Ficus benghalensis latex-based combinatorial formulations and its use for control of wood weight loss and infestation caused by Indian forest termite *Odontotermes obesus*

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Abstract

In the present investigation, various bioassays were conducted to evaluate the anti-termite efficacy of plant latex-based formulations to control population of Indian white termite in sub-tropical soil. Crude latex and its combinatorial mixtures have shown significant anti-termites efficacy against. Indian forest termite (*Odontotermes obesus*). The LD_{50} values were found in range of 11.887–717.609 µg/g. In subsequent bioassays, crude latex and combinatorial mixtures have shown high toxicity against termites, that is, LD_{50} was obtained in range of 717.609 µg/g for CU-MLT-C against *O. obesus.* It was observed in thread binding assays, poison baits, and direct spray in field and garden saplings. Combinatorial mixtures of plant latex have shown synergistic activity against termites. Bait formulation (0.125% w/v) was also used which significantly cut down infestation of crop field termite in various crop field. Combinatorial mixtures CU-MLT-C repelled (80%) termites to the opposite arm in Y-shaped tube. Besides this, *Ficus benghalensis* latex has shown significant reduction (86%) in termite infestation in garden saplings. Same combinatorial mixtures were also used for wood seasoning of solid wood sticks and hollow wood sticks which gave very good results as test wood sticks have shown significantly 65–86% reductions in termite infestation. Plant latex-based combinatorial mixtures displayed significant protection to garden saplings and resisted against wood invasion in field experiments. In field experiments, significant protection was seen in maize and millet crops after using latex-based combinatorial mixtures.

Key words: *Ficus benghalensis*, Latexes, *Odontotermes obesus*, Termiticides

INTRODUCTION

Termites are highly destructive polyphagous insect pests. These attack crop plants such as sugarcane, maize, and wheat[1,2] and cause 50–100% yield losses in these crops.[3,4] Termites severely infest garden trees, wood, building materials, stored products, papers and clothes, and cause enormous economic losses to them. Termites infest at various stages of plant growth. Termites play a key role as ecosystem engineers in numerous ecological processes, though they degrade the wood in tropical dry forests, particularly the level of the crown canopy. Termites invade standing dead trees in forests. Termites make huge mounds for colonization and protection from environmental stress and hide from predators.[5] Termites also play a key role in the decomposition of above ground dead wood, mediating the incorporation of suspended and standing dead wood into the soil.[6]

However, for controlling termite attack, harmful synthetic chemical pesticides are extensively applied.[7] For controlling termite on crop plants, various synthetic pesticides such as cyclodiene,[8,9] cypermethrin,[10] hydroquinone, and indoxcarb[11] have been used. Dursban spray found highly effective in the management of wood destroying termites[12]

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Thiamethoxam shows high mortality in Asian subterranean termite *Coptotermes gestroi* workers after 1–3 days exposure time.[13] These chemicals put serious deleterious effects on non-targeted biotic and abiotic factors of environment.[14] Although, chemical insecticides are highly effective against termite, they are hazardous to non-target organisms in the ecosystem.[15] It is 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. However, to replace these highly toxic synthetic chemical fungal insecticides, bioinsecticides and botanicals were found suitable.[16,17]

Plant latexes and some botanicals from plant species *Calotropis procera, Ipomoea fistulosa, Maesa lanceolata, Croton macrostachyus, Targets minuta, Datura stramonium,* and *Azadirachta indica* are used for the management of termites.[18-20] There are many plant natural products which act effectively against termites in crop fields and gardens. Imidacloprid shows higher water solubility can be used with less water-soluble termiticides in baits.[21] Some botanical insecticides, such as *A. indica* oil, severely affect the immune system of various insect species.[22] Serine proteases are metabolic enzymes found in the midgut of red palm weevil, *Rhynchophorus ferrugineus* Olivier, they act as feeding inhibitors.[23] Latex is a type of sticky endogenous fluids derived from diverse plants. It contains unique compounds mainly defense molecules which protect plants against microbes and herbivores.[24] Many plants secrete latex exudates to protect from herbivores (kotan kono 20k).[25] Latex of *Euphorbia obtusifolia* obstructs mitochondrial functions in mammals.[26] There are so natural products, that is, extracts, latexes, essential oils, and bio-organic compounds from several plant species such as *Diospyros sylvatica*[27] *C. procera*, *A. indica,* and *M. lanceolata* were found to be effective in controlling termite.[9] Neem-based preparations are used to control and repel termites in crop fields.[28,29] Few natural products such as flavonoids,[30] sesquiterpenes,[31] and thiophenes[32] isolated from different plants species were found effective against termites. The present article signifies insecticidal potential and target specificity of plant latexes and its synergists effects to obstruct feeding, tunneling,[33] and reproductive behavior in termites. This article also suggests development of promising and highly effective low-cost formulations.

MATERIALS AND METHODS

Collection of Termites

Termite, *Odontotermes obesus* (Rambur), both soldier and workers were collected from the University garden and a temporary culture was maintained in the laboratory at 37°C ± 2°C at 80% RH by providing green leaves as food material. Termite culture was protected from light illumination, using black paper sheets wrapped around the glass containers (12 × 9 inch). Insects were provided fresh food material and it was changed regularly after 24 h. Termites were provided fresh food material and it was changed regularly after 24 h. The LD_{50} after 24 h of exposure to each was calculated using Probit analysis tested using the method of Finney (1971).

Latex Collection and its Anti-Termitic Effects

Latex was collected from *Ficus benghalensis* Banyan or Bargad Tree (Family: Moraceae) located in Deen Dayal Upadhyaya Gorakhpur University Garden, living specimen is photographed [Photoplate 1a and b]. This specimen was authenticated by an expert of botany and help was taken from Taxonomy of Indian Angiosperms. The herbarium specimen is healthy and preserved in Botanical garden of Gorakhpur University for future references. This plant is extensively used for shade, nutritional, and therapeutic purposes by local people not only in India but also in Southeast Asia. Fresh latex was collected by making a incision or cut mark with knife over tree trunk. Collected plant latex samples were lyophilized and powdered in vacuum in cold. Lyophilized latex was extracted with different solvents by changing the polarity. Active fractions from the latex were portioned between different solvents on the basis of their polarity. For better fractionation, solvent extraction was performed using polar and non-polar solvents. Latex was allowed to evaporate in a SpeedVac vacuum concentrators to get residue. It was dried and weighed and re-dissolved in known volume of distilled water. Dissolved residues were stored in cold at 4°C for experimental purpose. All chemicals used in this study were purchased from CDH-laboratory chemicals suppliers India supplied by Eastern Scientific Company, Gorakhpur.

Determination of LD Values in Extracts and Combinatorial Mixtures

Toxicity bioassay

For evaluation of dose response relationship of different latex extracts, different doses (w/v), that is, 8, 16, 32, 64, 128, 256, 512, 768, and 1024 µg of different latex extracts were loaded on separate Whatmann paper strips (1 × 1 cm^2) and air dried to remove the solvent. These pre-coated solvent free strips were placed in the center of separate Petri dishes (42 mm diameter) as tests and uncoated as control. Twenty worker termites

Photoplate 1: (a and b) Vegetative parts of *Ficus benghalensis* plant

were released in the Petri dish to observe the mortality. After setting the experiment, green leaves were provided as food for both tests and control insects and containers were covered with black paper sheets. Mortality was recorded on the basis of dead and living termites and observations were made in triplicate for each extract and pure compounds up to 24 h. Insects were treated as dead when become immobile and have shown no further activity to the external stimuli. TheLD_{50} after 24 h of exposure to each was calculated using Probit analysis tested using the method of Finney.

