ORIGINAL ARTICLE

Effects of *Tagetes erecta* essential oil-based formulations on wood weight loss and termite infestation in garden saplings

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Abstract

Introduction: Since last ten decades various synthetic pesticides were used for insect control but most of them were found highly toxic and harmful to non-target living beings. These also persist for longer time inside soil as bound residue and enter into food chain and reach to human body and evoke certain diseases. Plant species synthesize thousands of dynamic biomolecules and so numerous of them have been extracted and distinguished for its insecticidal exercises. **Objective:** In the present investigation, various field bioassays were performed to observe the anti-termites efficacy of plant essential oils based formulations to control population of Indian white termite. **Method:** Combinatorial mixtures of *Tagetes erecta* essential oils have shown synergistic activity against termites. LD50 values obtained in mixtures B-AST- and S-AST-C were in a range of 261.930 to 507–666. For the control of termite infestation, thread-binding assay was found quite effective to control garden termite. Bait formulation was also used which cut down termite infestation in various crop fields. *T. erecta* extract with different combinatorial mixtures provides sustainable way of termite control. **Result:** Use of crude essential oils, its active components, and combinatorial mixtures have shown significant anti-termites efficacy against *Odontotermes obesus*. **Conclusion:** In subsequent bioassays, both crude essential oils, its active components and combinatorial mixtures, have shown high toxicity and repellency against *Odontotermes obesus*.

Key words: *Odontotermes obesus*, Plant essential oils, Poison bait, *Tagetes erecta*, Termiticides, Wood seasoning

INTRODUCTION

Termites are social insects belong to class *Insecta* (order: *Isoptera*). These are exceedingly damaging polyphagous insects which voraciously feed on trees, crop plants and building materials, cereal grains, wood filaments, cloths, and papers.[1,2] These too harm green foliage, seedlings, wood, strands, and other family cellulose-based materials. Termites intensely overrun post-harvest put away items, cereal grains, wood strands, cloths, and papers. In forest land woodlands, gardens termites make burrows, termitarium, tunnels to hide during the day and feed on green biomass, vegetation, or crops of adjoining areas.

Termites colonize in rainy tropical areas of the world; they build huge termitarium and digest plant litter, soft wood, and other cellulose-based fabric materials. Most of the termite species assault edit plants that come about in lessening of trim surrender and biomass generation essentially. There are roughly 2800 termite species which have been distinguished and have a place to 282 genera around the world. Termites do decay of over ground dead wood, intervening the consolidation of suspended, and standing dead wood into the soil.[3] These are break down forest and farming biomass in environment and help in various environmental forms. Termites scavenge in timberlands primarily green takes off and make gigantic hills. These attacks standing dead trees in tropical timberlands and debases the wood, and diminishes its quality which are utilized by a few tribes for memorial service as well as otherworldly viewpoints.[4] Few common items such as flavonoids,[5] sesquiterpenes,[6] and thiophenes[7] disconnected from distinctive plants species were found successful against termites. In final 100, a long time manufactured pesticides such as chlordane,[8] cypermethrin,[9] hydroquinone, and

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indoxacarb were enormously utilized for termite control.[10] In spite of the fact that, these cause high lethality in termites but were demonstrated exceedingly harmful to non-target living beings.

Plant species synthesize thousands of dynamic biomolecules and so numerous of them have been extricated and distinguished for its insecticidal exercises.[11] Few of them act at cellular and physiological level.[12]. Plant-derived compounds are utilized as an elective of manufactured pesticides for termite control. Both crude oil and its combinatorial mixtures were utilized to control the Indian white termite *Odontotermes obesus*[13] and put away grain creepy crawlies[14] appeared string termiticidal impacts. Plant characteristic items are utilized in eco-friendly control of termites and its administration.[15] For termite control, few bait formulation have been given more extensive control against underground termite colonies.[16] Noviflumuron is utilized in harm baits to murder molting specialist populace of Formosan underground termite, *Coptotermes formosanus Shiraki* interior settle.[17] Polyacrylamide/attapulgite composite snares are, moreover, connected in agrarian soils. These draw in termite populace for scrounging.[18] Systemic harms or pesticides can be included to these gels to slaughter termites. Carbon dioxide discharge pull in termites. It moves forward adequacy of an spray to kill termites in homes.[19] Termite specialist populace can be controlled or smother by utilizing harm trap and slaughtered in pre-adult formative stages.[20] The capital of *Chrysanthemum roseum* and *C. cinerariaefolium* are dried, powdered, and utilized as insecticide.[21]

The fundamental oil of *L. sidoides* and its major components appeared a tall potential to control *Cryptotermes brevis pseudergates*.[22] Basic oil of hiba wood encompasses a unwinding woody scent and antifungal properties.[23] Vetiver oil could be a promising novel termiticide with diminished natural affect for utilize against underground termites.[24] *Tagetes erecta* combinatorial mixtures have significantly repelled termites in two-choice bioassays. These also have shown strong antifeedant action in termite workers.[25] New alternates in the form of various natural plant products such as essential oils, poison baits, deterrents, repellents, fumigants, insecticidal bio-organic chemicals, and genetic and biological control methods should employ to control this white termite.[26] The present study signifies insecticidal potential and target specificity of plant essential oil against termite.

MATERIALS AND METHODS

Collection of Termites

Workers and soldiers of *O. obesus* (Indian white termite) were collected from infested wood and plant material found at the University Garden of Gorakhpur University, and nearby forest area of East 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, 75 ± 5 RH for temporary culture. Termites were provided to feed on green leaves. 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), Table 1.

Preparation of Tagetes erecta extracts for anti-termitic effect

T. erecta belongs to family: *Asteraceae* (Sunflower family). Its flowers were collected from Deen Dayal Upadhyaya Gorakhpur University garden; living specimen is photographed [Figure 1a and b]. This is also called African marigold, Aztec marigold, American marigold, or big marigold, is native to Mexico and Central America. 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 ornamental and therapeutic purposes in India. Flowers were used for the preparation of combinatorial mixture w/v in distilled water. Flowers were weighed and extract was prepared in distilled water in a power mixture and grinder. The extract obtained was dried in rotatory evaporator and kept in refrigerator for further use. All chemicals used in this study were purchased from CDH-laboratory chemicals suppliers India supplied by Eastern Scientific Company, Gorakhpur.

