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TOXICITY AND REPELLENCY OF *Ficus benghalensis* LATEX BASED COMBINATORIAL FORMULATIONS IN *Odontotermes obesus* THE INDIAN WHITE TERMITE

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ABSTRACT

In present research investigations toxicity and repellency in *Ficus benghalensis* plant latexes was seen in Indian white termite (*Odontotermes obesus*). In various anti-termite bioassays crude plant latex and extracts were used in different combinatorial mixtures for treatment of termites. In treatments LD50 values obtained at 24 h were in a range of 11.887– 717.609µg/gm. These have shown significant toxicity and repellency in workers and soldier termites. Addition of inorganic and other materials to latex induced synergistic action against termites. From results it is clear that plant latex based combinatorial formulations have shown very high lethality in *Odontotermes obesus*. This is proved by very low LD 50 values obtained in each case. These latex based formulations might be highly useful in control of not only termites but also for the control of insect pest of field crops and garden trees. These are less hazardous, non persistent, eco-friendly and provide long lasting insect control.

KEYWORDS: *Odontotermes obesus*; *Ficus benghalensis* latexes; combinatorial formulations, toxicity; repellency and synergistic effects.

INTRODUCTION

Termites are major destroyers of field crops, household and building materials. Termite workers and soldier possess cutting and chewing mouthparts that is used to chew commercial wood, fibers, cellulose, sheets, papers, clothes, woolens and mats, and infests green foliages, and cereals stored in go downs. There are about 2000 known termite species in the world. Termites attack a variety of crops at any stage of development and cause great loss to agriculture production. Termite infestation reduces crop yield up to a threshold level.[1] Termites are causing great economic losses annually that is approximately estimated to $30 billion worldwide.[2] Termites show enormous biodiversity throughout the world. There are 3,106 species of termites have been reported worldwide and out of which 337 species found in India. Among them, 35 reported to responsible for significant damage in agricultural crops and buildings. The major damaging species of termites in India are *Odontotermes*, *Coptotermes*, *Heterotermes*, *Microtermes*, *Microcerotermes* and *Trinervitermes*. Among them *Odontotermes* is responsible for major loss infesting crops and building structures.[3]

The Indian white termite, *Odontotermes obesus* (Rambur) (Isoptera: Odontotermitidae), is highly destructive polyphagous insect pest, it lives in huge mounds, feeds on cellulose material and almost anything which contains carbohydrate. Both worker and soldier termites harm non seasoned commercial wood and its formed materials. Whether it is a rural area or an urban domestic site, termite menace is everywhere. Termites are major decomposers of garden leafy biomass and convert ground wood into the soil.[4] Termites dare used in bait formation to catch birds and fish. Termites invade standing dead tree in forests and make huge mounds having gallaries and chamber. They make holes in mounds to control inside temperature to provide cool air to the colony members. *C. oculatus* construct mushroom shaped mounds for thermal regulation within the nests/mounds.[5] Termites belong to family Termitidae build huge great up to 5 m high. Termite mounds have ethnological value as these are used as burying places for dead bodies. Mounds and soil of termites are used as fertilizer, making bricks, geochemical prosperity, pottery and plastering of houses. Termites are used as feed for poultry. Fungus gardens and soil of termiterium in used to culture mushrooms. Colonies are composed of casts: a queen, a king, soldiers and workers. Some species of termite cultivate specialized fungi to digest cellulose. Termites cultivate specialized fungi and other gut symbionts to digest wood cellulose and hemicelluloses.[6] Termites constitute 10% of all animal biomass in the tropics.

There are four major destroyer species of termites found all over the world i.e: (1) subterranean (2) drywood (3) dampwood (4) powderpost. Subterranean termites are responsible for about 95 percent of the damage in all over world. Similarly at the global level in both tropical sub-tropical countries the infestation of drywood termite *Cryptotermes brevis* (Kalotermitidae) is one of the most important wood structural pest in the world. In Tarai belt of Gorakhpur termites menace is seeing in different local regions mainly in crop fields, house hold and forests. In this area the main species of termite is *Odontotermes obesus* (Indian white termite and red termite *Coptotermes* sp).