Repellency bioassay

Repellent responses were observed in a glass Y-tube olfactometer using serial concentrations 3.5, 7.0, 14, 21, 28, and 35 µg of different crude latex/fractions/formulations loaded on separate Whatman paper strips (1 × 1 cm^2) and air dried to remove the solvent. These pre-coated solvent free strips were placed in the right arm of Y-tube olfactometer (16 mm diameter × 90 cm length) as tests, while similar strips uncoated were placed in the left arm as control. Twenty worker termites were released inside the opposite tri-arm to observe the repellent activity. After introduction of termites, tube openings were closed by Teflon tape and number of termites oriented toward uncoated strips or non-scented area were counted as repelled. Individuals that did not enter at least one of the arms were scored as unresponsive. Tests were conducted for 18 h at 27°C temperature. Same tests were conducted after reversing the arms to test directional bias. A Chi-square test was used to compare the number of termites responding to the olfaction generated by *C. procera* active fractions. Number of repelled termites in presence of each latex extract were counted after 30 min of treatment with five different concentrations (1.0, 2.0, 4.0, 8.0, and 16.0 µg/g) of each latex extract which were used. The ED50values that repelled 50% of termite population were calculated.

Combinatorial Formulations

Ficus benghalensis latex and other ingredients were used in the preparation of combinatorial mixtures. The details of all combinatorial mixtures are mention in table 1.

FIELD EXPERIMENTS

Wood Seasoning

In wood seasoning experiments, various types of solid and hollow bamboo wood sticks were planted in crop fields and garden soil, etc. These wood sticks were treated with various combinatorial mixtures.

Treatment of solid wood sticks

In these experiments, dried solid wood sticks of Sagwan (*Tectona grandis*) (Family: *Lamiaceae*) having 1 ft and ~50 mm average diameter were used. These solid wood sticks were seasoned with different combinatorial mixtures of *Ficus benghalensis* for 24 h. Following sets were made:

Set no I

In set no.1, solid wood sticks were submerged for 24 h in S-MLT-A, S-MLT-B, and S-MLT-C mixtures for its seasoning. These were prepared using 03 g sulfur as an inorganic substance [Table 1]. For each test mixture, experiments were continued up to 6 months and infestation was noted down.

Set no II

In another set of experiment, solid wood sticks were seasoned with B-MLT-A, B-MLT-B, and B-MLT-C combinatorial mixtures. These were prepared by mixing 03 g borate powder while rest of the content were same as above [Table 1].

Set no III

In third experiment, wood sticks were seasoned with C-MLT-A, C-MLT-B, and C-MLT-C mixtures which were prepared by mixing 03 g of copper sulfate. Other components were similar as above [Table 1].

Set no IV

In fourth set of experiment, solid wood sticks were seasoned with CU-MLT-A, CU-MLT-B, and CU-MLT-C mixtures [Table 1].

Set no V

In fifth set of experiment, inorganic compounds such as malathion, fipronil, and thiamethoxam were used for the wood seasoning [Table 1].

All the above seasoned wood sticks were planted inside soil by making pits of 0.75 ft depth. Separate pits were used for each stick. For comparison unseasoned, wood sticks of similar size and diameter were used as control. For observations, one wood stick each from control and test was dug out after 30 days interval and weighed. These wood sticks were marked with paint to identify. Experiments were continued up to 6 months [Photoplate 2].

Treatment of solid wood sticks

In these experiment, wood seasoning was done by dipping wood stick overnight in a combinatorial mixture of *F. benghalensis* Latex, *F. benghalensis* Latex + sulfur, and *F. benghalensis* Latex +sulfur + cow urine. Sticks were 3 ft in length and 1.25 inches in diameter were planted in soil at one meter distance from each. In each row, the six stick of *Tectona grandis* were planted. Control was also planted in a row with fixed solid wood stick as control. After one month, wood weight loss, % of infestation and termite population were observed in bamboo sticks and these observation was follow up to six month. Besides above experiments, hollow bamboo wood sticks were also used for wood seasoning to observe the tunneling activity of termites in presence of various mixtures [Photoplate 3].

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 g) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Sulfur (3 g) + Water (5 l)
2.	S-MLT-B	*Ficus benghalensis* latexes (12 g) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Sulfur (3 g) + Water (5 l)
3.	S-MLT-C	*Ficus benghalensis* latexes (18 g) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol (50 ml) + Sulfur (3 g) + Water (5 l)
4.	B-MLT-A	*Ficus benghalensis* latexes (9 g) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Borate (3 g) + Water (5 l)
5.	B-MLT-B	*Ficus benghalensis* latexes (12 g) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Borate (3 g) + Water (5 l)
6.	B-MLT-C	*Ficus benghalensis* latexes (18 g) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Borate (3 g) + Water (5 l)
7.	C-MLT-A	*Ficus benghalensis* latexes (9 g) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Copper (3 g) + Water (5 l)
8.	C-MLT-B	*Ficus benghalensis* latexes peels (12 g) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Copper (3 g) + Water (5 l)
9.	C-MLT-C	*Ficus benghalensis* latexes (18 g) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Copper (3 g) + Water (5 l)
10.	CU-MLT-A	*Ficus benghalensis* latexes (9 g) + Photoactivated Cow urine (10 g/L) + Water (5 l)
11.	CU-MLT-B	*Ficus benghalensis* latexes (12 g) + Photoactivated Cow urine (10 g/L) + Water (5 l)
12.	CU-MLT-C	*Ficus benghalensis* latexes (18 g) + Photo activated Cow urine (10 g/L) + Water (5 l)
13.	AQ-MLT	*Ficus benghalensis* latexes (40 g) + Water (200 ml)
14.	A-MLT	*Ficus benghalensis* latexes (40 g) + Acetone (200 ml)
15.	H-MLT	*Ficus benghalensis* latexes (40 g) + Hexane (200 ml)
16.	P-MLT	*Ficus benghalensis* latexes (40 g) + Petroleum Ether (200 ml)
17.	EA-MLT	*Ficus benghalensis* latexes (40 g) + Ethyl Alcohol (200 ml)
18.	Malathion	Malathion powder (7.5 g/l) + Water (5 l)
19.	Fipronil	Fipronil powder (7.5 g/l) + Water (5 l)
20.	Thiomethaxam	Thiomethaxam powder (7.5 g/l) + Water (5 l)

Photoplate 2: Showing solid wood sticks experiment set up

Photoplate 3: Showing solid wood sticks experiment set up

Treatment of hollow wood sticks

For evaluation of toxic and repellent properties of *F. benghalensis* combinatorial mixtures, bamboo wood sticks (1 ft and ~50 mm diameter) were used for the treatment. For this purpose, obstructions present at each internode were made by iron rod to fill agar impregnated combinatorial mixture [Photoplate 4].

Photoplate 4: Showing hollow wood sticks experiment set up

Set no I

In the first experiment MLT-1, MLT-2, RMLT-3, MLT-4, MLT-5, and MLT-6 combinatorial mixture (prepared from Latex [9 g, 10 g, 12 g, 14 g, 16 g, and 18 g, respectively]+Ash [100g]) [Table 2].

Set no II

In second experiment, hollow bamboo sticks were treated with REAT-CU-1, REAT-CU-2, MLT-CU-3, MLT-CU-4, MLT-CU-5, and MLT-CU-6 mixture (prepared from Latex [9 g, 10 g, 12 g, 14 g, 16 g, and 18 g, respectively] + Cow Urine [90 ml, 100 ml, 120 ml, 140 ml, 160 ml, and 180 ml, respectively] + Ash [100 g]) [Table 2].

