* found in termitaria of termite *Odontotermes* wallonensis

Sr. No.	Treatments	Dose (ppm)	Mean diameter growth of *Termitomyces* * (cm)			Mean	Mean per cent suppression of fungus*			Mean
			5th day **	10th day	15th day		5th day **	10th day	15th day	
1.	Tomatine	1000	0.65a	1.53a	2.03a	1.41ab	89.94ab	82.96a	77.41a	83.44ab
			(1.07)	(1.43)	(1.59)	(1.36)	(72.02)	(66.02)	(61.97)	(66.67)
2.	Tomatine	900	0.70a	1.70a	2.17a	1.52b	89.21ab	81.11a	75.93a	82.08b
			(1.10)	(1.48)	(1.63)	(1.40)	(71.3)	(64.62)	(60.96)	(65.62)
3.	Tomatine	800	0.82a	2.10b	2.80b	1.91c	87.39b	76.67b	69.26b	77.77c
			(1.15)	(1.61)	(1.82)	(1.52)	(69.65)	(61.47)	(56.42)	(62.51)
4.	Tomatine	700	1.27b	2.10b	3.50c	2.29d	80. 63c	76.67b	61.11c	72.80d
			(1.33)	(1.61)	(1.99)	(1.65)	(64.29)	(61.51)	(51.52)	(59.11)
5.	Tomatine	600	2.07c	2.90c	3.90d	2.96e	68.37d	68.15c	56.67d	64.39e
			(1.58)	(1.84)	(2.1)	(1.84)	(56.77)	(55.72)	(49.12)	(53.87)
6.	Carbendazim	500	0.55a	1.47a	1.93a	1.32a	91.55a	83.70a	78.52a	84.59a
			(1.02)	(1.4)	(1.56)	(1.33)	(73.57)	(66.57)	(62.74)	(67.63)
7.	Untreated check	-	6.50d	9.00d	9.00e	8.17f	0.00e	0.00d	0.00e	0.00f
			(2.64)	(3.08)	(3.08)	(2.94)	(4.05)	(4.05)	(4.05)	(4.05)
	SED		0.07	0.05	0.04	0.03	1.66	1.12	0.93	0.74
	C.D.(P=0.05)		0.14	0.10	0.08	0.06	3.56	2.40	2.00	1.49

*Mean of 3 replications **Days after inoculation

Figures in parentheses are $\sqrt{0+0.5}$ and x + 0.5 arc sin transformed values

Means followed by same letter(s) are not significantly different at 5 per cent level by DMRT

Reasons for toxicity of tomatine to fungus was reported by McKee (1959) who indicated that toxicity of tomatine increased with increasing concentrations of sodium and potassium ions and decreased in the presence of calcium ions, was dependent on the sodium ion/calcium ion ratio, and correlated with the hemolytic activities of the compounds. He suggested that toxicity increased with pH of the compounds, that it is the non-protonated forms of the compounds which disrupt cell membranes by penetrating the cholesterol monolayers of the membranes of the fungus *Fusarium caereleum*. Robert *et al*. (1997) suggested that toxicity of tomatine to broad range of fungus was due to binding of tomatine to 3 β- hydroxyl sterols in fungal membrane.

The *Termitomyces* fungus was collected from *Odontotermes* mound. The fungus was cultured using *Difco* media mixed with various concentrations of tomatine under *in vitro* condition to evaluate the fungistatic activity. The fungal suppression by tomatine was very effective one (83.44%). The chances of direct interactions between tomatine with fungus inside the fungal comb was discussed. This might be through salivary secretions, regurgitations and excretions of termites after ingested with tomatine. Tomatine exposed to fungal garden through bait might have caused the fungistatic activity by disrupting the fungal cell membrane. It was concluded that tomatine could be used as a termiticide to control fungus growing termites.

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