However, control of termite population in the field, various synthetic pesticides such as chlordane.[7]cypermethrin[8] hydroquinone, and indoxacarb[9] have been used. But all such synthetic pesticides are highly poisonous, as they enter into food chain and kill non target organisms. Due to their longer residual persistence in the environment, these have been banned and its new alternatives are discovered in form of natural pesticides.

Few natural products such as flavonoids[10] sesquiterpenes[11] and thiophenes[12] isolated from different plants species were found effective against termites. In addition, for enhancing the insecticidal potential of plant essential oils and its target specificity, few synergists will be applied in form of poison baits which successfully exploit feeding, tunneling[13] and reproductive behavior in termites.[14] However, for controlling termite attack harmful synthetic chemical pesticides are extensively applied.[15] For controlling termite on crop plants various synthetic pesticides such as cyclodiene[16][17], cypermethrin, hydroquinone and indoxcarb[18] have been used. Dursban spray found highly effective in the management of wood destroying termites.[19] These chemicals put serious deleterious effect on non-targeted biotic and abiotic factors of environment.[20] Though, chemical insecticides are highly effective against termite but they are hazardous to non-target organisms in the ecosystem.[21] It's bound residues persists for longer duration in the environment, and through various trophic levels they entered into the food chain. They are occasionally associated with severe damage to rangeland vegetation, particularly, in degraded arid and semi-arid ecosystems. Present research article investigated toxic and repellent action of *Ficus benghalensis* plant latex and its various formulations on Indian white termite *Odontotermes obesus*.

MATERIALS AND METHODS

Collection of termites

Termite *Odontotermes obesus* was collected from infested logs found at the University Garden of Gorakhpur University, and nearby forest area of eastern Uttar Pradesh, India. Termites removed from plant biomass and logs collected in glass jars (height 24", diameter 10") and kept under complete dark conditions at 28 ± 2 C ^{o}, 75 ± 5 RH for temporary culture. Termites were provided to feed on green leaves.

Isolation of Plant latex

The crude latexes was collected from *Ficus benghalensis* by making small incision with sharp razor on stem from University Garden, it was diluted by addition of equal volume of water to check its coagulation.

Combinatorial formulations

Various ingredients were used in preparation of combinatorial mixtures. These have been explained in table 1 as following. In all preparation of various combinatorial formulations known volumes of latex (w/v) and some natural and other inorganic materials were used was used for testing the synergistic action of plant latex on worker and soldier termites in various bioassays.

Table. 1: *Ficus benghalensis* and other ingredients used in preparation of combinatorial mixtures.

S. No.	Combinatorial Mixtures	Ingredients
1.	S-MLT-A	*Ficus benghalensis* latexes (9 gm) + Coconut oil (17ml) + Terpene oil (17ml) + Glycerol (17 ml) + Sulphur (3 gm) + Water (5 liter)
2.	S-MLT-B	*Ficus benghalensis* latexes(12 gm) + Coconut oil (17ml) + Terpene oil (17ml) + Glycerol (17 ml) + Sulphur (3gm) + Water (5 liter)
3.	S-MLT-C	*Ficus benghalensis* latexes (18 gm) + Coconut oil (50ml) + Terpene oil (50ml) + Glycerol (50 ml) + Sulphur (3gm) + Water (5 liter)
4.	B-MLT-A	*Ficus benghalensis* latexes (9 gm) + Coconut oil (17ml) + Terpene oil (17ml) + Glycerol (17 ml) + Borate (3 gm) + Water (5 liter)
5.	B-MLT-B	*Ficus benghalensis* latexes (12 gm) + Coconut oil (17ml) + Terpene oil (17ml) + Glycerol (17 ml) + Borate (3 gm) + Water (5 liter)
6.	B-MLT-C	*Ficus benghalensis* latexes (18 gm) + Coconut oil (17ml) + Terpene oil (17ml) + Glycerol (17 ml) + Borate (3 gm) + Water (5 liter)
7.	Cu-MLT-A	*Ficus benghalensis* latexes (9 gm) + Coconut oil (17ml) + Terpene oil (17ml) + Glycerol (17 ml) + Copper (3 gm) + Water (5 liter)
8.	Cu-MLT-B	*Ficus benghalensis* latexes (12 gm) + Coconut oil (17ml) + Terpene oil (17ml) + Glycerol (17 ml) + Copper (3 gm) + Water (5 liter)
9.	Cu-MLT-C	*Ficus benghalensis* latexes (18 gm) + Coconut oil (17ml) + Terpene oil (17ml) + Glycerol (17 ml) + Copper (3gm) + Water (5 liter)
10.	Cow-MLT-A	*Ficus benghalensis* latexes (9 gm) + Photoactivated Cow urine