Field Experiments

Determination of LD values in extracts and combinatorial mixtures

Toxicity bioassay

For the evaluation of dose–response relationship of essential oil, different doses (w/v), that is, 60, 120, 240, 360, 480, and 600 μg of different extracts, were 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 center of separate Petri dishes (42 mm diameter) as tests and uncoated as control. Twenty worker termites 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 termites 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. The LD_{50} after 24 h of exposure to each was calculated using probit analysis tested using the method of Finney[20] [Table 1].

Repellency bioassay

Repellent responses were observed in a glass Y-tube olfactometer using serial concentrations 10, 20, 40, 60, 80, and 100 of different crude latex/fractions/formulations loaded on separate Whatman paper strips (1 × 1 cm^2) and air-dried to

Table 1: LD_{50} value of different combinatorial mixture

S. No.	Name of extract/ combinatorial mixture	LD_{50} μg/g	0.95 confidence limit UCL-LCL	Chi-square	Slope function	Degree of freedom	Heterogeneity	LD_{40} μg/g	LD_{20} μg/g
1.	S-AST-A	337.839	521.443–243.460	7.2207	−0.131622	4	1.8052	135.1	67.56
2.	S-AST-B	370.722	485.216–280.403	5.6770	−0.136581	4	1.4192	148.28	74.14
3.	S-AST-C	507.666	691.192–371.138	6.7542	−0.141483	4	1.6886	203.06	101.53
4.	B-AST-A	261.930	352.270–194.957	6.2448	−0.129716	4	1.5612	104.77	52.38
5.	B-AST-B	364.898	434.773–305.138	3.584	−0.123795	4	0.896	145.9	72.97
6	B-AST-C	494.352	683.442–352.598	7.1354	−0.140405	4	1.7839	197.74	98.87
7	Cu-AST-A	307.838	430.957–230.441	10.717	−0.133137	4	2.6792	123.1	61.5
8	Cu-AST-B	358.599	475.526–266.342	5.6228	−0.129255	4	1.4057	143.43	71.7
9	Cu-AST-C	531.550	715.288–397.738	10.489	−0.144219	4	2.6222	212.62	106.31
10	Cow-AST-A	236.106	328.254–164.877	9.0054	−0.130733	4	2.2513	94.44	47.22
11	Cow-AST-B	377.816	478.518–297.051	4.0440	−0.133482	4	1.0110	151.1	75.56
12	Cow-AST-C	577.159	734.248–459.912	4.1740	−0.154133	4	1.0435	230.86	115.43
13	AQ-AST	30.147	36.071–24.929	1.077	−0.708375	4	0.269	12.05	6.02
14	A-AST	30.212	36.543–25.045	2.400	−0.684713E	4	0.600	12.08	6.04
15	H-AST	50.601	90.739–34.517	8.6803	−0.822924	4	2.1701	20.24	10.12
16	P-AST	-	-	67.670	−0.458164	4	16.917	-	-

Figure 1: (a and b) Vegetative parts of *Tagetes erecta* plant

remove the solvent. These pre-coated solvent-free strips were placed in 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 to toward uncoated strips or non-scented area was 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 *T. erecta* essential oil components. Number of repelled termites in the presence of each extract was counted after 30 min of treatment with six different concentrations (10, 20, 40, 60, 80, and 100 μg/g) of each *T. erecta* extract which were used. The ED50values that repelled 50% of termite population were calculated.

Wood Seasoning

In wood seasoning bioassay we planted in each row ,the six solid wood stick and hollow bamboo wood sticks under dick in different places such as crop fields and gardens. These wood sticks were treated with various combinatorial mixtures.

Treatment of solid wood sticks

In these experiments, dried solid wood sticks of Sangwan (*Tectona grandis*) family *Verbenaceae* having 1 foot and 50 mm average diameter were used. These solid wood sticks were seasoning with different combinatorial mixtures of *T. erecta* for 24 h. Following sets were made.

- Set no. 1: In set no. 1, solid wood sticks were undergird for 24 h in S-AST-A, S-AST-B, and S-AST-C mixtures. These were prepared using 0.3 g sulfur as in inorganic substance [Table 2]. For each mixture, six wood sticks were seasoned for 6 months observation
- Set no. 2: In another set of experiment, solid wood sticks were seasoned with B-AST-A, B-AST-B, and B-AST-C mixtures, these were prepared using 0.3 g borate; other components were similar as above [Table 2]
- Set no. 3: In the third experiments, solid wood sticks were seasoned with C-AST-A, C-AST-B, and C-AST-C mixtures, these were prepared using 0.3 g copper sulfate; other components were similar as above [Table 2]
- Set no. 4: In the fourth set of experiment, solid wood sticks were seasoned with CU-AST-A, CU-AST-B, and CU-AST-C mixtures, these were prepared using 0.3 g borate; other components were similar as above [Table 2]

Table 2: *Tagetes erecta* and other ingredients used in preparation of combinatorial mixture