Set no III

In third experiment, hollow bamboo sticks were treated with REAT-CU-CD -1, REAT-CU-CD-2, MLT-CU-CD-3, MLT-CU-CD-4, MLT-CU-C-5, and MLT-CU-CD-6 mixtures (prepared from Latex (9 g, 10 g, 12 g, 14 g, 16 g, and 18 g, respectively)+ Cow Urine (90 ml, 100 ml, 120 ml, 140 ml, 160 ml, and 180 ml, respectively)+ Cow Dung [90 g, 100 g, 120 g, 140 g, 160 g, and 180 g, respectively] +Ash [100 g]) [Table 2].

Set no IV

In this experiment, control was prepared with the mixing of cow urine and cow dung. (Cow Urine [90 ml, 100 ml, 120 ml, 140 ml, 160 ml, and 180 ml, respectively]+Cow Dung [90 g, 100 g, 120 g, 140 g, 160 g, and 180 g, respectively]+ Ash [100 g]) [Table 2].

Set no V

In this experiment, fipronil was mixed with different amount of cow dung (Fipronil [9 g, 10 g, 12 g, 14 g, 16 g, and 18 g, respectively] +Cow Dung [90 g, 100 g, 120 g, 140 g, 160 g, and 180 g, respectively]+ Ash [100 g]) [Table 2].

For above experiment, Maize crop (Krishna cultiver) was sown on in middle of March 2021 in the field. For this purposes, loam soil was selected, field was prepared by three consecutive ploughing and watered after 3 days. Field was made weed free and soil texture fine. The field size was 8 × 3 m (W × L); both control and test are arranged in the form of six regular replicate planted at a distance of 1 m. Stick to stick difference was kept 1.5 feet. A control was set using six PVC pipes (1 ft length and 1.25 inch diameter). PVC pipes were planted in the soil to 3 inches (up to humus region of the soil) so that active ingredient could come out and diffuse in the soil and its intake become possible to the plants through absorption.

Humidity, temperature and day of sowing were noted down. All climatic regimes were continuously noted as additional parameters. Average humidity noted was 64–70%, the day temperature noted was 23°C, and night temperature was 12.3°C, Dew was moderate, Day period 6.05 AM–6.45 PM, and sowing day was rainy cloudy day. In the end, % response of ingredient (% control), % infestation, and wood weight loss were enumerated that six random soil sample are investigated after 1 month interval. In the end of experiment crop loss, % yield and termite infestation versus termiticidal efficacy of latex were determined/calculated.

Treatment of hollow wood sticks

In these experiment, Wood seasoning was done by fixed *F. benghalensis* latex, *F. benghalensis* Latex + sulfur, and *F. benghalensis* Latex +sulfur + cow urine for overnight. Stick size was 3 feet in length and 1.25 inches in diameter were planted in soil at one meter distance from each. In each row, the six stick of bamboo were planted. Control was also planted in a row with fixed solid wood stick as control. After one month, wood weight loss, % of infestation and termite population were observed in bamboo sticks and these observation was follow up to six month [Photoplate 5].

Poison Bait Experiment

For controlling field, termite homemade baits were prepared using Multani earth and organic ash + loam soil (2:1:2). Disk shape baits were prepared using iron bottle lid of 22 mm diameter. In the cavity of it, *F. benghalensis* latex ingredient was filled, these was air dried inside room condition. In first set, above soil mixture was used and the active ingredient was *F. benghalensis* latex 1.4 g. In second set, 1.4 g *F. benghalensis* latex added with 1.25 g sulfur in each bait. In third set, bait is prepared by 1.4 g *F. benghalensis* latex, 1.25 g sulfur in each bait, and 10 ml cow urine in each bait. Similar field size was prepared and maize seed was used for sowing. Soil condition was same as used in the past experiment. Experiments were conducted for 6 months. Millet and maize were sown in separate fields. Termite number was counted from each wood stick just completion of 30 days. Both plastic pipe and hollow wood sticks were used for testing the infestation. Dried wood sticks were weighed for weight loss [Photoplate 6].

Table 2: *Ficus benghalensis* and other ingredients used in preparation of combinatorial mixtures

S. No.	Combinatorial mixtures	Ingredients
1.	MLST-1	Latexes (9 g)+Ash (100 g)
2.	MLST-2	Latexes (10 g)+Ash (100 g)
3.	MLST-3	Latexes (12 g)+Ash (100 g)
4.	MLST-4	Latexes (14 g)+Ash (100 g)
5.	MLST-5	Latexes (16 g)+Ash (100 g)
6.	MLST-6	Latexes (18 g)+Ash (100 g)
7.	ML-CU-1	Latexes (9 g)+Cow Urine (90 ml)+Ash (100 g)
8.	ML-CU -2	Latexes 10 g)+Cow Urine (100 ml)+Ash (100 g)
9.	ML-CU -3	Latexes (12 g)+Cow Urine (120 ml)+Ash (100 g)
10.	ML-CU -4	Latexes (14 g)+Cow Urine (140 ml)+Ash (100 g)
11.	ML-CU -5	Latexes (16 g)+Cow Urine (160 ml)+Ash (100 g)
12.	ML-CU -6	Latexes (18 g)+Cow Urine (180 ml)+Ash (100 g)
13.	ML-CU-CD -1	Latexes 9 g)+ Cow Urine (90ml)+Cow Dung (90 g)+ Ash (100 g)
14.	ML-CU-CD -2	Latexes 10 g)+ Cow Urine (100ml)+Cow Dung (100 g)+ Ash (100 g)
15.	ML-CU-CD -3	Latexes (12 g)+ Cow Urine (120ml)+Cow Dung (120 g)+ Ash (100 g)
16.	ML-CU-CD -4	Latexes (14 g)+ Cow Urine (140ml)+Cow Dung (140 g)+ Ash (100 g)
17.	ML-CU-CD -5	Latexes (16 g)+ Cow Urine (160ml)+Cow Dung (160 g)+ Ash (100 g)
18.	ML-CU-CD -6	Latexes (18 g)+ Cow Urine (1800ml)+Cow Dung (180 g)+ Ash (100 g)
19.	Control-1(PVC)	Cow Urine (90ml)+Cow Dung (90 g)
20.	Control-2(PVC)	Cow Urine (100ml)+Cow Dung (100 g)
21.	Control-3(PVC)	Cow Urine (120ml)+Cow Dung (120 g)
22.	Control-4(PVC)	Cow Urine (140ml)+Cow Dung (140 g)
23.	Control-5(PVC)	Cow Urine (160ml)+Cow Dung (160 g)
24.	Control-6(PVC)	Cow Urine (180ml)+Cow Dung (180 g)
25.	Control-1	Fipronil (9 g) +Cow Dung (90 g)+ Ash (100 g)
26.	Control-2	Fipronil (10 g)+Cow Dung (100 g)+ Ash (100 g)
27.	Control-3	Fipronil (12 g)+ Cow Dung (120 g)+ Ash (100 g)
28.	Control-4	Fipronil (14 g)+ Cow Dung (140 g)+ Ash (100 g)
29.	Control-5	Fipronil (16 g)+Cow Dung (160 g)+ Ash (100 g)
30.	Control-6	Fipronil (18 g) +Cow Dung (180 g)+ Ash (100 g)

Thread Binding Bioassay

To evaluate the efficacy of *F. benghalensis,* latex cotton threads were soaked in different combinatorial mixture for 30 min. After drying, the threads were tagged around the trunks of infested trees at an average height of 5–6 ft above the ground. In control, the same thread was tagged at the same height without coating any active fraction for comparison. Few important observations such as tunneling and foraging behaviors were significantly noted to evaluate the termite infestation [Photoplate 7].