		(10g/L) + Water (5 liter)
11.	Cow-MLT-B	*Ficus benghalensis* latexes (12 gm) + Photoactivated Cow urine (10g/L) + Water (5 liter)
12.	Cow-MLT-C	*Ficus benghalensis* latexes (18 gm) + Photoactivated Cow urine (10g/L) + Water (5 liter)
13.	AQ-MLT	*Ficus benghalensis* latexes (40 gm) + Water (200 ml)
14.	A-MLT	*Ficus benghalensis* latexes (40 gm) + Acetone (200 ml)
15.	H-MLT	*Ficus benghalensis* latexes (40 gm) + Hexane (200 ml)
16.	P-MLT	*Ficus benghalensis* latexes (40 gm) + Petroleum Ether (200 ml)
17.	EA-MLT	*Ficus benghalensis* latexes (40 gm) + Ethyl Alcohol (200 ml)
18.	Malathion	Malathion powder (7.5 gm/liter) + Water (5 liter)
19.	Fipronil	Fipronil powder (7.5 gm/liter) + Water (5 liter)
20.	Thiamethoxam	Thiamethoxam powder (7.5 gm/liter) + Water (5 liter)

Toxicity bioassays

Toxicity bioassays were conducted in the laboratory and LD_{50} of each mixture was determined separately. Toxicity experiments were conducted by using increasing concentration of each combinatorial mixture i.e. 10, 20, 40, 60, 80, and 100µl. For this purpose various combinatorial mixtures were coated on cellulose paper (size 1X1 cm^2), air dried and kept in the central area of Petri dish. Both tests and controls were tested in six replicate for each mixture. In each Petri dishes 10 termites were released and termite mortality and survival were observed at different periods. Dead termites were separated from the alive on the basis of body movement. For comparison parallel controls were set in each experiment. LD_{50} values were determined by Probit method (Finney, 1971). LD_{50} values were calculated in µg/gm body weight of termites at 16 h.

Feeding inhibition and Repellency

For evaluation of repellency action various concentrations of each combinatorial mixture were coated on Whatmann paper no. 1 (1X1 cm^2). These pre-coated dried strips were employed in the centre of Petri dishes (42mm diameter).Ten worker termites were released in each Petri dish to observe the repellent activity. Same experiment was repeated six times to maintain the precision and accuracy in repellent activity. Numbers of repelled insects which are away from scented zone or remain scare off from scented zone were counted.

Statistical Analysis

Standard deviations chi-square, t-significance, correlation, and ANOVA were calculated from the means of two replicate using three equal sub samples from each replicate by using method of Sokal and (Rohlf and Sokal, 1973). In the experiments analysis of variance (ANOVA) was done whenever two means were obtained at a multiple test range and $p < 0.05$

probability level. The LD50 after 24 hrs of exposure were calculated by applying POLO program (Russell, 1977).

