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Anti-termite efficacy of *Capparis decidua* and its combinatorial mixtures for the control of Indian white termite *Odontotermes obesus* (Isoptera: Odontotermitidae) in Indian soil

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ABSTRACT: *Capparis deciduas* and its combinatorial mixtures were evaluated to observe the anti-termite efficacy against Indian white termite *Odontotermes obesus*. These have shown very high termiticidal activity and wood protection in the soil. It is proved by very low LD_{50} values i.e. 0.0218mg/g and 0.021mg/g obtained in stem fractions CDS3 and CDS7 respectively. When other ingredients, were used to prepare combined mixtures with *C. decidua* these have shown synergistic action and caused very high termite mortality in termites. Besides this, both tag binding and wood seasoning experiments significantly cut down ($p<0.05$ and 0.01 level) in termite infestation in garden saplings and in seasoned dry wood planted in the garden soil. Above treatments have successfully obstructed the ascending and descending movements of termites and prohibited mud plastering and tunnel formation in workers. It was found statistically significant at the level 0.05. @JASEM

Termites are highly destructive polyphagous insect pests, which largely damage house hold materials, finished goods, plants and agricultural crops such as sugarcane, millet, barley and paddy. Termite colonies also heavily attack fodder crops and make tunnels in subtropical and tropical soil. For termite control in the field various synthetic chemicals such as cyclodiene (Sim *et al.,* 1998), cypermethrin (Valles and Woodson, 2002), hydroquinone and indoxocarb (Hu, 2006) have been used. But these pesticides were found cost effective, detrimental to environment and kill non-target organisms. Due to their longer residual persistence and very high toxicity, many synthetic pesticides are banned. Hence, their new alternatives have been discovered in form of natural or plant origin pesticides. These plant-origin pesticides are found much safer and easily biodegradable in the soil in comparison to synthetic pesticides. In the past for termite control few plant species such as *Pseudotusuga menziesii, Lysitoma seemnii, Tabebina guaycan, Diospyros sylvatica* (Ganapaty *et al.,* 2004), *Curcuma aromatica* and *Euphorbia kansuii* (Shi *et al.,* 2008), have been explored for their anti-feedant and insecticidal activities. These extracts heavily deter termite feeding and reduce their survival (Boue and Raina, 2003). Besides crude extracts, few active anti-termite compounds have also been isolated from plants (Kinyanjui *et al.,* 2000) and their diverse biological activities such as anti-feedent, repellent and toxic activities have been established in field termites (Motohashi *et al.,* 2000; Blaske and Hertel, 2001; Blaske *et al.,* 2003). However, to enhance the lethality and target specificity of natural pesticides few synergists are also used. These synergists in form of poison baits more efficiently exploit feeding and tunneling behavior in termites. In the present study, anti-termite efficacy of various solvent fractions of *C decidua* was determined. For this purpose various toxicity, repellency, wood seasoning and tag binding bioassays were conducted in the laboratory and field.

MATERIALS AND METHODS

Termite *O. obesus* were collected from infested logs found at the University of Gorakhpur U.P. India and near by forest area of Eastern Uttar Pradesh, India. Termites removed from plant biomass and logs were maintained in glass jars (height-24”, diameter 10”) in complete dark conditions at 28 ± 2^0C, 75±5 RH. Termites were fed on green leaves. Stem, leaves, flowers and fruits of *Capparis* were chopped in small pieces, milled to make powder, and extracted sequentially with acetone, methanol, chloroform and water. Solvent was evaporated to prepare residues. The extracts were filtered with Whatman paper No. 1 and were concentrated under vacuum (30^0C) to make about one third of original volume or until most of the solvent is removed. The remaining aqueous solution was partitioned with chloroform to remove non-polar compounds. The aqueous extracts were combined and concentrated under vacuum and freeze-dried. Preliminary trials were made in the laboratory to test the anti-termite efficacy of *C. decidua*. For toxicity bioassay, various serial concentrations of different extracts were loaded on Whatmann paper strips (1x1 cm^2) and air dried to remove the solvent. These pre-coated solvent free strips were placed in the center separate Petri dishes (42mm diameter) as tests and uncoated as control. Twenty-five worker termites were released in the Petri dish to observe the mortality and feeding behavior. Besides this, in field experiments solid wood sticks (3 ft length X 3.2’ diameter) seasoned in various mixtures for 48 hrs were planted in garden soil in separate pits of 2.75 feet depth. Separate pits