Statistical Analysis

For calculation of LD50 value, POLO (Probit Or LOgit), a computer program was used. Data were analyzed for calculation of dose response (LD_{50} and degrees of freedom (df)), heterogeneity, and Chi-square goodness of fit test using POLO (Probit Or LOgit) computer program. This is specifically developed to analyze data obtained from insecticide bioassays (Russell and Robertson (1979). Dosage-response lines may be compared for parallelism or equality by means of likelihood ratio tests. Statistical features of the program, suggestions for the design of experiments that provide data for analysis, and formats for data input and output are described in detail. Standard deviations Chi-square, t-significance, correlation, and analysis of variance (ANOVA) were calculated from the means using Sokal and Rohlf method (1973). In the experiments, ANOVA was done whenever two means were obtained at a multiple test range and $P < 0.05$ probability level.

Photoplate 5: Showing hollow wood sticks experiment set up

Photoplate 6: Showing poison bait experiments

Photoplate 7: Showing thread binding assay

RESULTS

Determination of Toxicity and LD_{50} Value

All the combinatorial fractions of *F. benghalensis* have shown very high insecticidal activity. It is proved by very low LD_{50} values obtained in each mixture, that is, 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/g body weight of termites for S-MLT-A, S-MLT-B, S-MLT-C, B-MLT-A, B-MLT-B, B-MLT-C, C-MLT-A, C-MLT-B, C-MLT-C, CU-MLT-A, CU-MLT-B, CU-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/g LD_{50} for malathion, fipronil, and thiamethoxam The lowest LD_{50} was obtained in H-MLT mixture, that is, 11.887 µg/g body weight of termite. The upper and lower confidence limits obtained were ranged from 550.006 to 276.516, 1000.726 to 342.461, 914.105 to 587.428, 757.042 to 191.141, 582.976 to 312.699, 992.442 to 495.051, 494.773 to 195.430, 1049.929 to 368.438, 927.577 to 538.238, 479.273 to 238.499, 1402.248 to 343.231, 1353.574 to 481.076, 48.771 to 19.287, 24.405 to 15.488, 37.163 to 6.857, 39.564 to 19.464, 19.602 to 10.885, 95.511 to 52.909, 58.871 to 18.100, and 63.329 to 41.833 for S-MLT-A, S-MLT-B, S-MLT-C, B-MLT-A, B-MLT-B, B-MLT-C, C-MLT-A, C-MLT-B, C-MLT-C, CU-MLT-A, CU-MLT-B, CU-MLT-C, AQ-MLT, A-MLT, H-MLT, P-MLT, EA-MLT, malathion, fipronil, and thiamethoxam, respectively [Table 3]. These combinatorial formulations have 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).

Wood Seasoning

For solid wood sticks

In these experiments, seasoned wood sticks were planted in the soil at an equal distance. It was found that *F. benghalensis* latex and its combinatorial mixture have shown anti-termitic activity in seasoned wood sticks which were planted in the soil. Results showed that wood seasoning protected wood weight losses and termite infestation in comparison to unseasoned wood sticks.

Set no I

Very high anti-termitic activity was obtained in S-MLT-A, S-MLT-B, and S-MLT-C seasoned wood sticks in the garden soil. In this set of experiment, both percent weight loss and termite infestation were found to be significantly reduced in comparison to control. However, S-MLT-A mixture showed 54–90% wood protection and it effectively prevented termite infestation in comparison to control. Similarly, S-MLT-B mixture caused significant decrease both in percent weight loss 50–93%, S-MLT-C mixture gave better wood protection approximately 65–85%, and a very significant decrease in termites infestation [Figures 1-9].

Set no II

In another experiment, wood seasoning was done using B-MLT-A, B-MLT-B, and B-MLT-C mixtures. Out of which,

Table 3: Toxicity experiment

S. No.	Name of Latex/ combinatorial mixture	LD_{50} µg/g	LD40 µg/g	LD20 µg/g	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	C-MLT-A	295.197	118.07	59.03	494.773–195.430	7.2238	−0.109047	4	1.8060
8	C-MLT-B	548.854	219.54	109.77	1049.929–368.438	7.9930	−0.126025	4	1.9983
9	C-MLT-C	717.439	286.97	143.48	927.577–538.238	3.231	−0.129877	4	0.808
10	CU-MLT-A	323.776	129.51	64.75	479.273–238.499	4.1271	−0.110143	4	1.0318
11	CU-MLT-B	555.295	222.11	111.05	1402.248–343.231	10.558	−0.124327	4	2.6396
12	CU-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

