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TOXIC & REPELLENT ACTIVITY OF *TAGETES ERECTA* ESSENTIAL OIL BASED COMBINATORIAL FORMULATIONS ON INDIAN WHITE TERMITE *ODONTOTERMES OBESUS*

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ABSTRACT

In present investigation *Tagetes erecta* and its combinatorial formulations were evaluated to observe the anti-termite efficacy against Indian white termite *Odontotermes obesus*. These have shown very high termiticidal activity and repellency against present termite species. It was proved by very low LD_{50} values obtained i.e. B-AST-A 261.930 and S-AST-C 507.666 respectively. *Tagetes erecta* termiticidal potential was enhanced when other bio-material were added to them in a defined ratio. This synergistic action caused very high mortality in termites. Besides this, combinatorial mixtures significantly repelled termites in two Choice bioassays. These also have shown strong anti-feedant action in termite workers.

KEYWORDS: *Odontotermes obesus*; *Tagetes erecta*; Combinatorial formulations, Toxicity; Repellency and Synergistic effects**.**

INTRODUCTION

Termites are detrivorous social insects live in colonies belong to class Insecta and family Termitidae. Termite workers and soldiers possess cutting and chewing mouthparts and heavily eat upon cellulose containing plant materials and crop plants. These house endosymbiotic protozoan inside their gut that secrete eat cellulose enzyme that assists in digestion of woody materials. Termites massively feed on field crops and make burrows and huge mounds in sub tropical loam soil. There are 1231 species of termites belong to families. In Indian 112 species have reported. Among which *Odontotermes obesus* is highly damaging. *Reticulitermes* species is highly destructive polyphagous pest reported from China.[1]

For termite control various synthetic pesticides such as cyclodiene,[2] cypermethrin,[3] hydro quinone and indoxocarb.[4] have been used at large scale. But these pesticides persist for longer duration and non-target organisms and enter into food chain and under go bio-magnification. Heptachloro applied for termite control agents toxicity, many synthetic pesticides are banned.[5] Hence, their new alternatives have been discovered in form of natural or plant origin pesticides. Chlorpyrifos and thiamethoxam are used to kill underground termites.[6,7]

In comparison to synthetic pesticides plant-origin pesticides are safer and biodegradable within the soil and show no residual effect. Few plant species such as *Pseudotusuga menziesii, Lysitoma seemnii, Tabebina guaycan, Diospyros sylvatica,*[8] *Artemisia Chrysanthemum roseum, and C. cinerriefolium*[9] *Curcuma fragrant and Euphorbia kansuii*[10] have been investigated for their anti-feedant and insecticidal activity. These significantly cut down fecundity and survival of insect pests[11,12] These are low toxic and effective as they showed anti-feedent, repellent in field termites.[13,14] Few plant Essential Oils such as *L. Alba posses 1, 8 cineole* that show efficacy against termite *Nasutitermes corniger* and *maize weevil Sitophilus zeamais* at (LC50: 0.297 μL/g)[15] *Artemisia dracunculus L.* (tarragon), Asteraceae, contains components as flavonoids, phenolic acids, coumarins and alkamides, and show anti-temrite activity. Curcuma essential oils, and potential security concerns of Curcuma crucial oils and their components.[16] Essential oil of hiba wood includes a loosening up woody fragrance and antifungal properties[17] However, to enhance the lethality and target specific to natural pesticides few synergists are also used. These synergists in form of poison baits more efficiently exploit feeding and tunnelling behavior in termites. Bio – control agent *metarhizium anisopliae* is, to used underground termite *Coptotermes curvignathus*[18] Besides this, plant-based Nano formulations are which massively target termites as stomach posion[19] Similarly novaluron snare is used against subterranean termites, *Coptotermes* infestations.[20,21] In the present study,anti-termite efficacy of various solvent fractions of *Tagetes erectas* was determined. For this purpose various toxicity, repellency, wood seasoning and tag binding bioassays were conducted in the laboratory and field.

MATERIALS AND METHODS

Collection of Termites

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

Collection of plant material: Flowers of *Tagetes erecta* Asteraceae family were collected from different places of Gorakhpur Uttar Pradesh, India. The solubilized extracts were filtered with Whatmann paper. No 1 and were concentrated under vacuum (30^{0}C). After evaporation of water, it was weighed and solubilized in known volume of double distilled water.

Extraction and Isolation of Essential Oil: Flowers *of Tagetes erecta* were grounded by using domestic mixer and powdered material was hydro-distilled in Clevenger apparatus continuously for 5 hrs to yield essential oil. The crude powder was extracted with pure methanol twice and dried residue will dissolved in known volume of fresh solvent (w/v) before testing the anti-termite activity.