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were used for different concentration at a distance of 3 feet. Six control and 54 tests wood sticks were applied in these experiments. These treated wood sticks were dug out after each one-month duration. Experiments were run up to 180 days for observation of termite infestation on wood sticks. Weight loss/infestation, exposure period and concentration of ingredients were considered for determination of anti-termite activity in garden soil. Besides this, cotton threads pre-soaked in *C. decidua* aqueous extracts were tagged around the tree trunk at a high at of 5-6 feet above the ground. For tests 8 different rows of sagwan trees (*Tectona grandis*) having 24 plants were chosen and each was tagged with the cotton threads. In control, the same thread was tagged at the same height without coating any active fraction. These garden saplings were also sprayed with *C. decidua* aqueous extract. The LD_{50} after 24 hrs of exposure to each was calculated by using Probit analysis tested using the method of Finney (1971).

Table-1 Anti-termite efficacy of solvent extracts of *C. decidua* against Indian white termite *Odontotermes obesus*

Fraction/Extract	LD_{50} (mg/gm) body wt	UCL-LCL*	Slope function
CD S1 (Stem)	0.0812	3.45-1.95	2.213
CD S2 (Stem)	0.0193	2.54-0.19	3.885
CD S3 (Stem)	0.0218	0.278-0.205	4.01
CD S4 (Stem)	0.017	2.10-0.20	3.89
CD S5 (Stem)	0.18	2.22-0.19	3.66
CD S6 (Stem)	0.00652	0.78-0.087	2.66
CD S7 (Stem)	0.021	1.94-0.45	2.07
CD S8 (Stem)	0.025	2.6-0.30	3.41
Acetone extract (root)	0.028	0.24-0.02	1.455
Acetone extract (fruit)	0.031	0.30-0.030	3.10
Acetone extract (flower)	0.040	0.31-0.03	2.40
Combinatorial mixtures			
C. deciduas T1	27.0	7.78-2.60	1.87
C. decidua T2	5.5	2.20-0.547	2.22
C. decidua T3	22.68	3.73-1.69	1.57
Borate T1	18.56	3.79-1.14	1.75
Borate T2	53.6	13.65-4.45	1.89
Borate T3	13.0	20.0-0.430	1.64
Copper T1	1.88	1.78-0.495	2.08
Copper T2	0.84	0.980-0.179	2.65
Copper T3	1.56	1.18-0.521	1.62
Pure cow urine	19.20	1.45-0.631	1.66
Cow urine T1	35.2	2.65-0.347	3.19
Cow urine T2	50.7	9.55-1.58	2.79
Cow urine T3	15.68	6.04-0.829	3.16
Malathion	11.50	13.42-9.85	1.86
Fipronil	7.75	8.52-7.05	1.46
Thiomethoxam	9.0	10.36-7.82	1.77

* Values are mean + SE of 6 replicates. * Three different concentrations i.e.T1 6gm/l; T2 9-gm/l; and T3 contain 12gm/l *Capparis decidua* extract.CD S* denotes fraction prepared from stem.,UCL-LCL* Upper confidence limit and lower confidence limit

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

RESULTS AND DISCUSSION

In the present study, toxicity bioassays of *C. decidua* solvent fractions CDS3, CDS6, CDS2 and CDS7 were found to be highly toxic to the termites (*Odontotermes obesus*). It is proved by very low LD_{50} values obtained i.e. 0.218-0.021 mg/gm (Table-1). Besides this, combinatorial mixtures have shown synergistic activity as high mortality was observed in each combination (Table-3 & 4). LD_{50} values noted in them were very low between 0.84-27.0 mg/g-body weights of termites. Both *C. decidua* and C-CoT1, T2 and T3 mixtures have shown significantly much lower LD_{50} values in comparison to other mixtures (Table-1). Further, *C. decidua* active ingredients coated on tags were tied over tree trunks. It has shown significant reduction in search operations made by termites for feeding. Moreover, toxic and repellent action of combined mixtures has successfully checked the deposition of mud plastering and tunnel formation. It has also obstructed the ascending and descending movements of termites. It was found statistically significant at the level 0.05 and 0.01 as F-value shows that experiments are quite successful for random control of termites in the groups. [$F_{0.05}$= 2.895, $F_{0.01}$=4.455], F is significant for X value while for Y values it is non-significant and Fxy=5.38. It was also tried to adjust the values by computation for adjustment of SS for Y that shows termite killing was significant (Table-2) [df= 31, $t_{0.05}$ =2.04, $t_{0.01}$ =2.75]. Besides this, in another experiment, tag binging was done and plants were treated with *C. decidua* aqueous extract by spraying. In this experiment the number of termites, % infestation and % tunneling activity were significantly (p<0.01) reduced up to 14.07%, 11.68% and 19.82% respectively in trees both sprayed and tagged (Table-2).