RESULTS

Toxicity bioassay

All the combinatorial fractions of *Ficus benghalensis* have shown very high insecticidal activity. It is proved by very low LD_{50} values obtained in each mixture i.e. 368.529, 521.701, 716.570, 323.034, 421.634, 670.349, 295.197, 548.854, 717.439, 323.776, 555.295, 717.609, 27.719, 19.078, 11.887, 25.634 and 13.902 µg/gm body weight of termites for S-MLT-A, S-MLT-B, S-MLT-C, B-MLT-A, B-MLT-B, B-MLT-C, Cu-MLT-A, Cu-MLT-B, Cu-MLT-C, Cow-MLT-A, Cow-MLT-B, Cow-MLT-C, AQ-MLT, A-MLT, H-MLT, P-MLT and EA-MLT respectively. Besides this, toxixity of synthetic pesticides was also determined which showed 67.026, 27.891 and 50.255 µg/gm LD_{50} for malathion, fipronil and thiamethoxam The lowest LD_{50} was obtained in H-MLT mixture i.e. 11.887 µg/gm body weight of termite. The upper and lower confidence limits obtained were ranged from 550.006-276.516, 1000.726-342.461, 914.105-587.428, 757.042-191.141, 582.976-312.699, 992.442-495.051, 494.773-195.430, 1049.929-368.438, 927.577-538.238, 479.273-238.499, 1402.248-343.231, 1353.574-481.076, 48.771-19.287, 24.405-15.488, 37.163-6.857, 39.564-19.464, 19.602-10.885, 95.511-52.909, 58.871-18.100 and 63.329-41.833 for S-MLT-A, S-MLT-B, S-MLT-C, B-MLT-A, B-MLT-B, B-MLT-C, Cu-MLT-A, Cu-MLT-B, Cu-MLT-C, Cow-MLT-A, Cow-MLT-B, Cow-MLT-C, AQ-MLT, A-MLT, H-MLT, P-MLT, EA- MLT, malathion, fipronil and thiamethoxam respectively (Table-2). These combinatorial formulations much better toxicity than synthetic pesticides. These have shown time and dose dependent toxicity in termites. Besides this Chi- Square, Slope function, Degree of freedom and Heterogeneity were also calculated to find upper and lower limits of toxicity and its level significance (significant at < 0.05).

Feeding inhibition and Repellency

Combinatorial mixtures of *Ficus benghalensis have* shown very high repellent activity against termites. The percent repellency observed in highest repellency was obtained in S-MLT-A (76%), Cu-MLT-A (76%), Cow-MLT-B(76%) and P-MLT (73%) (Table-3). Similarly photo-activated cow urine has shown very high percent repellency i.e. Cow-MLT-C (80%) against termites. Contrary to this, inorganic pesticides have shown very low repellent activity against termites (Table-3).

Table-2: Toxicity Experiment.

S. N.	Name ofLatex/Combinatorial Mixture	LD50µg /gm	LD40µg/gm	LD20µg /gm	0.95 confidence limit UCL-LCL	Chi-Square	Slope function	Degree of freedom	Heterogeneity
1.	S-MLT-A	368.529	147.41	73.70	550.006-276.516	4.0815	-0.118078	4	1.0204
2.	S-MLT-B	521.701	208.68	104.34	1000.726-342.461	10.476	-0.134144	4	2.6191
3.	S-MLT-C	716.570	286.62	143.31	914.105-587.428	3.996	-0.132971	4	0.999
4.	B-MLT-A	323.034	129.21	64.60	757.042-191.141	10.497	-0.108045	4	2.6244
5.	B-MLT-B	421.634	168.65	84.32	582.976-312.699	5.6838	-0.131763	4	1.4210
6	B-MLT-C	670.349	268.13	134.06	992.442-495.051	5.5602	-0.139478	4	1.3901
7	Cu-MLT-A	295.197	118.07	59.03	494.773-195.430	7.2238	-0.109047	4	1.8060
8	Cu-MLT-B	548.854	219.54	109.77	1049.929-368.438	7.9930	-0.126025	4	1.9983
9	Cu-MLT-C	717.439	286.97	143.48	927.577-538.238	3.231	-0.129877	4	0.808
10	Cow-MLT-A	323.776	129.51	64.75	479.273-238.499	4.1271	-0.110143	4	1.0318
11	Cow-MLT-B	555.295	222.11	111.05	1402.248-343.231	10.558	-0.124327	4	2.6396
12	Cow-MLT-C	717.609	287.04	143.52	1353.574-481.076	8.4164	-0.133737	4	2.1041
13	AQ-MLT	27.719	11.08	5.54	48.771-19.287	6.7998	-0.643967	4	1.7000
14	A-MLT	19.078	7.63	3.81	24.405-15.488	1.693	-0.542454	4	0.423
15	H-MLT	11.887	4.75	2.37	37.163-6.857	11.181	-0.417258	4	2.7953
16	P-MLT	25.634	10.25	5.12	39.564-19.464	1.793	-0.535656	4	0.448
17.	EA-MLT	13.902	5.56	2.78	19.602-10.885	3.592	-0.430078	4	0.898
18.	Malathion	67.026	26.81	13.40	95.511-52.909	2.083	-0.875498	4	0.521
19.	Fipronil	27.891	11.15	5.57	58.871-18.100	11.839	-0.715511	4	2.9597
20.	Thiamethoxam	50.255	20.10	10.05	63.329-41.833	2.844	-0.872107	4	0.711

Table-3: Percent repellency obtained in different combinatorial mixtures against termites.