Preparation of combinatorial formulations

Various ingredients were used in preparation of combinatorial mixtures. These have been explained in Table 1 as following. In all formulations *Tagetes erecta* fixed essential oil was added to see its termiticidal potential and synergistic action of some natural and other inorganic materials.

Toxicity Bioassay

For evaluation of dose response relationship of different essential oil extracts, different doses (w/v), *i.e.* 10,20,40,60,80, and 100 µl of different extracts were loaded on separate Whatmann paper strips (1 × 1 cm2) and air dried to remove the solvent. These pre-coated solvent free strips were placed in the centre of separate Petri dishes (42 mm diameter) as tests and uncoated as control. Ten 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 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. The LD50 after 24 h of exposure to each was calculated by using Probit analysis tested using the method of Finney.[14]

Feeding inhibition and Repellency

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

STATISTICAL ANALYSIS

Standard deviations chi-square, t-significance, correlation, and ANOVA were calculated from the means of two replicate using three equal sub samples from each replicate by using method of Sokal and Rohfl.[23] In the experiments analysis of variance (ANOVA) was done whenever two means were obtained at a multiple test range and $p < 0.05$ probability level. The LD50 after 24 hrs of exposure were calculated by applying POLO pro- gram.[24]

RESULTS

Toxic and repellent responses of various essential oil fractions, crude essential oil and its various combinatorial formulations were applied against Indian white termite *O. obesus* in the crop field and laboratory. For evaluation of toxicity and essential oil generated effects, insects were treated with increasing dose of various essential oil fractions, crude essential oil and its various combinatorial formulations separately. *Tagetes erecta* and other ingredients used in preparation of combinatorial mixture shown in (Table 1) The mortality rate was found dose and time dependent as it was found to be increase with an increase with an increase in dose and exposure period. The LD50 values for different essential oil fractions of 24 h are given in (Table 2). Solvent extracts have shown LD50 in a range of 30.147- 50.601 µg/mg while combined mixtures of have shown synergistic activity against termites and caused comparably high mortality with LD50 577.159 - 236.106 µg/mg (Table 2).

Among all the fractions, hexane fraction has shown highest toxicity in comparison to other fractions. It has shown very high anti-termite potential against *O. obesus* with an LD50 value of 50.601 µg/mg (Table 2). Among the combinatorial formulations have shown significantly much higher toxicity to the *O. obesus* as the LD 50 obtained was the lowest one, *i.e.* 236.106 μg/mg (Table 2). It is highly noticeable that *Tagetes erecta* fractions in termites remain active for longer duration and cause high lethality. The index of toxicity estimation indicates that the mean value was within the limit at all probabilities (90%, 95% and 99%) as it is less than 0.05 values of t-ratio. Besides this, regression was also found significant. The steep slope values indicate that even small increase in the dose causes high mortality. Values of the heterogeneity less than 2.0 denotes that in the replicate test of random sample, the dose response time would fall within 95% confidence limit and thus the model fits the data adequately (Table 2). The percent repellency observed in highest repellency was obtained in S-AST-B (82%), Cu-AST-A (78%), and Cow-AST-A (80%) (Table 3). Similarly photo-activated cow urine has shown very high percent repellency i.e. Cow-AST-B (82%) against termites contrary to this, inorganic pesticides have shown very low repellent activity against termites. (Table 3)

Both *Tagetes erecta* crude essential oil and combinatorial mixture have shown high repellency at a very low concentration range.

Table 1: Tagetes erecta and other ingredients used in preparation of combinatorial mixture.

S.NO	Combinatorial mixture	Ingredients
1	AST- S – A	*Tagetes erecta* Extract (90 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Sulphur (30gm) + Water (15 litre)
2	AST - S – B	*Tagetes erecta* Extract (120 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Sulphur (30gm) + Water (15 litre)
3	AST - S – C	*Tagetes erecta* Extract (180 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Sulphur (30gm) + Water (15 litre)
4	AST -B – A	*Tagetes erecta* Extract (90 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Borate (30gm) + Water (15 litre)
5	AST- B – B	*Tagetes erecta* Extract (120 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Borate (30gm) + Water (15 litre)
6	AST –B – C	*Tagetes erecta* Extract (180 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Borate (30gm) + Water (15 litre)
7	C-AST – A	*Tagetes erecta* Extract (90 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Copper (30gm) + Water (15 litre)