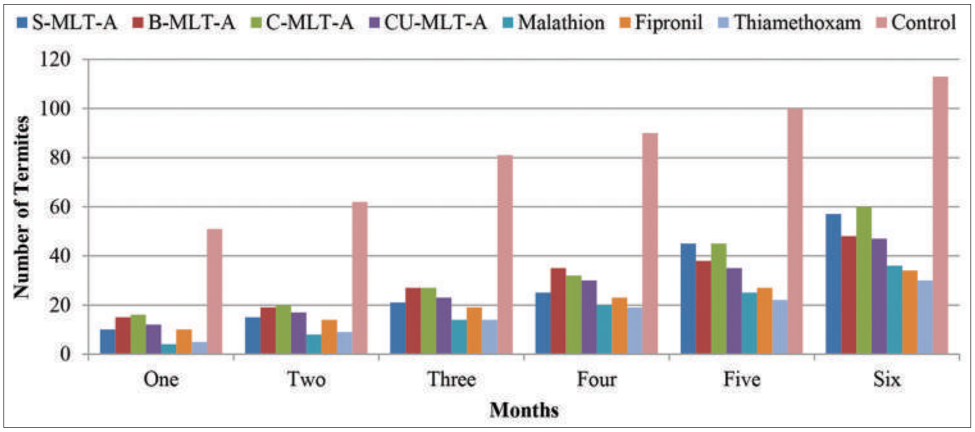

Figure 1: Number of termites in solid wood sticks

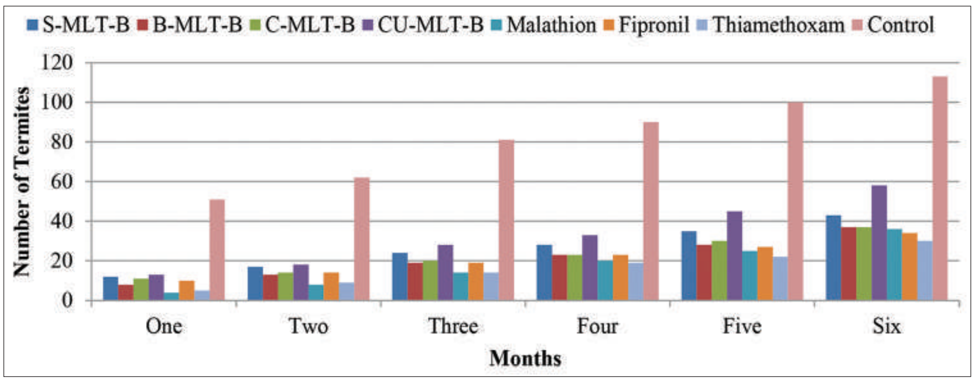

Figure 2: Number of termites in solid wood sticks

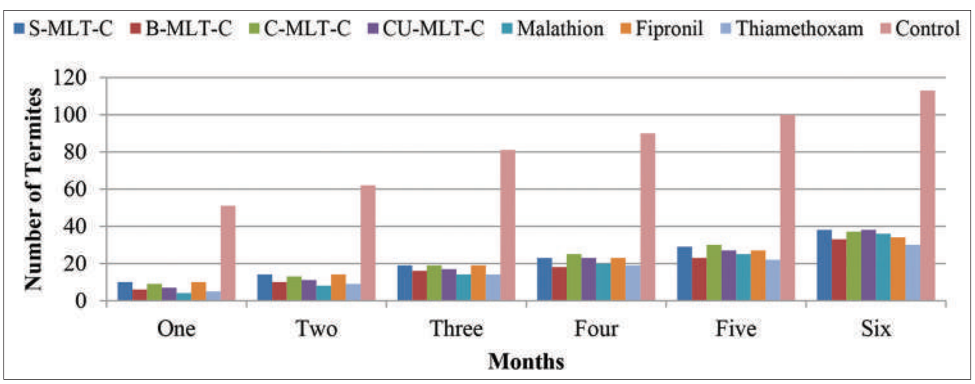

Figure 3: Number of termites in solid wood sticks

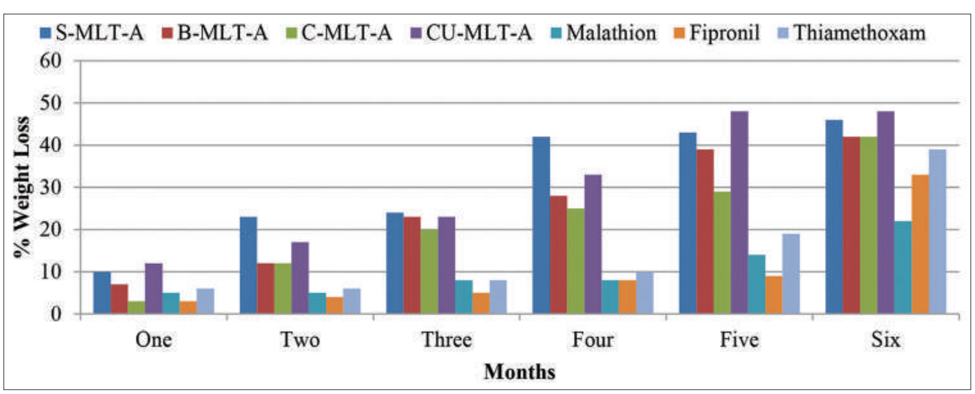

Figure 4: Percent weight loss in one feet solid wood sticks

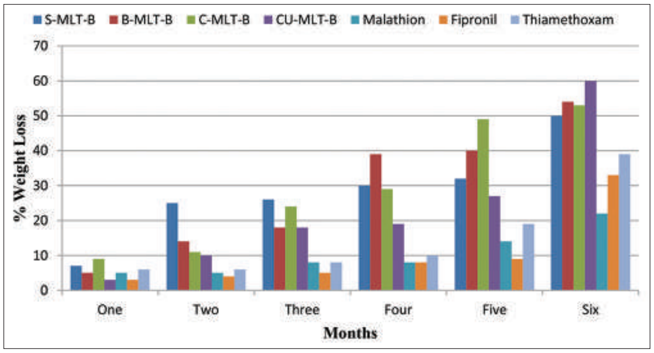

Figure 5: Percent weight loss in solid wood sticks

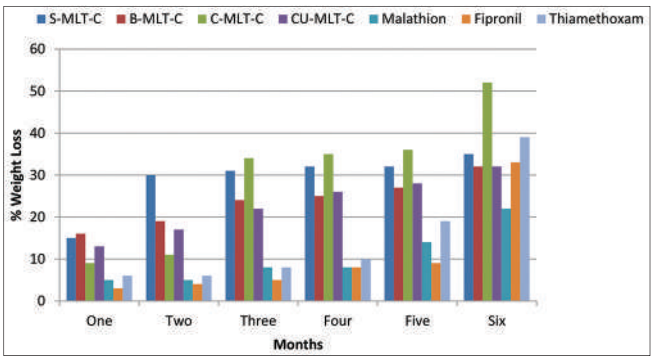

Figure 6: Percent weight loss in solid wood sticks

B-MLT-C mixture was found more effective in comparison to other mixtures. The percent weight loss in wood sticks seasoned with B-MLT-A mixture was obtained in a range of 07–42%, while termite infestation was obtained in a range of 29–42%. Similarly, both B-MLT-B and B-MLT-C mixtures have shown very high wood protection, that is, 46 and 95% a significant decrease in termite infestation, respectively [Figures 1-9 and Photoplate 2].

Set no III

In this experiment, C-MLT-A, C-MLT-B, and C-MLT-C mixtures were used to evaluate the termiticidal and repellent properties of isolated mixtures. Highest protection in wood weight loss was obtained in C-MLT-A mixture, that is, 03–42% after 180 days. Similar activity was obtained in C-MLT-B and C-MLT-C mixtures. Above mixture was also successfully controlled the termite infestation in treated wood sticks as percent termite infestation recorded was very low, that is, 17 to 32% in presence of C-MLT-C mixture, 21–32% in presence of C-MLT-B mixture, and 31–53% in presence of C-MLT-A mixture, respectively [Figures 1-9].

Set no IV

In this experiment, photoactivated cow urine was used for wood seasoning. It has also cut down weight loss up to 03–60% up to 6 months [Figures 1-9].

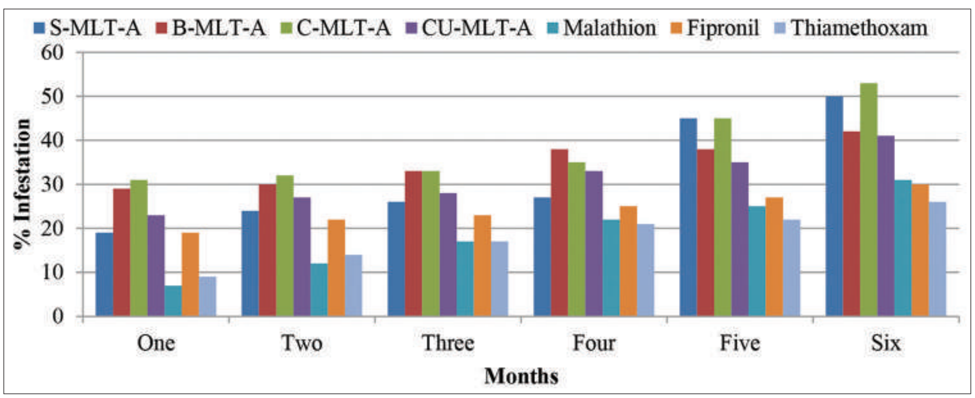

Figure 7: Percent infestation in solid wood sticks

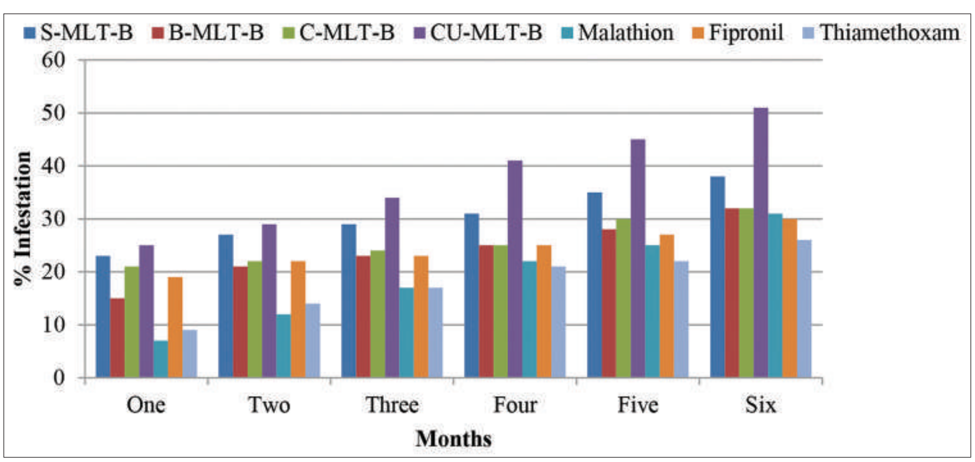

Figure 8: Percent infestation in solid wood sticks

Set no V

Percent weight loss in presence of inorganic pesticides such as malathion, fipronil, and thiamethoxam was also recorded. Malathion seasoned wood sticks have shown 05–22% weight loss and 07–31% infestation. Fipronil has shown significant reduction in weight loss (33%) and termite infestation (30%). Weight loss of thiamethoxam seasoned wood sticks ranges between 06% and 39%, while percent termite infestation ranges between 09% and 26% [Figures 1-9 and Photoplate 2].