Table-2 Termite management after employment of tag binding and spray on infested garden plants. Different rows of early saplings of sagaun were treated with active fractions, Observations were made at every 15 days time interval, Significant at p<0.01 levels.

	Number of termites Mean±SE	% infestation Mean±SE	% inhibition in tunneling activity Mean±SE			
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Spray	21.50±0.021 (100)	15.5±0.017 (41.89)	76.75±0.14 (100)	19.0±0.03 (19.8)	55.4±0.01 (100)	21.73±0.04 (28.17)
Tag binding	19.4±0.019 (100)	10.75±0.09 (35.65)	63.2±0.07 (100)	11.25±0.02 (15.11)	38.6±0.05 (100)	13.75±0.01 (26.26)
Spray and tag binding	20.6±0.03 (100)	3.375±0.004 (14.07)	67.8±0.07 (100)	8.97±0.01 (11.68)	37.2±0.06 (100)	9.2±0.08 (19.82)

After one month total termite infestation was subsided and mud plastering get shed off from the infested trees by tag binding and spray treatment. In seasoned wood sticks C. decidua mixtures T1, T2 and T3 have shown long term protection of wood. As combined mixtures have shown significant correlation between tests and control, as the values of correlation were found positive in the weight loss and infestation, while it was found negative for malathion treatment (0.716 to 0.322). Similarly both fipronil and thiomethoxam treated wood sticks have shown greater weight loss than treated with combinatorial mixtures (Table-3). In few wood sticks weight loss was not due to termite attack but due to soil activity. There was observed a significant very low termite count in seasoned wood sticks in comparison to un-seasoned or controls.. Contrary to this, more number of termites was found in malathion treated wood sticks in comparison to bioorganic fraction treatment. It indicates that *C. decidua* and it various combinations are more protective to seasoned wood sticks in comparison to un-seasoned. Similar results were obtained in Borate (T3) and copper (T3) mixtures which have shown wood protection upto six months. Besides this, solid wood sticks treated with inorganic fraction cause mild percent weight loss and % infestation made the termite were between 2.97% to 26.61%. Similar results were obtained in pure cow urine seasoned wood sticks as they effectively control % weight loss i.e. 16.63 to 75.23% up to six month. While cow urine mixed with *C. decidua* infestation and minimum weight loss obtained and protect seasoned wood sticks from infestation was 29.20% up to five month in T1 fraction. From the experiments, it was noticed that % infestation is directly related to % weight loss (Table-3). It is highly noticeable that *C. decidua* fractions remain active for longer duration, significantly deter termites, and cause high lethality. It shows that active components present in mixtures of *C decidua* significantly repelled more number of termites from treated trees and no further infestation was observed on these treated saplings even after six months. It remarkably shows that wood seasoning is effective method to control termite damage. The index of toxicity estimation indicate that the mean value was with in the limit at all probabilities (90, 95 and 99%) as it is less than 0.05 values of t-ratio greater (p<0.05). Besides this regression was also found significant. The steep slope values indicate that even small increase in the dose cause high mortality. Values of the heterogeneity less than 1.0 denotes that in the replicate test of random sample, the dose response time would fall with in 95% confidence limit and thus the model fits the data adequately.

Results confirms that *C. decidua* combinatorial mixtures have successfully obstructed tunneling, feeding and penetration of termites in the garden soil (Boue and Raina, 2003; Blaske and Hertel, 2001). Similarly *Diospyros sylvatica*, (Ganapaty *et al.,* 2004), *Polygonum hydropiper* (L) and *Pogostemon paviflorus* (Benth) (Rehman *et al.,* 2005) *Aleurits fordii* (Tung tree) extracts (Hutchins, 2006) garlic *Allium sativum and Euphorbia kansuii* (Shi *et al.,* 2008) have shown antitermite activity. Similar termiticidal activity occurs in Cajput oil (*Melunuca cajputi*) against *Coptotermes formosanus* due to presence of monoterpene, diterpenes sesquiterpene and hydrocarbons (Cornelius and Grace, 1997, Kim *et al.,* 2006). Similarly, both Heartwood and sapwood of *Taiwania cryptomerioides* were found highly effective against *C. formosanus* at 10 mg/g (Chang *et al.,* 2001). Similar results were obtained by wood seasoning against *Anacanthotermes ochreceus* (Kaakeh, 2005). It was assessed from the results that present plant species may have few active compounds with diverse biological activity, which could be successfully used for termite control. Active compounds from this plant certainly deter feeding, orientation and tunneling behavior in termites. However, for elimination of field termite's natural pesticides are applied in form of baits, fumigants and soil treatment toxicants use of natural pesticides strengthen the cost benefit model developed for agro economic fields. Such methods are more feasible for sustainable agricultural development and are much able to check the environmental deterioration.