S. No.	Combinatorial mixture	Ingredients
1	AST-S-A	*T. erecta* extract (9 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+sulfur (3 g)+water (5 L)
2	AST-S-B	*T. erecta* extract (12 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+sulfur (3 g)+water (5 L)
3	AST-S-C	*T. erecta* extract (18 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+sulfur (3 g)+water (5 L)
4	AST-B-A	*T. erecta* extract (9 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+borate (3 g)+water (5 L)
5	AST-B-B	*T. erecta* extract (12 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+borate (3 g)+water (5 L)
6	AST-B-C	*T. erecta* extract (18 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+borate (3 g)+water (5 L)
7	CU-AST-A	*T. erecta* extract (9 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+copper (3 g)+water (5 L)
8	CU-AST-B	*T. extract* (12 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+copper (3 g)+water (5 L)
9	CU-AST-C	*T. erecta* extract (18 g)+coconut oil (17 ml)+terpene oil (17 ml)+glycerol (17 ml)+copper (3 g)+water (5 L)
10	CW-AST-A	*T. erecta* extract (9 g)+photoactivated cow urine (10 g/L)+water (5 L)
11	CW-AST-B	*T. erecta* extract (12 g)+photoactivated cow urine (10 g/L)+water (5 L)
12	CW-AST-C	*T. erecta* extract (18 g)+Photoactivated cow urine (10 g/L)+water (5 L)
13	Fipronil	Fipronil (7.5 g\L) water (5 L)
14	Thiomethoxide	Thiomethoxide (7.5 g\L) water (5 L)
15	Malathion	Malathion (7.5 g\L) water (5 L)

- Set no. 5: In the fifth set of experiments, inorganic compounds such as malathion, fipronil, and thiamethoxam were used for the wood seasoning [Table 2].

All the above seasoned wood sticks were planted inside soil by making pits of 2.75 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.

Treatment of solid wood sticks

In these experiments, wood seasoning was done by fixed *T. erecta* family *Verbenaceae essential* oil extract, fixed oil extract + sulfur, and fixed oil extract + sulfur + cow urine mixtures for overnight stick size was 3 ft in length and 1.25 inches in diameter were planted in soil at 1 m distance from each. In each row fixed solid wood stick of *T. grandis* family *Verbenaceae*. 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 termite in the presence of various mixtures.

Treatment of hollow bamboo wood sticks

For the evaluation of toxic and repellent properties of *T. erecta* combinatorial mixtures, bamboo wood sticks (1 ft and 50 mm diameter) were used for the treatment. For this purpose, inside obstructions present at each internode were made free by iron rod to fill agar impregnated combinatorial mixtures.

- Set no. 1: In the first experiment, AST-1, AST-2, AST-3, AST-4, AST-5, and AST-6 combinatorial mixture (prepared from extract 9 g, 10 g, 12 g, 14 g, 16 g, and 18 g respectively) + Ash (100 g) [Table 3]
- Set no. 2: In the second experiment, hollow bamboo sticks were treated with AST-CU-1, AST-CU-2, AST-CU-3, AST-CU-4, AST-CU-5, and AST-CU-6 mixture (prepared from extract 9 g, 10 g, 12 g, 14 g, 16 g, and 18 g + Cow urine 90 ml, 100 ml, 120 ml, 140 ml, 160 ml, and 180 ml, respectively) + Ash (100 g) [Table 3]
- Set no. 3: In the third experiment, hollow bamboo sticks were treated with AST-CU-CD-1, AST-CU-CD-2, AST-CU-CD-3, AST-CU-CD-4, AST-CU-CD-5, and AST-CU-CD-6 mixtures (prepared from extract 9 g, 10 g, 12 g, 14 g, 16 g, and 18 g + Cow urine 90 ml, 100 ml, 120 ml, 140 ml, 160 ml, and 180 ml, respectively)

Table 3: *Tagetes erecta* and other ingredients used in preparation of combinatorial mixtures

S. No.	Combinatorial mixtures	Ingredients
1	AST-1	Extract (9 g)+Ash (100 g)
2	AST-2	Extract (10 g)+Ash (100 g)
3	AST-3	Extract (12 g)+Ash (100 g)
4	AST-4	Extract (14 g)+Ash (100 g)
5	AST-5	Extract (16 g)+Ash (100 g)
6	AST-6	Extract (18 g)+Ash (100 g)
7	AST-CU-1	Extract (9 g)+cow urine (90 ml)+Ash (100 g)
8	AST-CU-2	Extract (10 g)+cow urine (100 ml)+Ash (100 g)
9	AST-CU-3	Extract (12 g)+cow urine (120 ml)+Ash (100 g)
10	AST-CU-4	Extract (14 g)+cow urine (140 ml)+Ash (100 g)
11	AST-CU-5	Extract (16 g)+cow urine (160 ml)+Ash (100 g)
12	AST-CU-6	Extract (18 g)+cow urine (180 ml)+Ash (100 g)
13	AST-CU-CD-1	Extract (9 g)+cow urine (90 ml)+cow dung (90 g)+Ash (100 g)
14	AST-CU-CD-2	Extract (10 g)+cow urine (100 ml)+cow dung (100 g)+Ash (100 g)
15	AST-CU-CD-3	Extract (12 g)+cow urine (120 ml)+cow dung (120 g)+Ash (100 g)
16	AST-CU-CD-4	Extract (14 g)+cow urine (140 ml)+cow dung (140 g)+Ash (100 g)
17	AST-CU-CD-5	Extract (16 g)+cow urine (160 ml)+cow dung (160 g)+Ash (100 g)
18	AST-CU-CD-6	Extract (18 g)+cow urine (180 ml)+cow dung (180 g)+Ash (100 g)
19	Control-1 (PVC)	Cow urine (90 ml)+cow dung (90 g)
20	Control-2 (PVC)	Cow urine (100 ml)+cow dung (100 g)
21	Control-3 (PVC)	Cow urine (120 ml)+cow dung (120 g)
22	Control-4 (PVC)	Cow urine (140 ml)+cow dung (140 g)
23	Control-5 (PVC)	Cow urine (160 ml)+cow dung (160 g)
24	Control-6 (PVC)	Cow urine (180 ml)+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 (1800 g)+Ash (100 g)

+ Cow dung (90 g, 100 g, 120 g, 140 g, 160 g, and 180 g, respectively) + Ash (100 g) [Table 3]

- Set no. 4: 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 3]
- Set no. 5: In this experiment, inorganic termiticide 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 3].

For above experiment, maize crop (Krishna cultivar) was sown on March 13, 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 make the soil texture fine. The field size was 8 × 3 meter (W × L) both control and test are arranged in the form of six regular replicate planted at a distance of 1 m. Each at the width of (stick to stick distance) was kept 1.5 feet. A control was set using six PVC pipes (1 ft length and 1.25 inch in 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 became 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°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; six random soil samples are investigated after 1 month interval. In the end of experiment, crop loss % yield and termite infestation versus termiticidal efficacy of essential oil were determined/calculated.

