



Research Article

EFFECT OF TOMATINE ON SUBTERRANEAN TERMITES, NEMATODES AND SOIL BORNE FUNGUS UNDER LABORATORY CONDITIONS

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Abstract: Laboratory experiments were conducted to evaluate the effect of Tomatine on subterranean termites, nematodes and soil borne pathogens. At 12 HAT (Hours After Treatment) Chlorpyrifos 1000 ppm recorded the highest mortality (100 %) of subterranean termite, *Odontotermes wallonensis* followed by Tomatine 1000 ppm (99.00 % mortality). Cent percent mortality of *Meloidogyne incognita juveniles* (j2) was observed in Tomatine (1 g / lit) at 36 HAT. Tomatine 1.0 g / lit recorded nil percent hatching of eggs upto 30 HAT. The same treatment recorded the lowest egg hatching of 1.45 percent at 36 HAT. Lowest mean mycelial growth of soil borne pathogens, *Sclerotium rolfsii* (5.10 %), *Fusarium solani* (4.00%) and *Pythium aphanidermatum* (5.60%) was recorded in Tomatine 1.0 % treatment.

Keywords: Tomatine, Termite, Nematode, Fungus

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Introduction

Termites cause serious damage to agricultural and horticultural crops grown in all types of soil in India. They attack crops in seedling stage, developmental stage, tree trunk and wooden logs [1]. *Odontotermes obesus* has been found to cause 10-15 percent yield loss in ground nut due to pod damage in Western Rajasthan [2]. In sugarcane high mortality of termites *Odontotermes wallonensis* was caused by Chlorpyrifos (2 ml / lit) [3]. Many crops are attacked by nematodes and wilt disease caused by fungi. To manage these pests nematicides and fungicides are applied in soil. The currently used pesticides to manage subterranean pests were found to adversely affect the soil properties, soil flora and soil fauna. Hence there is a need to evaluate the ecologically safe and potent plant alkaloids like tomatine against subterranean pests. Tomatine reduced the leaf consumption and altered behavior patterns of newly emerged beetles of Colorado Potato Beetle *Leptinotarsa decemlineata* when fed with diet [4]. Laboratory experiments were conducted to evaluate the efficacy of tomatine against subterranean pests.

Materials and Methods

Extraction of Tomatine

One Kg unripe tomatoes were washed well with distilled water. The tissues were extracted by maceration with 5 % acetic acid (15-20 parts) and filtered using ordinary nylon filter paper to remove the cellular debris. The filtered extract was heated at 70°C and added with NH₄OH 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 10,000rpm with 1% NH₄OH. The precipitate (tomatine) was collected, dried and weighed. Approximately 2.0 g of tomatine was obtained from 1.0 kg of unripe tomato. The obtained tomatine was confirmed using Wagner's reagent.

Preparation of stock solution

A stock solution of tomatine was prepared by dissolving tomatine (10 mg) in

ethanol (100 ml). From this stock solution six concentrations viz., 1000, 800, 600, 400, 250, 200 and 100 ppm were prepared by serial dilutions and used for further laboratory experiments.

Effect of Tomatine on Termites *Odontotermes wallonensis*

A laboratory experiment was conducted to test the efficacy of tomatine on termites *Odontotermes wallonensis* at Department of Agricultural Entomology, Agricultural College & Research Institute, Killikulam, Tamil Nadu during January 2014. Nine cm dia corrugated cardboard sheets were prepared and moistened with distilled water and shade dried for one hour. Then the sheets were thoroughly immersed in the tomatine stock solution (1000, 800, 600, 400 and 200ppm) and kept aside for 12 hr in the open to evaporate the ethanol. Chlorpyrifos 1000 ppm solution was the standard check. The treated sheet was placed in the Petri plate and 50 nos. of termite workers collected from the field using bait stations were released and covered and then kept in the BOD incubator at 25±1°C. The mortality was observed at 12 hours interval for up to 96 hour after treatment. There were eight treatments and three replications.

$$\text{Percent Mortality} = \frac{\text{No of termites dead}}{\text{Total no. of termites released}} \times 100$$