For solid wood sticks

In these experiment, fixed *F. benghalensis* latex mixture was shown weight loss 29–55%, *F. benghalensis* Latex + sulfur is 06–58%, and *F. benghalensis* Latex +sulfur + cow urine mixtures is 21–45% in 6-month duration. Their maximum % of infestation was 33%, 63%, and 32%, respectively. Fipronil was shown 47% termite infestation [Figures 10-12 and Photoplate 3].

For hollow wood sticks

In this set of experiment, bamboo wood sticks were seasoned with highest concentration of combinatorial mixtures. Therefore, various combinatorial mixtures, that is, MLT-6, MLT-CU-6, and MLT-CU-CD-6 were used for wood treatment. For comparison of termiticidal activity present in above mixtures and inorganic pesticides, bamboo wood sticks were also treated with inorganic pesticides fipronil.

Set no. I

MLST-3 treated bamboo sticks very significantly cut down percent weight loss and recorded 32% in 3rd month of experiment after which 28% termite infestation was observed in any bamboo sticks [Figures 13-15].

Set no. II

Similarly, ML-CU-4 treated bamboo wood sticks have shown a significant decrease in percent weight loss and recorded 39%, while percent infestation was significantly decreased up to 26%, respectively [Figures 13-15 and Photoplate 4].

Set no. III

In ML-CU-CD-5 treated, bamboo wood sticks percent weight loss was decreased and recorded 22%, while almost 23% termite infestation was observed in these wood sticks. In this experiment, wood weight loss and percent termite infestation were found significantly reduced up to 5 months [Figures 13-15].

Set no IV

In this experiment, control mixture of cow urine and cow dung was shown high termite infestation in comparison to combinatorial mixtures [Figures 13-15].

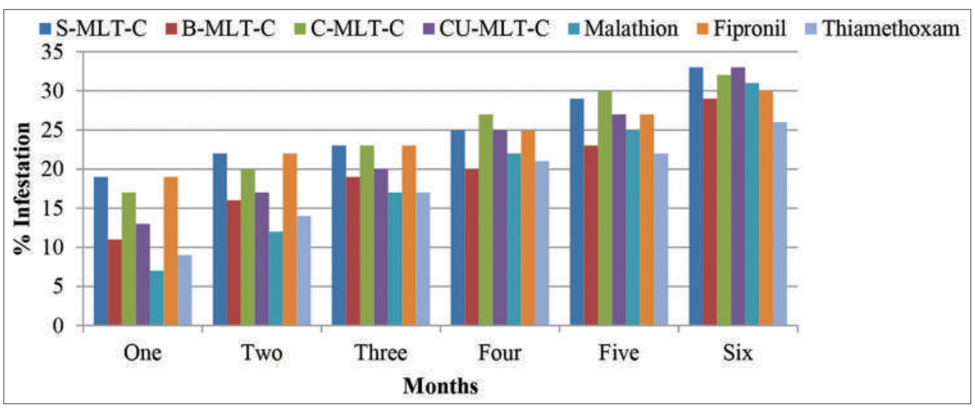

Figure 9: Percent infestation in 1 ft solid wood sticks

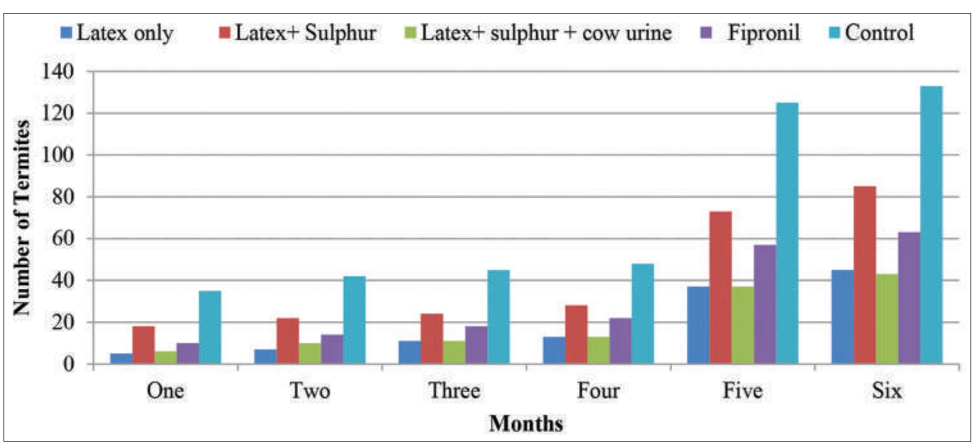

Figure 10: Number of termites in solid wood sticks

Set no. V

Percent weight loss was found high in case of fipronil seasoned bamboo wood sticks, that is, 03–20% and high termite infestation was observed in fipronil, that is, 06–32% [Figures 13–15 and Photoplate 4].

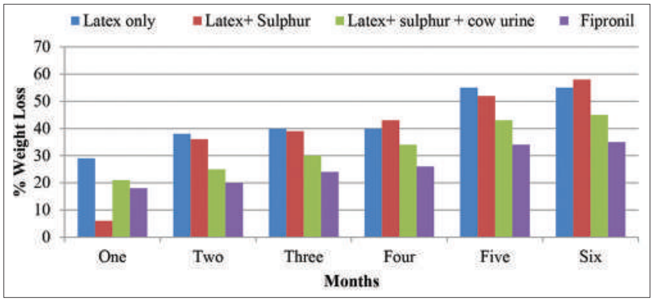

Figure 11: Percent weight loss in solid wood sticks

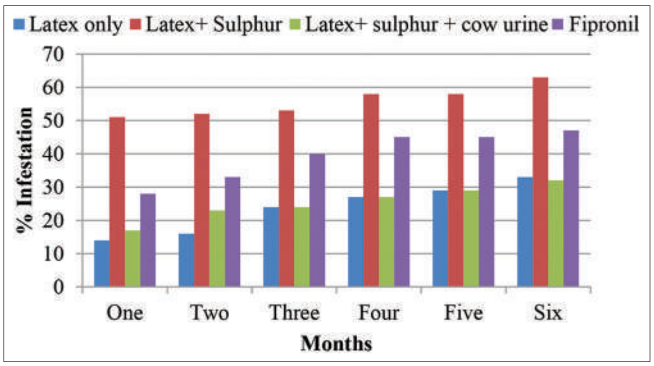

Figure 12: Percent infestation in solid wood sticks

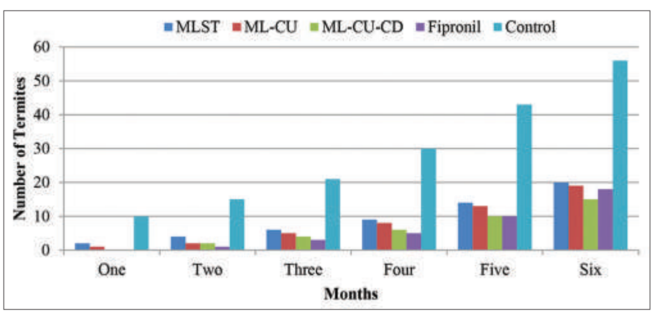

Figure 13: Number of termites in hollow bamboo wood sticks

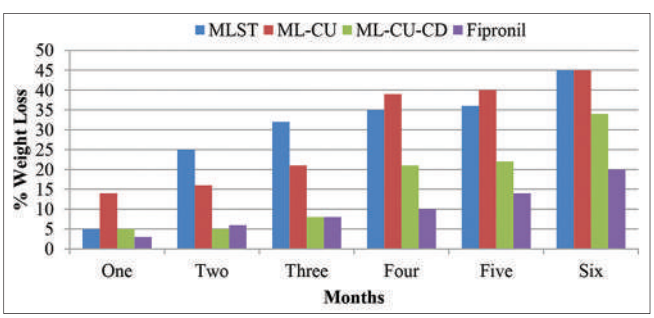

Figure 14: Percent weight loss in hollow bamboo wood sticks

For hollow wood sticks

In these bioassay, *F. benghalensis* latex mixture was shown weight loss 35–37%, *F. benghalensis* Latex + sulfur is 11–37%, and *F. benghalensis* Latex +sulfur + cow urine mixtures is 18–40% in 6-month duration. Their maximum % of infestation is 48%, 51%, and 28%, respectively. Fipronil was shown 40% termite infestation [Figures 16-18 and Photoplate 5].