S.No.	Mixture	Doses (µl)	Percentage	Mean ± SE
1.	S-MLT-A	10	23	2.33± 0.272
		20	30	3.0± 0.471
		40	40	4.0± 0.471
		60	50	5.0± 0.471
		80	60	6.00± 0.471
		100	76	7.66± 0.272
2.	S-MLT-B	10	23	2.33 ± 0.272
		20	33	3.33 ± 0.272
		40	30	3.0 ± 0.471
		60	46	4.66 ± 0.272
		80	53	5.33 ± 0.272
		100	73	7.33 ± 0.272
3.	S-MLT-C	10	23	2.33 ± 0.272
		20	33	3.33 ± 0.272
		40	60	6.0 ± 0.720
		60	36	3.66± 0.471
		80	63	6.33± 0.471
		100	73	7.33 ± 0.272
4.	B-MLT-A	10	10	1.00± 0.471
		20	23	2.33± 0.272
		40	36	3.66± 0.272

		60 80 100	50 56 70	5.0± 0.471 5.66± 0.272 7.0± 0.471
5.	B-MLT-B	10 20 40 60 80 100	10 30 40 53 63 73	1.0± 0.471 3.0± 0.471 4.0± 0.471 5.33± 0.272 6.33± 0.272 7.33± 0.272
6	B-MLT-C	10 20 40 60 80 100	10 20 33 46 60 76	1.0± 0.471 2.0± 0.471 3.33± 0.272 4.66± 0.272 6.0± 0.471 7.66± 0.272
7	Cu-MLT-A	10 20 40 60 80 100	10 20 26 40 56 76	1.00± 0.471 2.0± 0.471 2.66± 0.720 4.0± 0.471 5.66± 0.720 7.33± 0.272
8	Cu-MLT-B	10 20 40 60 80 100	16 23 33 43 53 70	1.66± 0.272 2.33± 0.544 3.33± 0.272 4.33± 0.272 5.33± 0.272 7.0± 0.471
9	Cu-MLT-C	10 20 40 60 80 100	20 33 40 53 56 70	2.0± 0.471 3.33± 0.272 4.0± 0.471 5.33± 0.272 5.66± 0.720 7.0± 0.471
10	Cow-MLT-A	10 20 40 60 80 100	16 30 43 50 63 76	1.66± 0.272 3.00± 0.471 4.33± 0.272 5.0± 0.471 6.33± 0.272 7.66± 0.272
11	Cow-MLT-B	10 20 40 60 80 100	20 23 30 40 56 76	2.0± 0.471 2.33± 0.272 3.0± 0.471 4.00± 0.471 5.66± 0.272 7.66± 0.272
12	Cow-MLT-C	10 20 40 60 80	20 30 40 50 76	2.0± 0.471 3.0± 0.471 4.0± 0.471 5.0± 0.471 7.66± 272

		100	80	8.0± 0.471
13	AQ-MLT	10 20 40 60 80 100	26 33 40 50 60 73	2.66± 0.272 3.33± 0.272 4.0± 0.471 5.0± 0.471 6.00± 0.471 7.33± 0.272
14	A-MLT	10 20 40 60 80 100	20 30 40 50 60 66	2.0± 0.471 3.0± 0.471 4.0± 0.471 5.0± 0.471 6.00± 0.471 6.66± 0.720
15	H-MLT	10 20 40 60 80 100	20 30 40 50 50 70	2.0± 0.471 3.0± 0.471 4.0± 0.471 5.00± 0.471 5.00± 0.471 7.0± 0.471
16	P-MLT	10 20 40 60 80 100	23 30 40 50 63 73	2.33± 0.272 3.00± 0.471 4.00± 0.471 5.0± 0.471 6.33± 0.272 7.33± 0.272
17.	EA- MLT	10 20 40 60 80 100	06 13 20 33 43 63	0.66± 0.272 1.33± 0.272 2.0± 00 3.33± 0.272 4.33± 0.272 6.33± 0.272
18.	Malathion	10 20 40 60 80 100	06 20 16 26 40 53	0.66± 0.272 2.0± 00 1.66± 0.272 2.66± 0.272 4.0± 00 5.33± 0.544
19.	Fipronil	10 20 40 60 80 100	10 16 23 26 46 66	1.00± 0.0 1.66± 0.272 2.33± 0.272 2.66± 0.544 4.66± 0.272 6.66± 0.272
20.	Thiamethoxam	10 20 40 60 80 100	13 20 26 36 40 66	1.33± 0.272 2.0± 00 2.66± 0.272 3.66± 0.272 4.00± 0.471 6.66± 0.272