Effect of tomatine on survival of *Meloidogyne incognita juveniles* (j2)

Pure tomatine of the respected quantity (0.10, 0.20 0.50 and 1.0g) was dissolved in a litre of water. One hundred infective juveniles (j2) of *Meloidogyne incognita* were handpicked from the pure culture maintained in the glass house using stereo zoom microscope at Nematology unit, Department of Entomology, Agricultural College & Research Institute, Madurai during 2014. The j2 were placed in Syracuse dish containing respective concentrations of tomatine. Observations on mortality of the juveniles were made at 6, 12,18,24,30 and 36 hours interval. The deaths of the juveniles were confirmed by placing them in distilled water.

Table-1 Effect of Tomatine on Termites, *Odontotermes wallonensis*

SN	Treatments	Dose (ppm)	Mean percent mortality *					Mean
			1 HAT**	2 HAT	4 HAT	6 HAT	12 HAT	
1	Tomatine	1000	16.67 ^b (23.09)	46.33 ^a (42.90)	61.67 ^a (52.31)	82.00 ^a (65.00)	99.00 ^a (86.95)	61.13 ^b (51.48)
2	Tomatine	800	12.33 ^b (21.13)	36.33 ^b (37.07)	62.33 ^a (52.17)	84.00 ^b (66.97)	98.33 ^a (83.71)	58.66 ^{bc} (50.01)
3	Tomatine	600	15.33 ^b (22.97)	40.00 ^b (39.82)	63.00 ^b (52.21)	84.00 ^b (69.94)	97.00 ^a (79.94)	59.87 ^b (50.89)
4	Tomatine	400	9.00 ^b (16.36)	31.67 ^{bc} (34.25)	55.33 ^{bcd} (48.21)	68.00 ^c (55.58)	92.33 ^{ab} (73.82)	51.27 ^{cd} (46.11)
5	Tomatine	200	13.33 ^b (20.61)	31.33 ^{bc} (34.04)	53.00 ^{cd} (47.15)	67.67 ^c (55.05)	91.67 ^{ab} (72.91)	51.40 ^{cd} (45.95)
6	Tomatine	100	9.33 ^b (17.76)	30.33 ^{bc} (33.42)	45.67 ^d (42.03)	66.33 ^c (54.69)	91.67 ^{ab} (72.37)	48.70 ^d (44.32)
7	Chlorpyrifos 20 EC	1000	68.00 ^a (56.00)	97.67 ^a (83.47)	100.00 ^a (89.72)	100.00 ^a (89.72)	100.00 ^a (89.72)	93.13 ^a (75.00)
8	Untreated check	-	6.33 ^c (13.91)	15.67 ^c (23.31)	27.67 ^c (31.79)	35.00 ^c (36.13)	61.33 ^b (57.11)	29.20 ^e (33.05)
SED			5.10	6.72	3.09	2.66	8.81	2.38
CD(P=0.05)			10.79	14.21	6.29	5.69	18.76	5.09

*Mean of 3 replications, **HAT - Hours After Treatment, Figures in parentheses are arc sin transformed values
Means followed by same letter(s) are not significantly different at 5% level by DMRT

Table-2 Mean percent mortality of *Meloidogyne incognita* juveniles (j2) at different concentration of tomatine and at different intervals

Concentration of tomatine g/l	Time in Hours					
	6	12	18	24	30	36
0.10	6.33 ^a (14.57)	13.86 ^a (21.85)	22.26 ^a (28.15)	32.46 ^a (34.73)	40.65 ^a (39.61)	50.32 ^a (45.18)
0.20	23.41 ^c (28.93)	32.11 ^c (34.51)	46.41 ^c (42.94)	53.16 ^c (46.81)	67.14 ^c (55.02)	70.16 ^c (56.89)
0.5	32.18 ^b (34.56)	45.25 ^b (42.27)	63.62 ^b (52.90)	78.25 ^b (62.20)	84.17 ^b (66.55)	98.29 ^b (82.48)
1.0	52.36 ^a (46.35)	64.47 ^a (53.41)	75.64 ^a (60.42)	88.17 ^a (69.88)	98.45 ^a (82.85)	100.00 ^a (89.71)
CD (0.5)	6.65					