8	C-AST – B	*Tagetes erecta* Extract (120 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Copper (30gm) + Water (15 litre)
9	C-AST – C	*Tagetes erecta* Extract (180gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol(50 ml) + Copper (30gm) + Water (15 litre)
10	CW- AST – A	*Tagetes erecta* Extract (90 gm) + Photo-activated cow urine (120ML)+ Water (15 litre)
11	CW- AST –B	Extract (120 gm) + Photo-activated cow urine (120ML)+ Water (15 litre)
12	CW -AST – C	*Tagetes erecta* Extract (180 gm) + Photo-activated cow urine (120ML)+ Water (15 litre)
13	Fipronil	Fibronil (7.5 g\L) Water (15 litre)
14	Thiomethoxine	Thiomethoxine (7.5 g\L) Water (15 litre)
15	Melathion	Melathion (7.5 g\L) Water (15 litre)
16	AQ- AST	*Tagetes erecta* extract (40gm) + water (200 ml)
17	A-AST	*Tagetes erecta* extract (40gm) + Acetone (200 ml)
18	H-AST	*Tagetes erecta* extract (40gm) + Hexane (200 ml)
19	P-AST	*Tagetes erecta* extract (40gm) + Petroleum ether (200 ml)

Table 2: Different combinatorial mixture data analysis.

S. N.	Name of Extract/Combinatorial Mixture	LD50 µg/gm	0.95 confidence limit UCL-LCL	Chi-Square	Slope function	Degree of freedom	Heterogeneity	LD40µ g/gm	LD20 µg/gm
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	-	-

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

S.NO	Mixtures	Doses Ul	Percentage	Mean ± SE
1	S-AST-A	10	09	1 ±0.2582
		20	17	2.16 ±0.3073
		40	38	3.166±0.3073
		60	59	4.5±0.4282
		80	67	6±0.3651
		100	79	8±0.3651
		10	08	**1.**33±0.3333

2	S-AST-B	20	18	2.33±0.3333
		40	35	4.16±0.3073
		60	60	5.66±0.3333
		80	72	7.83±0.3073
		100	82	8.5±0.2236
3	S-AST-C	10	4	1.33±0.3333
		20	13	2.5±0.2236
		40	27	3.71±0.1844
		60	40	6±0.3651
		80	52	7.16±0.6009
		100	70	8.66±0.2108
4	B-AST-A	10	10	1.16±0.3073
		20	14	2.83±0.3073
		40	28	3.83±0.3073
		60	45	5.33±0.2108
		80	62	7.33±0.4216
		100	76	8.5±0.2236
5	B-AST-B	10	7	1.5±0.2236
		20	13	3±0.3651
		40	25	4.33±0.3333
		60	38	5.66±0.2108
		80	56	6.5±0.4282
		100	68	8.33±0.3333
6	B-AST-C	10	3	1±0.3651
		20	12	3.33±0.3336
		40	23	4.16±0.3073
		60	34	5.33±0.3333
		80	49	7.5±0.4282
		100	68	8.5±0.2236
7	Cu-AST-A	10	08	1.16±0.1667
		20	19	2±0.2582
		40	32	3.16±0.3073
		60	48	4.66±0.4944
		80	64	6.16±0.3073
		100	78	8.5±0.2236
8	Cu-AST-B	10	06	1.5±0.2236
		20	13	2.66±0.3333
		40	26	4.33±0.2108
		60	36	5.66±0.4216
		80	48	7.5±0.2236
		100	64	8.66±0.2108
9	Cu-AST-C	10	07	1.33±0.2108
		20	15	2.16±0.3073
		40	28	3.5±0.2236
		60	45	5.33±0.3333
		80	60	7.1±0.3073
		100	74	8.83±0.3073
10	Cow-AST-A	10	05	1.33±0.3333
		20	13	2.33±0.33333
		40	27	3.8 ±0.4773

		60	39	6±0.3651
		80	58	7.6±0.3333
		100	80	9.16±0.3073
11	Cow-AST-B	10	08	1.5±0.2236
		20	17	2.83±0.3073
		40	28	4.16±0.6009
		60	42	6±0.5774
		80	58	7.33±0.4216
		100	82	8.5±0.2236
12	Cow-AST-C	10	09	0.83±0.3073
		20	19	0.83±3073
		40	30	2.33±0.3333
		60	45	3.83±0.4014
		80	60	5.16±0.3073
		100	80	6.66±0.7149
				8.33±0.3333
13	AQ-AST	10	10	1.5±0.2236
		20	22	3.33±0.4216
		40	38	5.16±0.3073
		60	56	6.16±0.3073
		80	65	7.5±0.2236
		100	78	8.16±0.3073
14	A-AST	10	07	1.5±0.2236
		20	14	9.4±6.1693
		40	30	5.16±0.3073
		60	45	6.16±0.3073
		80	58	7.5±0.2236
		100	72	7.83±0.3073
15	H-AST	10	12	1.16±0.3073
		20	25	2.16±0.3073
		40	36	2.5±0.2236
		60	48	4.5±0.4282
		80	60	5.5±0.2236
		100	75	7.5±0.2236
16	P-AST	10	06	1.5±0.2236
		20	14	1.66±0.2108
		40	27	4.33±0.3333
		60	38	4.66±0.3333
		80	55	5.83±0.3073
		100	68	7.3±0.33333