Treatment of hollow bamboo wood sticks

In these experiments, wood seasoning was done by fixed *T. erecta* essential oil extract, fixed oil extract + sulfur, and fixed oil extract + sulfur + cow urine mixtures for overnight stick size was 3 ft in length and 1.25 inches in diameter were planted in soil at one meter distance from each. In each row, fixed hollow wood stick of bamboo. 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.

Poison Baits Experiments

For controlling field, termite homemade baits were prepared using Multani soil 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, fixed oil ingredient was filled, these was air-dried inside room condition, in the first set, above soil mixture was used and the active ingredient was fixed oil extract 1.4 g. In the second set, 1.4 g fixed oil extract added with 1.25 g sulfur in each bait. In the third set, bait is prepared by 1.4 g fixed oil extract, 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 last experiments.

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.

Thread Binding Bioassay

To evaluate the efficacy of *T. erecta* extract, 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.

Statistical Analysis

For calculation of LD50 value, Probit or LOgit (POLO), a computer program was used for the analysis of dose response in various bioassays. Data were analyzed for the calculation of dose response (LD_{50} and degrees of freedom), heterogeneity, and Chi-squared goodness-of-fit test using POLO computer program. This is specifically developed to analyze data obtained from insecticide bioassays (Russell and Robertson, 1979). Dose–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 $P < 0.05$ [Table 1].

RESULTS

Wood Seasoning

Solid wood sticks

In these experiments, seasoned wood sticks were planted in the soil at an equal distance. It was found that *T. erecta* extract and its combinatorial mixture have shown anti-termitic activity in seasoned wood sticks which planted in the soil [Graph 1]. Results showed that wood seasoning protected wood weight losses and termite infestation in comparison to unseasoned wood sticks.

- Set no. 1: Very high anti-termitic activity was obtained in S-AST-A, S-AST-B, and S-AST-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-AST-A mixture showed 34–63% wood protection and it effectively prevented termite infestation in comparison to control. Similarly, S-AST-B mixture caused significant decrease both in percent weight loss 51–86%, S-AST-C mixture gave better wood protection approximately 73–82%, and a very significant decrease in termites infestation [Table 4 and Figure 2a-c]
- Set no. 2: In another experiment, wood seasoning was done using B-AST-A, B-AST-B, and B-AST-C mixtures. Out of which B-AST-C mixture was found more effective in comparison to other mixtures. The percent weight loss in wood sticks seasoned with B-AST-A mixture was obtained in a range of 57–78% [Table 4] while termite infestation was obtained in a range of 32–56%. Similarly, both B-AST-B and B-AST-C mixtures have shown very high wood protection, that is, 34 and 76% a significant decrease in termite infestation, respectively [Table 4 and Figure 2a-c]
- Set no. 3: In this experiment, C-AST-A, C-AST-B, and C-AST-C mixtures were used to evaluate the termiticidal and repellent properties of isolated mixtures. Highest protection in wood weight loss was observed in C-AST-C mixture, that is, 09–39% after 180 days. Similar activity was obtained in C-AST-A and C-AST-B mixtures. Above

Table 4: Estimation of weight, termite, and infestation of 1 ft solid wood sticks experiment up to 6 months

S. No.	Combination	Conc. variation	Month	Control termite count	Termite count	Initial weight (g)	Final weight (g)	Weight loss (g)	% infestation
	S-AST	A	1	45	25	560	510	050	34 (66)
			2	60	38	390	340	050	39 (61)
			3	88	30	380	320	055	53 (47)
			4	86	46	480	425	060	55 (45)
			5	95	57	540	400	140	60 (40)
			6	105	41	570	420	150	63 (37)
		B	1	45	27	520	440	080	33 (67)
			2	60	25	460	380	080	35 (65)
			3	88	35	470	390	080	39 (61)
			4	86	44	440	350	090	41 (59)
			5	95	32	750	590	120	51 (49)
			6	105	37	530	410	160	60 (40)
		C	1	45	13	530	410	050	17 (83)
			2	60	14	430	380	070	21 (79)
			3	88	19	560	480	080	23 (77)
			4	86	21	590	460	090	24 (76)
			5	95	17	480	390	120	27 (73)
			6	105	29	470	400	130	28 (72)
	C-AST	A	1	45	47	590	500	070	10 (90)
			2	60	48	460	390	080	35 (65)
			3	88	31	390	310	090	48 (52)
			4	86	42	710	580	130	49 (51)
			5	95	47	590	450	140	53 (47)
			6	105	56	740	590	150	80 (20)
		B	1	45	25	750	640	030	25 (75)
			2	60	17	520	490	070	28 (72)
			3	88	22	510	440	090	34 (66)
			4	86	33	590	500	110	38 (62)
			5	95	33	530	420	110	40 (60)
			6	105	42	870	710	160	55 (45)
		C	1	45	22	510	430	060	16 (84)
			2	60	13	570	490	070	19 (81)
			3	88	17	660	540	080	20 (80)
			4	86	14	500	430	080	21 (79)
			5	95	19	680	620	120	23 (77)
			6	105	25	490	370	120	48 (52)
	B-AST	A	1	45	27	470	410	050	37 (63)
			2	60	33	670	610	060	45 (55)
			3	88	40	320	180	140	55 (45)
			4	86	52	540	360	150	60 (40)
			5	95	36	820	670	150	60 (40)
			6	105	46	850	640	210	72 (28)
		B	1	45	20	580	510	060	18 (82)

(Contd...)