*Mean of Five replications; C- Concentration; T-Time, Figures in parentheses are arc sin transformed values

Table-3 Mean percent egg hatching of *Meloidogyne incognita* at different concentration of tomatine and at different intervals

Concentration of Infective Juveniles/l	Time in Hours					
	6	12	18	24	30	36
0.10	4.38 ^a (0.7307)	6.32 ^a (0.8645)	10.46 ^a (1.0591)	18.12 ^a (1.2814)	19.36 ^a (1.3087)	20.24 ^a (1.3271)
0.20	1.16 ^b (0.3344)	2.16 ^b (0.4996)	4.18 ^b (0.7143)	6.35 ^b (0.8662)	9.86 ^b (1.0358)	10.48 ^b (1.0599)
0.5	0.00 ^a (0.3010)	0.00 ^a (0.3010)	1.12 ^a (0.3263)	1.12 ^a (0.3263)	2.63 ^b (0.5599)	2.63 ^b (0.5599)
1.0	0.00 ^a (0.3010)	0.00 ^a (0.3010)	0.00 ^a (0.3010)	0.00 ^a (0.3010)	0.00 ^a (0.3010)	1.45 ^a (0.3891)
CD(0.5)	0.0731					

*Mean of Five replications; C- Concentration; T-Time, Figures in parentheses are log transformed values

Table-4 Effect of Tomatine against soil borne plant pathogens

S	Tomatine Conc.	<i>Sclerotium rolfsii</i>		<i>Fusarium solani</i>		<i>Pythium aphanidermatum</i>	
		Mean mycelial growth (cm)	% reduction over control (%)	Mean mycelial growth (cm)	% reduction over control (%)	Mean mycelial growth (cm)	% reduction over control (%)
1	0.2 %	8.06	10.44	7.90	12.22	8.00	11.11
2	0.4 %	7.80	13.33	6.90	23.33	7.80	13.33
3	0.6 %	7.00	22.22	6.06	32.66	7.20	20.00
4	0.8 %	6.40	28.83	5.83	35.22	6.00	33.33
5	1.0 %	5.10	43.33	4.00	55.50	5.60	37.77
6	Control	9.00		9.00		9.00	
CD (P = 0.05)		0.37		0.32		0.48	

Effect of tomatine on egg hatching of *Meloidogyne incognita*

Pure tomatine of the respected quantity (0.10, 0.20 0.50 and 1.0g) was dissolved in a litre of water. Five egg masses of *Meloidogyne incognita* were collected from the pure culture maintained in the glass house using stereo zoom microscope. The egg masses were placed in Syracuse dish containing respective concentrations of tomatine. Observations on hatching of the eggs were made at 6, 12, 18, 24, 30 and 36 hours interval. Unhatched eggs were transferred to distilled water after 36 h and death of the eggs were confirmed.

Effect of Tomatine against soil borne pathogens

In vitro experiments on efficacy of tomatine against soil borne pathogen were tested by poisoned food technique at Department of Plant Pathology, Agricultural College & Research Institute, Madurai during 2014. Soil borne pathogens viz., rhizome rot of turmeric (*Pythium aphanidermatum*), wilt of Tomato (*Fusarium solani*) and stem rot of groundnut (*Sclerotium rolfsii*) were tested. In this experiment tomatine at different concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0% were prepared and tested against the above pathogens by poisoned food technique.

Results and Discussion

Effect of Tomatine on Termites, *Odontotermes wallonensis*

Data collected on the mortality of termites with tomatine is presented in Table 1.