DISCUSSION

In present investigation toxic and repellent effects *Tagetes erecta* essential oil (fixed oil) based combinatorial formulations were tested against Indian white termite *Odontotermes obesus*. *Tagetes erecta* solvent extracts have shown very high lethality which is proved by very low LD 50 value, that is, 30.147- 50.106 μg/mg obtained. (Table 2) Further, addition of coconut and terpene oil, glycerol, and sulphur in *Tagetes erecta* have shown synergistic activity against termites. (Table 1) In such combinations, LD50 was found in arrange of 236.106–577.159µg/mg (Table 2). Highest repellency was obtained in S-AST-B (82%), Cu-AST-A (78%), Cu-AST-A (78) and Cow-AST-A (80%) (Table 3). Similar toxic and repellent activity of plant products have been reported on subterranean termites by Blaske et al,[25] Besides this, *Chamaecyparis nootkatensis, Sequoia sempervirens*, and *Pseudotsuga menziesii* also showed high antifeedant and toxic activities against termites.[26] It is fact that synthetic pesticides i.e. thiamethoxam and Chlorpyrifos show high toxicity against termites but these cause serious damage to environment, human health and kill non target organisms).[27] Poison baits were also used to kill Formosan underground termites *(Isoptera: Rhinotermitidae*) by using chitin amalgamation inhibitor traps.[28]

Similarly, *Aleurites fordii* have shown anti-termite potential (Tung tree) extracts against *Reticulitermes flavipes* at 0.1 to 5.0% w/w[29] acetonaphthone also obstructed tunneling and feeding behavior in Formosan subterranean termite *Coptotermes formosanus Shiraki* at 8.33 mg/kg concentration[30] Similarly, in a filter paper-based bioassay for termiticide, *guineesine*, a minor constituent isolated from *Piper nigrum*. This natural product has shown >90% mortality in termites at 1% wt/wt application. *Pongamia pinnata* wood extracts also showed anti-termite activity against *Coptotermes heimi (Wasmann)* at three different concentrations no-choice tests.[31]

These formulations also have repelled termites at a very concentration of each formulations tested in two choice bioassays. Repellency provided extra advantage to wood seasoning and increased its protection from termite infestation and damage by *O. obesus* in the field. Similarly photo- activated cow urine has shown very high percent repellency i.e. Cow-AST-B (82%) against termites contrary to this, inorganic pesticides have shown very low repellent activity against termites. (Table 3) Simialrly, root extracts of *Diospyros sylvatica* impose significant repellent activity and cause high mortality in subterranean termite, *Odontotermes obesus* in filter paper disc bioassay due to presence of *plumbagin*, *isodiospyrin*, and *microphyllone* or *quinnones.*[32] Besides plant extracts, essential oils have also shown very strong repellent and toxic activity against *Formosan subterranean* termite due to presence of volatile compounds.[33] Similarly, patchouli oil and patchouli alcohol have shown high toxicity and repellency against same species.[34] Both have caused tissue destruction inside exoskeleton of the termites due to contact activity.[35]

Similarly, vetiver oil, nootkatone, and disodium octaborate tetrahydrate imposed feeding, inhibition and obstruction of wood digestion inside the termite gut.[36] Vetiver oil was found highly effective against subterranean termites without any side effect on environment.[37] Hexaflumuron and copper chloride are used in poison baits to control fungus-growing termite, Odontotermes formosanus.[38] These have been successfully prevented termite attack on garden vegetation mainly flowering plants.[39] Similarly, wood treated with copper II compounds tri and di alkylamine-boric acid complex showed lesser termite damage and acted as good preservatives.[40] *Nootkatone* affect wood consumption termite survival and affects growth of flagellate symbionts.[35] Similarly, in the present study, sulfur, added in *Tagetes erecta* combinatorial mixture significantly decreased the termite attack and damage. It may be due to action of ingredients on exoskeleton of termites and antimicrobial effect of sulfur on termite gut microflora. It is also possible that sulfur inhibits growth of microbes in the soil destroy microbial population found inside the termite gut. Termites do mud plastering on tree bark provides substratum for growth of fungi and bacteria. Presence of microbes and its secretions make hard wood soft due to enzyme activity. Presence of sulfur in natural products such as amide causes higher mortality in termites.[41] Though many commercial termiticides are available in the market to combat the destructive termites but none of them is entirely natural. The main purpose of present work was to contribute to the development of new termiticide from plant natural resource that may have better activity than synthetic termiticides. No doubt, *Tagetes erecta* possesses enough antitermite potential and its active components can be used for controlling the damage and termite infestation if used as spray, fumigant or in form of poison baits. Hence, strong recommendations are being made to develop ecofriendly ant-termite formulation from *Tagetes erecta* for effective control of field termites.

Conflict of Interests

The authors declare that they have no conflict of interests.

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