Table 4: *(Continued)*

S. No.	Combination	Conc. variation	Month	Control termite count	Termite count	Initial weight (g)	Final weight (g)	Weight loss (g)	% infestation
			2	60	22	390	330	060	26 (74)
			3	88	16	460	370	070	31 (69)
			4	86	27	640	480	090	33 (67)
			5	95	32	700	640	140	36 (64)
			6	105	28	690	550	160	44 (56)
		C	1	45	12	600	510	050	10 (90)
			2	60	14	450	400	090	13 (87)
			3	88	27	570	480	090	20 (80)
			4	86	09	610	520	090	23 (77)
			5	95	13	680	570	080	26 (74)
			6	105	22	450	370	110	30 (70)
	CU-AST	A	1	45	13	390	330	060	13 (87)
			2	60	24	390	320	070	23 (77)
			3	88	12	610	530	080	40 (60)
			4	86	43	390	200	160	50 (50)
			5	95	49	710	540	170	50 (50)
			6	105	53	690	530	190	51 (49)
		B	1	45	29	410	350	060	13 (87)
			2	60	14	350	290	060	20 (80)
			3	88	20	470	400	070	22 (78)
			4	86	12	650	520	130	22 (78)
			5	95	19	530	400	130	23 (77)
			6	105	24	590	430	160	64 (36)
		C	1	45	11	320	270	050	10 (90)
			2	60	09	440	370	070	10 (90)
			3	88	14	440	300	080	15 (85)
			4	86	09	410	300	090	15 (85)
			5	95	10	650	560	110	17 (83)
			6	105	18	610	530	140	24 (76)
	Malathion	-	1	45	5	360	310	050	11 (89)
			2	60	4	440	410	020	06 (94)
			3	88	8	270	250	030	09 (91)
			4	86	9	380	350	030	10 (90)
			5	95	12	460	420	030	12 (88)
			6	105	18	390	360	040	17 (83)
	Fipronil	-	1	45	10	410	380	020	11 (89)
			2	60	11	450	420	030	11 (89)
			3	88	10	340	310	030	16 (84)
			4	86	14	520	500	030	17 (83)
			5	95	11	350	320	030	18 (82)
			6	105	18	320	290	030	22 (78)

(Contd...)

Table 4: *(Continued)*

S. No.	Combination	Conc. variation	Month	Control termite count	Termite count	Initial weight (g)	Final weight (g)	Weight loss (g)	% infestation
	Thiamethoxam	-	1	45	03	350	320	030	06 (94)
			2	60	08	450	420	030	09 (91)
			3	88	11	360	320	030	10 (90)
			4	86	09	430	400	040	12 (88)
			5	95	14	310	270	040	13 (87)
			6	105	10	610	520	090	14 (86)

*Abbreviation (bracket) shows the % reduction

Figure 2: (a-i) Treatment of wood sticks and use of poison baits for control of field and garden termite

mixture was also successfully controlled the termite infestation in treated wood sticks as percent termite infestation recorded was very low, that is, 13–51% in the presence of C-AST-C mixture, 28–62% in the presence of C-AST-B mixture, and 25–76% in the presence of C-AST-A mixture, respectively [Table 4 and Figure 2a-c]

- Set no. 4: In this experiment, photoactivated cow urine was used for wood seasoning. It has also cut down weight loss up to 9–55% up to 6 months [Table 4 and Figure 2a-c]
- Set no. 5: Percent weight loss in the presence of inorganic pesticides such as *malathion*, fipronil, and *thiamethoxam* was also recorded. Malathion seasoned wood sticks have shown 04–24% weight loss and 06–14% percent infestation. Fipronil has shown significant reduction in weight loss (17%) and termite infestation (26%). Weight loss of thiamethoxam seasoned wood sticks ranges between 06% and 29% while percent termite infestation ranges between 06% and 23% [Table 4 and Figure 2].

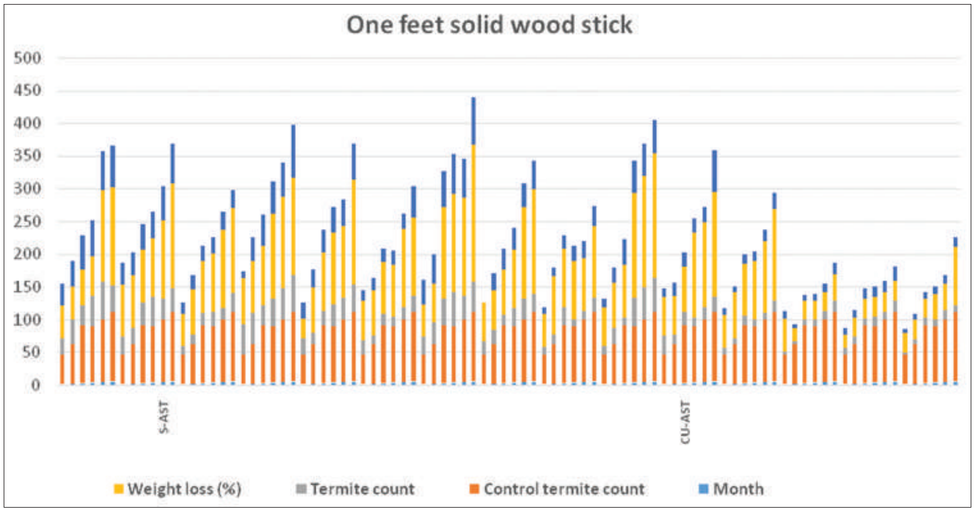
Solid wood sticks

In these experiments, fixed *T. erecta* essential oil extract mixture was shown weight loss 26–35%, fixed oil extract + sulfur is 08–26%, and fixed oil extract + sulfur + cow urine mixtures is 16–34% in 6 months duration. Their maximum % of infestation is 28%, 44%, and 36%, respectively. Fipronil was shown 47% termite infestation [Graph 2; Figure 2a and b].