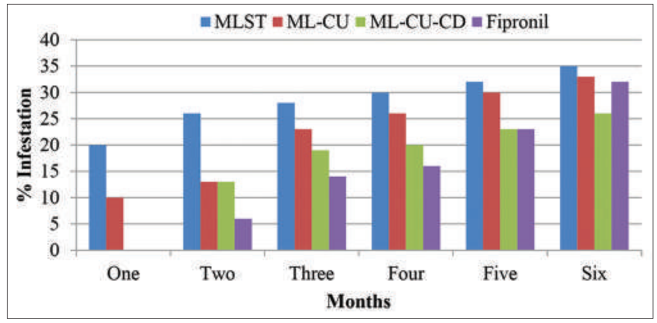

Figure 15: Percent infestation in hollow bamboo wood sticks

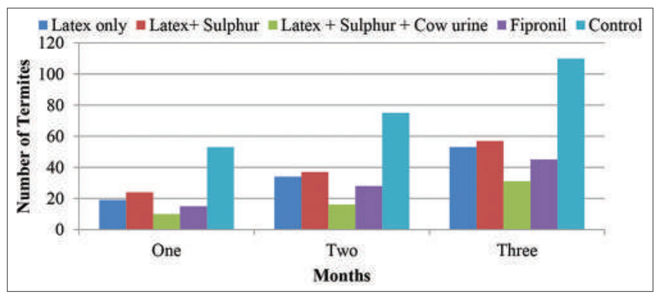

Figure 16: Number of termites in hollow bamboo wood sticks

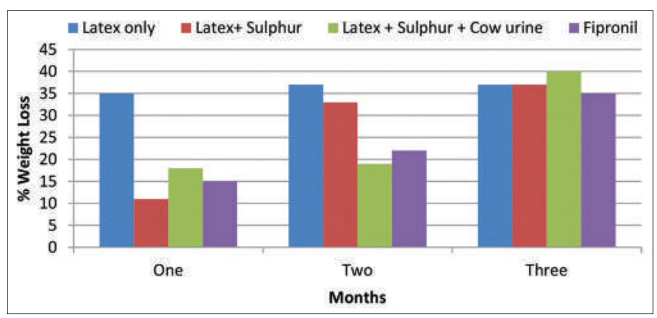

Figure 17: Percent weight loss in hollow bamboo wood sticks

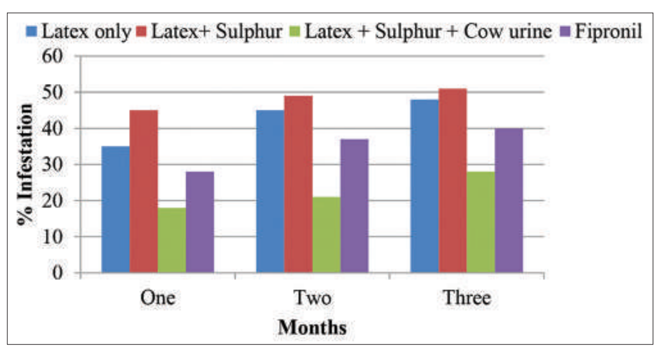

Figure 18: Percent infestation in hollow bamboo wood sticks

Poison Baits

In these bioassay, *F. benghalensis* Latex mixture was shown maximum infestation is 36% in 6th month, *F. benghalensis* Latex + sulfur is 58%, and *F. benghalensis* Latex +sulfur + cow urine mixtures is 40% in 6 month duration. Their maximum termite count is 36, 58, and 38, respectively, while Fipronil was shown 38% termite infestation and 40 termites [Figures 19 and 20, Photoplate 6].

Thread Binding Bioassays

For effective management of termites in garden, pre-soaked cotton threads were tagged around the tree trunks at a height of 5–6 ft above the ground. Termite infestation on these tagged trees was significantly decreased after 6 months of thread binding and mud plastering and tunnels were found shed off from the tree trunk. Lesser number of termites was observed on tested trees in comparison to untreated plants. Further, termite infestation was found to be significantly decreased after 6 months in comparison to control. An overall 88% control was observed in test plants. There was a significant decrease in number of infested plants and termite occurrence after 6 months [Figures 21 and 22, Photoplate 7].

DISCUSSION

In present investigation, *F. benghalensis* plant latex-based various combinatorial mixtures were found highly effective against termites [Tables 1 and 2]. These were tested in maize and millets crop field, garden soil, and trees. Above experiments were done on solid wood and hollow wood sticks. Firstly, all wood sticks were dipped in combinatorial mixtures overnight separately. After that emerging all wood sticks from the mixtures and had kept for dried at room temperature up to 24 h. These were planted in garden soil at a fix distance in different rows. As in the test and control wood sticks termite number was counted and weight loss was measured after digging out wood sticks and dried them for 72 h.

In wood seasoning experiments 07–50%, weight loss was obtained in S-MLT-B and S-MLT-C treated wood sticks, respectively. Similarly, B-MLT-A mixture-treated wood sticks showed approximately 07% weight loss up to 30 days of the treatment, but, later on, it was increased up to 42% after 5th month of the treatment. Again in a similar experiment reduction in wood, weight loss was obtained, that is, 13% in photoactivated cow urine (CU-MLT-C) seasoned wood sticks. Besides this, B-MLT-B, C-MLT-A, C-MLT-B, and CU-MLT-B mixtures have also shown similar activity and protected wood sticks from termite infestation very effectively in comparison to other mixtures. Contrary to this inorganic pesticides were not found able to protect wood as in such cases, wood weight loss was much higher than wood seasoned with *F. benghalensis* latex combinatorial mixtures, that is, 29–55% after 5th month of treatment. Results obtained from wood seasoning experiments indicate that *F. benghalensis* latex combinatorial mixtures favorably increased resistance in wood sticks against termite up to 6 month. Therefore, it can be said that *F. benghalensis* latex has few active components which can be used for development of a new termiticidal formulation that can be used as an effective wood preservative.[34]

In another experiment, anti-termite activity of all the above mixtures was tested in one foot hollow bamboo wood sticks. For this purpose, each combinatorial mixture was mixed with wood ash and poured inside hollow bamboo sticks and planted underground. MLST, ML-CU, and ML-CU-CD treated bamboo sticks have shown minimum weight loss, that is, 05–34% against termite. While in ML-CU and ML-CU-CD treated bamboo sticks approximately zero percent infestation were found in 1st month. Contrary to this, fipronil treated wood sticks have shown 20% weight loss up to 6th month of treatment. Besides this, various type of solid and hollow bamboo wood seasoning were setup in different garden location. Latex and sulfur combinatorial mixture of 3 ft solid and hollow bamboo wood sticks bioassay was showed minimum weight loss and termite infestation, i.e. 11–37% and 45 -51% respectively. Besides this, thread binding assays on infested saplings of *T. grandis* and poison bait