From the table it is observed that there was good progress of mortality, which was 16.33 percent during the initial period of observation and was 46.33, 61.67, 82.00 and 99.00 percent during 2 HAT (Hours After Treatment), 4 HAT, 6 HAT, and 12 HAT respectively in tomatine 1000 ppm. At 1 HAT, tomatine 1000 ppm (16.67 %), 800 ppm (12.33%), 600 ppm (15.33%), 400 ppm (9.00 %), 200 ppm (13.33%) and 100 ppm (9.33%), were on par with each other in recording maximum mortality. The standard check Chlorpyrifos 1000 ppm recorded the mortality of 68.00 percent. At 2 HAT Chlorpyrifos 1000 ppm recorded maximum mortality of 97.67 percent. This was followed by tomatine 1000 ppm (46.33 %), 600 ppm (40.00%) and 800 ppm (36.33%). The same trend was observed up to 6 HAT. But in 12 HAT, the treatments namely tomatine 1000 ppm (99.00%), 800 ppm (98.33%), 600 ppm (97.00%) were significantly on par with each other and with Chlorpyrifos 1000 ppm (100 %) in recording high mortality percent. While comparing the overall mean percent mortality due to tomatine, it was found that tomatine 1000 ppm (61.13 %) and 600 ppm (59.87 %) recorded the highest percent mortality against the workers of *Odontotermes wallonensis*. The present findings are in confirmation with that of Datchinamurthy, *et al.*, [5] who reported that Chlorpyrifos @ 1000 ppm recorded maximum mortality of 98.33 percent at two hours after treatment. Tomatine 1000 ppm caused 61.33 percent mortality to termites (*O.wallonensis*). Toxicity of tomatine against *Helicoverpa zea* and *Spodoptera exigua* already reported [6].

Effect of Tomatine on Root-knot Nematode *Meloidogyne incognita***(i) Effect of tomatine on survival of *Meloidogyne incognita* juveniles (j2)**

The results of the experiment are presented in Table 2 and from the table it is found that tomatine is capable of causing mortality of j2 of *Meloidogyne incognita* at lower concentration (0.2g / lit) within in 24 hrs. Tomatine at 1.08 / lit caused 52.36 percent mortality at 6 Hours after Treatment (HAT) and cent percent mortality was recorded at 36 HAT. A mortality percent of 98.29 was observed at 36 HAT in Tomatine 0.5 g / lit.

Effect of tomatine on egg hatching of *Meloidogyne incognita*

Data collected from the experiment are presented in Table 3 and the results revealed that tomatine is capable of inhibiting hatching of eggs of *Meloidogyne incognita* at lower concentration (0.5g/lit) even after 36 hrs after incubation. Hatching of eggs was nil till 30 HAT in Tomatine 1.0 g / lit and till 12 HAT in Tomatine 0.5 g / lit. The lowest percent egg hatching (1.45%) was observed in Tomatine 1.0 g / lit at 36 HAT. All the tomatine treatments recorded hatching percent ranged from 0.0 percent to 20.24 percent. Tomatine adversely affected hatching of *M. incognita* eggs and survival of juveniles. This is in accordance with the findings of Meher and Gaur (2003) [7] who reported that tomato accessions with higher tomatine content were resistant to root knot nematode *M. incognita*. Similarly, sesamin in aqueous sesame seed extract suppressed root knot nematode in okra and had the lowest infection index [8].

Effect of Tomatine against soil borne plant pathogens

Results of the experiment revealed that among the tomatine concentrations, tomatine 1.0 percent concentration effectively inhibited the mycelial growth of all soil borne pathogens under in vitro by poisoned food technique (Table 4). Lowest mean mycelial growth of 5.10 cm was recorded in tomatine 1.0 % treatment against *S.rolfsii*. The untreated check had the highest mean mycelial growth of 9.00 cm in all the soil borne fungus. The fungus *F.solani* was effectively controlled by tomatine 1.0 % recording lowest mean mycelial growth (4.00 cm). Among the pathogens tested *F.solani* causing wilt of tomato was significantly inhibited and recorded 55.55% reduction over control than *S.rolfsii* (43.33%) and *P.aphanidermatum* (37.77%). The present findings are in confirmation with that of Dow and Callow (1978) [9] who found 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*. Tomatine at 1000 ppm recorded reduced mycelial growth (1.41 cm dia) of *Termitomyces* fungus compared to untreated check (8.17 cm) [10].

Application of research: From the findings of the present study it is concluded that tomatine could be used as an alternate to insecticide, nematicide and fungicide to suppress termites, nematodes and soil borne fungi.

Research Category: Nematodes and soil borne pathogens

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