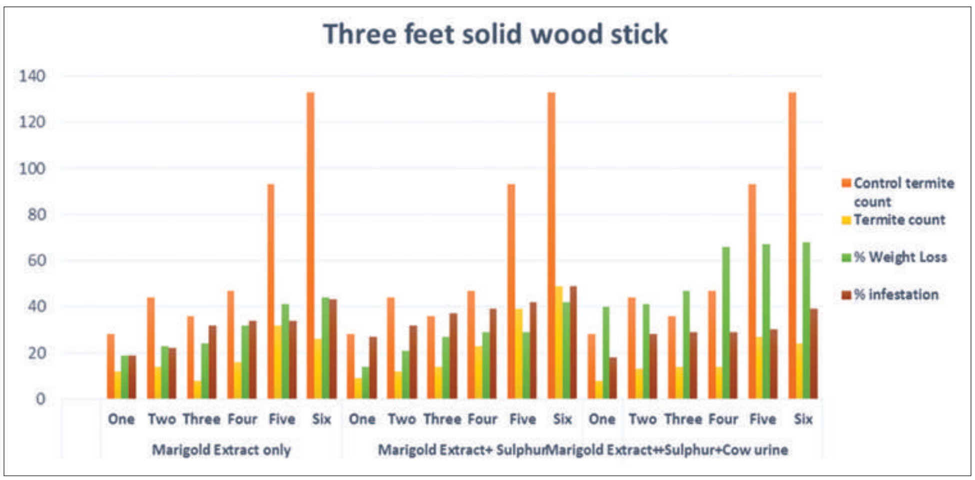
Hollow bamboo wood sticks

In this set of experiment, bamboo wood sticks were seasoned with highest concentration of combinatorial mixtures. Therefore, various combinatorial mixtures, that is, AST-Marigold Extract AST-Marigold Extract + Cow urine and AST-Marigold Extract Cow urine + Cow Dung 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.

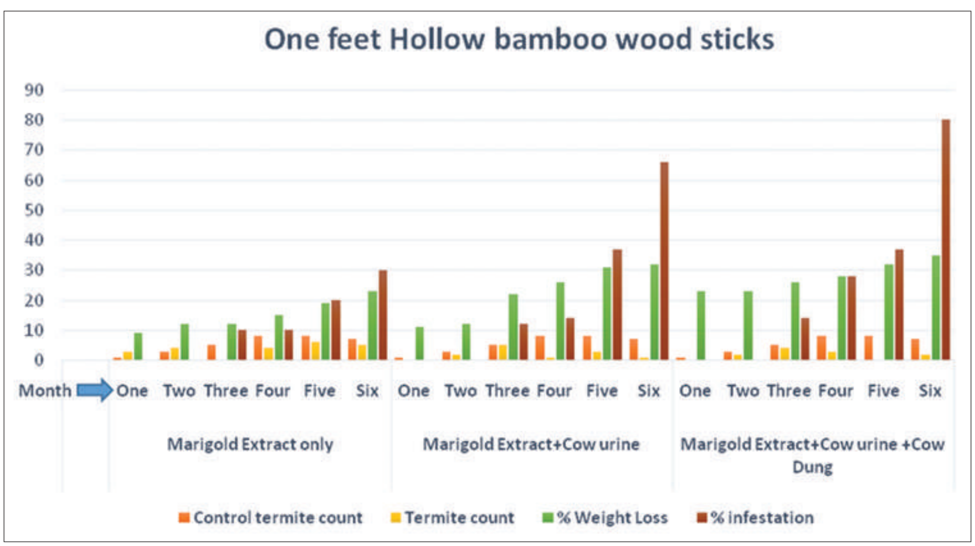
- Set no. 1: AST 6-Marigold Extract treated bamboo sticks very significantly cut down percent weight loss and recorded 10% in the 3rd month of experiment after which no termite infestation was observed in any bamboo sticks [Graph 3 and Figure 2]
- Set no. 2: Similarly, AST-Marigold Extract + Cow urine 4 treated bamboo wood sticks have shown a significant decrease in percent weight loss and recorded 19% while percent infestation was significantly decreased up to 15%, respectively [Graph 3 and Figure 2d and e]
- Set no. 3: Marigold Extract + Cow urine +Cow Dung-5 treated bamboo wood sticks percent weight loss was decreased and recorded 29% while almost no 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 [Graph 3]
- Set no. 4: In this experiment, control mixture of cow urine and cow dung was shown high termite infestation in comparison to combinatorial mixtures [Graph 3]



Graph 1: The % of weight loss, % infestation, and termite count



Graph 2: The % of weight loss, % infestation, and termite count



Graph 3: The % of weight loss, % infestation, and termite count

- Set no. 5: Percent weight loss was found high in case of fipronil seasoned bamboo wood sticks, that is, 3–20% and high termite infestation was observed in fipronil, that is, 25–80% [Graph 3 and Figure 2d-f].

Hollow bamboo wood sticks

In these bioassays, fixed *T. erecta* essential oil extract mixture was shown weight loss 10–35%, fixed oil extract + sulfur is 12–38%, and fixed oil extract +sulfur + cow urine mixtures is 06–15% in 6 months duration. Their maximum % of infestation is 28%, 29%, and 11%, respectively. Fipronil was shown 49% termite infestation [Graph 4 and Figure 2d-f].

Poison Bait

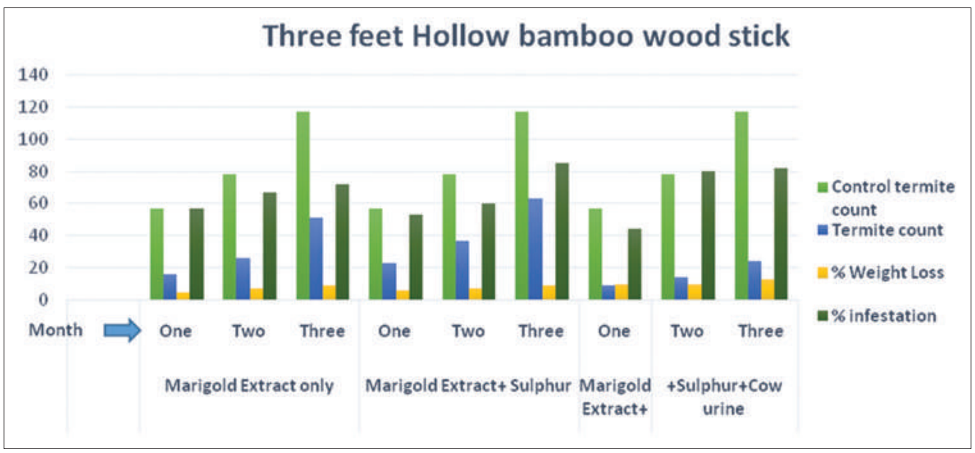
In these bioassays, fixed *T. erecta* essential oil extract mixture was shown maximum infestation is 39% in the 6th month, fixed oil extract + sulfur is 49%, and fixed oil extract + sulfur + cow urine mixtures is 43% in 6 months duration. Their maximum termite count is 36, 52, and 38, respectively. While fipronil was shown 38% termite infestation and 40 termites [Graph 5 and Figure 2g].