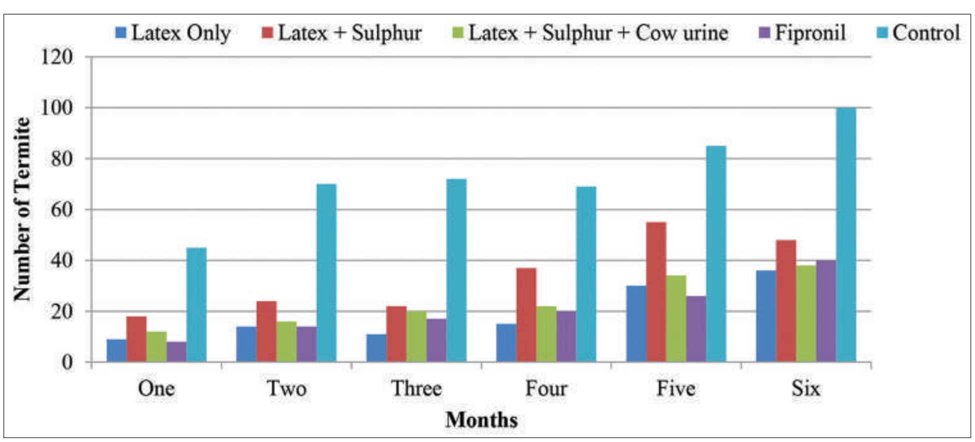

Figure 19: Number of termites in poison bait experiment

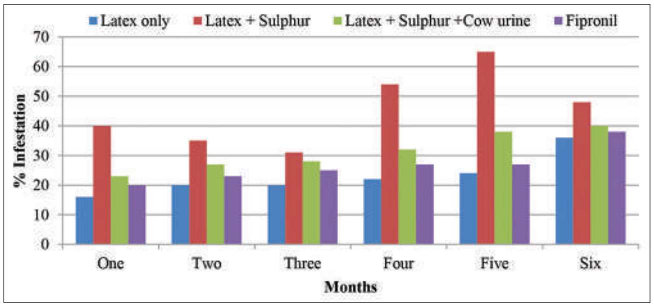

Figure 20: Percent infestation in poison bait experiment

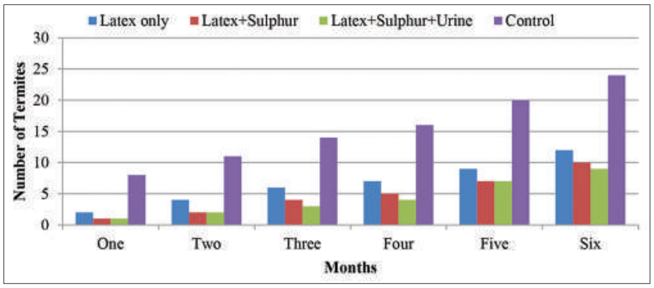

Figure 21: Number of termites in thread binding experiment

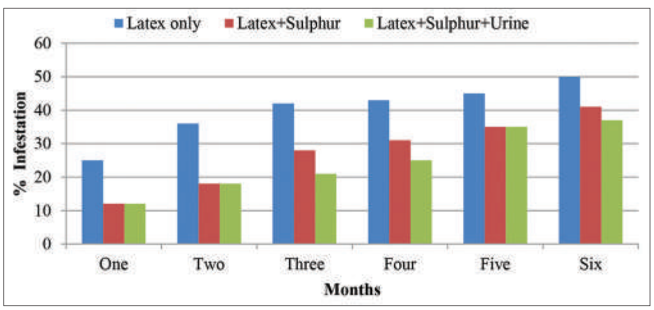

Figure 22: Percent infestation in thread binding experiment

bioassay in maize crop field were also conducted to check the mud plastering, tunneling activity, and up and downward movements of termites.

Poison bait was more significant to control termite infestation in crop field, because it reduced 64–84% infestation and the termite count in tested crop field and helpful in better yield of crop. Thread binding bioassay showed positive outcomes for controlling of termite infestation. In tested *T. grandis,* termite infestation was reduced approximately 50–88%. It was proved by reducing termite count and mud plastering around the plants.

From results, it is clear that *F. benghalensis* plant latex and its combinatorial mixtures significantly reduced the wood consumption/infestation by termites that controlled wood weight loss. It may be due to their fast leaching from wood to soil due to rain water. Results obtained from wood seasoning experiments indicate that combinatorial mixtures favorably increased resistance in wood sticks against termite up to 6 months. In test wood sticks percent, termite infestation was significantly reduced due to action of ingredients. This reduction was found to be concentration and time dependent. This activity of ingredients was remain up to 6 months, though it was little bit reduced later on. There is another reason that due to leaching of pesticides, wood infestation was found to be increased in underground wood sticks.[35] However, wood consumption is decided on the basis of wood softness due to humidity as termites feed more amounts of soft woods than hard wood.[36,37] Similar wood protection was obtained after wood seasoning done by applying heat and temperature.[38] Both these ecological factors significantly cut down termite infestation, weight loss, and provide decay resistance to the wood.[39]

Plant latex contains many secondary metabolites which are synthesized by plants in response to herbivorous insects.[40] This is a complex mixture of proteins, alkaloids, starch, sugars, oils, tannins, resins, and gums.[41] It is a natural plant polymer secreted by highly specialized cells known as laticifers. Many of latex components possess insecticidal property.[42] There is a possibility that its protein or volatile substances are responsible for repellent activity in insects.[43] These also act as antifeedants[44,45] and deter insects from feeding.[46] Latex components showed deleterious effects such as toxic, antifeedant, growth, and reproductive inhibitory in number of insect species.[47] However, *F. benghalensis* latex-based termite formulations were found effective against Indian white termite, *O. obesus* Rambur (*Isoptera*: *Odontotermitidae*). A significant protection was noted after wood seasoning, spray, tag binding, and soil treatments against termites. These have shown both insecticidal and repellent potential against termites. Latex components are highly selective, specific, biodegradable, and non-toxic products, do not kill non target organisms and are environment friendly.[48,49] These can used as plant-origin natural pesticides control wide range insect pests which might efficiently cut down the pest population if applied in field and store houses in very low quantity.[50] These are much safer, low cost, and easily biodegradable in the medium and show no residual effect. These are environmentally friendly and showed minimal mammalian toxicity.[51] These could be used development of new botanical insecticides.

CONCLUSION

Termites are the highly destructive polyphagous insect pests which inhabit in high humidity zones and severely infest and invade forest and garden tress and field crops. From the results, *F. benghalensis* latex based combinatorial formulations very efficiently controlled the wood weight loss and infestation caused by Indian forest termite *O. obesus.* These were found highly effective against termites after wood seasoning and tested in garden soil, trees, and various field experiments. These have provided enormous protection against termite infestation in seasoned wood sticks; these significantly have cut down tunneling and mud-plastering in termites. These have provided protection to seedlings and plant foliages in maize and millets crops in the agriculture crop field. From results, it is clear that *F. benghalensis* and its combinatorial mixtures significantly reduced the wood consumption/infestation by termites that controlled wood weight loss. In test wood sticks percent, termite infestation was significantly reduced due to action of ingredients. This reduction was found to be concentration and time dependent. This activity of ingredients was remain up to 6 months, though it was little bit reduced later on. However, active ingredients found in *F. benghalensis* latex can be used for development of a new termiticidal formulations and preservative of commercial wood.

ACKNOWLEDGMENTS

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