DISCUSSION

In the present study, *Ficus benghalensis* plant latex based formulations have shown a significant toxicity and repellency against termite workers of *Odontotermes obesus*. In toxicity bioassays crude latex, latex fractions and its various combinatorial formulations have shown very high lethality in termites, which is proved by very low LD 50 values obtained (Table 1 & 2). In various experiments certain additives mixed to the latex have improved the termiticidal potential and showed synergistic action against termites. LD 50 values obtained in toxicity bioassays were found in a range of 11.887– 717.609µg/gm (Table 2). It is highly noticeable that *Ficus benghalensis* fractions cause high lethality and show strong anti-feedant and repellent activity in termites. Values of the heterogeneity less than 4.0 denotes that in the replicate test of random sample, the dose response time fall within 95% confidence limit and thus the model fits the data adequately. Analyses of experimental data clear that *Ficus benghalensis latex* contains highly toxic components which display high toxicity that is dose and time dependent.

Similar but lower mortality toxicity and repellency was obtained in menadione in *C. formosanus* termiticidal 6 to 600.[22] In slow-acting toxic bait a moderate toxicity and repellent was noted in termites when wood or soil is treated with *Pseudomonas aeruginosa* of 10^9 CFU ml^{-1} concentrations.[23] *G. sulphureus* extract also showed similar insecticidal and repellent activity against M. gilvus, it was certainly due to presence of bioorganic compounds.[24] Termiticidal, repellent and antifeedant activities were also noted in extracts from *Pongamia pinnata* against *Coptotermes heimi* (Wasmann). More specifically, ethyl acetate-based extracts showed maximum repellency (100%) followed by petroleum ether extracts at 10 mg/ml and ethyl acetate at 5 mg/ml after 60 min of termite exposure .[25]

In addition, latex based formulations have shown deleterious effects on insects like anti-feedant, growth and reproductive inhibitory activities.[26] *C. procera* latex displayed toxic effects against *Culex quinquefasciat*,[27] *Sarcophaga haemorrhoidalis*[28] and *Musca domestica*,[29],[30] *Anopheles stephens*.[31] It successfully inhibits gonotrophic cycles, oviposition[32], egg hatching and larval development in *Aedes aegypti*.[33] Latexes from Asclepias humistrata (sandhill milkweed)[34], *Calotropis procera* and *Ficus racemosa* have shown larvicidal activity in insects.[35] *Parahancornia ampa* (Apocynaceae), latex shows effect on post embryonic development of blowfly *Chrysomya megacephala* (Diptera: Calliphoridae).[36] secMLTion an antitermite formulation shows toxic and repellent effect *Nasutitermes* spp. soldier.[37] A similar lethal dose (LD50) was obtained in *C. curvignathus* was 945 mg/kg, whereas LD50 value for *C. gestroi* was 1,102 mg/kg.[38] Though fipronil is a strong synthetic pesticide ad causes high mortality in termites but it is harmful for non target organisms. It's LD50s was found between 2.59- and 2.91 in tested termites workers. Though fipronil act as repellent to termite workers.[39] But it is harmful to environment and human health.[40] Hence natural pesticides must be preferred to save the environment and non target organisms. Therefore, use of synthetic pesticides should be minimized.

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CONFLICTS OF INTERES

The author declares no conflicts of interest regarding the publication of this paper.

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