Thread-Binding Bioassays

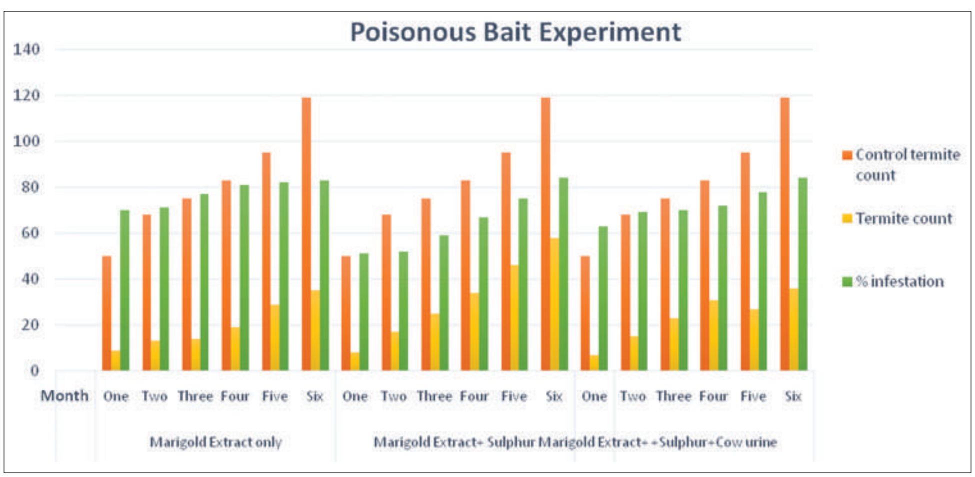
For effective management of termites in garden, pre-soaked cotton threads were tagged around the tree trunks at a height of 5–6 ft above the ground. Termite infestation on these tagged trees was significantly decreased after 6 months of thread binding and mud plastering and tunnels were found shed off 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 96% control was observed in test plants. There was a significant decrease in number of infested plants and termite occurrence after 6 months [Graph 6; Figure 2h and i].

DISCUSSION

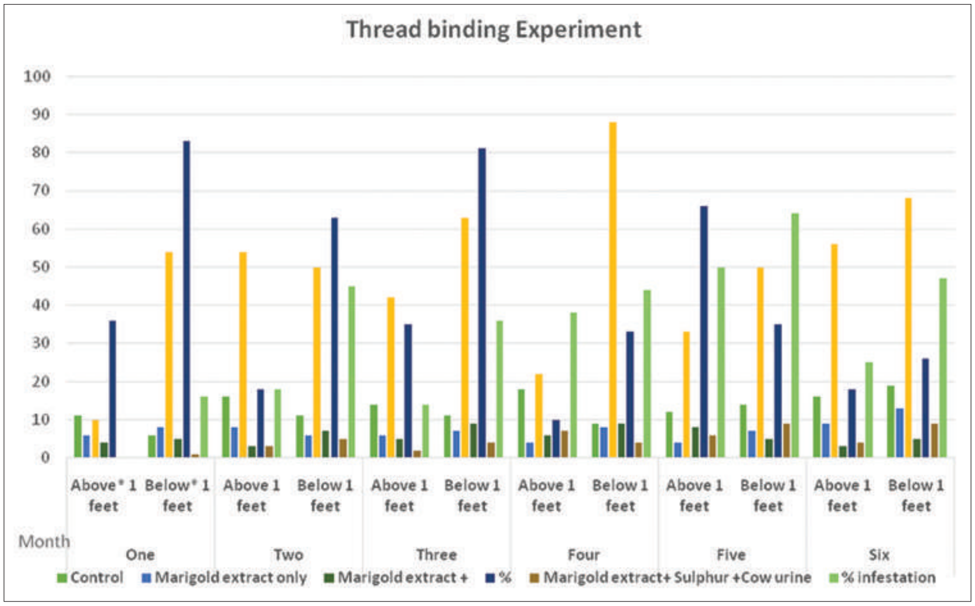
In this study, different solid wood sticks and hollow bamboo wood sticks were flavoring were setup totally different crop field and cultivate area conjointly sowing maize and millets along it. Other than this, string official measures on swarmed



Graph 4: The % of weight loss, % infestation and termite count



Graph 5: The % of weight loss, % infestation, and termite count



Graph 6: The % of weight loss, % infestation, and termite count

saplings of *T. grandis* (family *Verbenaceae*) and poison bait bioassay in maize edit field were also conducted to check the mud putting, tunneling movement, and up and descending developments of termites. Distinctive combinatorial blends of *T. erecta* were found exceedingly viable against termites. It were demonstrated by exceptionally moo percent of pervasion values such as S-AST-C, C-AST-C, and CU-AST-C blend has appeared 10%, 12%, and 14%, separately [Table 3, Figure 2a and b]. Crucial oils and/or their subordinates may be one of the potential sources of antischistosomal drugs.[27] The antimicrobial development was inspected against three microorganisms (*Staphylococcus aureus*, *Listeria monocytogenes,* and *Pseudomonas aeruginosa*). "*Sanguinello*" and "*Solarino Moro*" essential oils are basically energetic against *L. monocytogenes*, though "Valencia" hexanic removes against all the attempted microorganisms.[28]

Volatile Basic Oils Can Be Utilized to Progress the Adequacy of Warm Medications Focusing on the Western Drywood Termite: Prove from Recreated Entire House Warm Treatment Trials.[29] *A. trilobata* EO and its constituents, particularly the limonene, are promising for the control of *Nasutitermes corniger* due the tall poisonous quality, repellence, and conceivable unsettling influence within the colonies.[30] Basic oils and *ethanolic* extricate from *Cunninghamia konishii*, and their viable constituents, served as potential, eco-friendly termite-control agent,[31] fundamental oils from *Santolina chamaecyparissus*, *Ormenis multicaulis,* and *Eriocephalus punctulatus* (+)-α-pinene, (-)-limonene,(-)-α-pinene, β-pinene, and β-phellandrene against Japanese termite.[32] Essential oils isolated from various species of *Citrus* contain highly effective bio-organic components such as monoterpene, limonene, β-pinene, geranyl acetate, and verbenone from *T. erecta.*[33] *T. erecta* combinatorial mixtures were also evaluated in field. These seasoned wood sticks were planted in the soil after immersing them in various combinatorial mixtures separately for overnight. As in the tests wood sticks, less number of termites was observed. Besides this, *T. erecta* and its combinatorial mixtures significantly reduced the wood consumption and wood weight loss. Wood consumption is decided on the basis of wood softness as termites feed more amounts of soft woods than hard wood.[34,35] In the present study, percent termite infestation was also found to be reduced due to wood seasoning. Mostly, it was found to be decreased with increasing concentrations and time duration. However, due to leaching of pesticides, wood infestation was found to be increased in underground wood sticks.[36]

In wood seasoning experiments, 03–07% weight loss was obtained in S-AST-B and S-AST-C treated wood sticks, respectively. Similarly, B-AST-A mixture treated wood sticks showed approximately 05% weight loss up to 30 days of the treatment but later on, it was increased up to 53% after the 5th month of the treatment [Graph 5 and Figure 2g]. Again in a similar experiment, reduction in wood weight loss was obtained, that is, 04% in photoactivated cow urine (CU-AST-C) seasoned wood sticks. Besides this, B-AST-B, C-AST-A, C-AST-B, and CU-AST-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 *T. erecta* combinatorial mixtures. that is, 06–38% after the 5th month of treatment [Table 3]. Results obtained from wood seasoning experiments indicate that *T. erecta* combinatorial mixtures favorably increased resistance in wood sticks against termite up to 6 months. Therefore, it can be said that *T. erecta* have few active components which can be used for the development of a new termiticidal formulation that can be used as an effective wood preservative.[37] In another experiment, anti-termitic activity of all the above mixtures was tested in 1 ft hollow bamboo wood sticks. For this purpose, each combinatorial mixture was mixed with wood ash and poured inside hollow bamboo sticks and planted underground. AST-Marigold Extract, AST-Marigold Extract + Cow urine, and AST-Marigold Extract + cow urine + cow dung treated bamboo sticks have shown minimum weight loss, i.e.. Treated bamboo sticks have shown minimum weight loss, that is, 06–13% against termite [Graph 3; Figure 2d and e]. While in AST-Marigold Extract, AST-Marigold Extract + Cow urine, and AST-Marigold Extract + cow urine + cow dung, treated bamboo sticks approximately zero percent infestation was found in the 3rd, 1st, and 5th month, respectively. Contrary to this, fipronil treated wood sticks have shown 20% weight loss up to 6th month of treatment [Graph 3, Figure 2d and e]. In 3 ft solid and hollow bamboo wood sticks, *T. erecta*, sulfur, and photoactivated cow urine combinatorial mixture were showed minimum weight loss, that is, 06–49% and termite infestation was 13–38%.

Besides this, poison bait was also more significant to control termite infestation in crop field because it reduced 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. It was proved by reducing termite count and mud plastering around the plants. For wood protection, wood seasoning was also 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] Similarly, plant-derived natural products such as Vulgarone B (*Artemisia douglasiana)*, apiol (*Ligusticum hultenii*), and cinicin (*Centaurea maculosa)* significantly exhibited higher mortality than synthetic pesticides besides this, flavonoids also showed antifeedant activity against *C. formosanus* Shiraki.[40] Besides this, few low persistence toxic pesticides such as organophosphates, carbamates, and pyrethroids were also used for termite control in soil. But nowadays, many of these synthetic pesticides have been phased out because of health and environmental reasons. Although these pesticides work as repellents,[41] termites have developed the ability to detect them in soil when used as barrier. It was mostly found that on an average, *T. erecta* and its combinatorial mixtures have shown better toxicity than synthetic pesticides. Limonoids are a group of highly oxygenated terpenoid secondary metabolites found mostly in the seeds, fruits, and tissues of citrus fruits. Limonin, the aglycones, and glycosides of limonoids show insecticidal activity.[42] *C. sinensis* natural products are also found beneficial for human health and used to develop new drugs.[43] These plant-origin natural pesticides control wide range insect pests efficiently and cut down the pest population even applied in very low quantity.[44] These are much safer, low cost, and easily biodegradable in the medium and show no residual effect. This plant chemical diversity is also used to control specialized natural enemies. Hence, with the increasing spread of termite infestation, there is an increased need to discover and use effective, environmentally friendly, and safe termite control agents with minimal mammalian toxicity.[45]

CONCLUSION

Termites are the highly destructive polyphagous pests that inhabit in high humidity zones of forests and gardens field crops. Although, several methods are used to control termite population in the infield. From the results, various combinatorial mixtures of *T. erecta* essential oils were found active against termites. These were tested 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 foliage in maize and millets crops in the agriculture crop field. From results, it is clear that *Citrus maxima* 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. These also have shown synergistic activity in poison waits. This termite activity in essential oils of *T. erecta* is contributed by bio-organic components such as flavonoids sesquiterpenes and thiophenes. These active components found flowers of *T. erecta can* be used for the development of a new termiticidal formulations and preservative of commercial wood.

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