Table-3 Percent wood weight loss and % infestation in seasoned and unseasoned wood sticks up to six month

Mixture		1st month	2nd month	3rd month	4th month	5th month	6th month
Control	% Wt. loss	315 (100)	517 (100)	554 (100)	561 (100)	695(100)	755 (100)
	%infestation	245 (100)	451 (100)	500 (100)	501 (100)	809 (100)	1012 (100)
Capparis T1	% Wt. loss	231(73.33)*	273(52.81)*	341(56.68)*	385 68.66)*	543(78.13)*	563 (74.54)*
	%infestation	0 (00.00)	147(32.60)*	57 (11.40) *	0 (00.00)	20 (2.48) *	33 (7.32)*
Capparis T2	% Wt. loss	135 (42.86)*	247(47.78)*	293(52.22)*	365(65.57)*	481(26.05)*	563 (35.44)*
	%infestation	0 (00.00)	0 (00.00)	0 (00.00)	50 (9.99)*	42 (5.20)*	39 (3.86)*
Capparis T3	% Wt. loss	123 (34.05)*	179(34.69*)	205(37.01)*	311(55.44)*	433(62.31)*	490 (70.51)*
	%infestation	0 (00.00)	36 (7.99)	0 (00.00)	44 (8.79)*	40 (4.95)*	18 (1.78)*
Borate T1	% Wt. loss	346(109.84)*	368(71.17)*	376(67.87)*	402(71.65)*	438(63.02)*	528 (69.93)*
	%infestation	20 (8.17) *	80 (17.74)*	123(24.60)*	149(29.75)*	162(20.03)*	245 (24.21)*
Borate T2	% Wt. loss	204 (64.76) *	322(62.28)*	356(64.25)*	500(89.12)*	511(73.52)*	614 (81.32)*
	%infestation	23 (9.39)	40 (8.87)*	90 (18.00)*	80 (15.97)*	129(15.95)*	158 (15.62)*
Borate T3	% Wt. loss	76 (24.12) *	168(32.49)*	356(64.25)*	366(65.24)*	504(72.51)*	678 (89.80)*
	%infestation	28 (11.43) *	30 (6.67)*	265(53.00)*	270(53.90)*	50 (6.19)*	210 (20.76)*
Copper T1	% Wt. loss	82 (26.03) *	208 40.23)*	366(66.59)*	368(65.59)*	461 66.33)*	524 (69.40)*
	%infestation	10 (4.09)	23 (5.10)	35 (7.00)	44 (8.74)*	70 (8.66)*	200 (19.77)*
Copper T2	% Wt. loss	80 (25.39) *	108(20.88)*	152(27.43)*	472(84.13)*	550(79.13)*	784(103.84)*
	%infestation	21 (8.58) *	30 (6.66)	43 (8.60)*	40 (7.99)*	41 (8.19)*	50 (6.19)*
Copper T3	% Wt. loss	00 (0.00)	230(44.48)*	300(54.15)*	307(54.15)*	408(58.70)*	524 (69.40)*
	%infestation	12 (4.90)	20 (4.44)	83 (16.60)*	97 (19.37)*	112(13.85)*	130 (12.85)*
Pure cow urine	% Wt. loss	64 (20.31) *	86 (16.63)*	128(23.59)*	138(24.59)*	272(39.13)*	568 (75.23)*
	%infestation	14 (5.72)	16 (3.55)	28 (5.60)	19 (3.80)*	11 (1.36)*	16 (1.59)*
Cow urine T1	% Wt. loss	196 (62.23)*	172(33.27)*	187(33.75)*	188(33.51)*	203(29.20)*	288 (38.15)*
	%infestation	9 (3.68) *	0 (00.00)	2 (0.45)*	5 (1.00)*	0 (00.00)	10 (0.99)*
Cow urine T2	% Wt. loss	276(87.61)*	284(54.93)*	224(40.43)*	280(50.08)*	288(41.43)*	290 (39.60)*
	%infestation	0 (00.00)	0 (00.00)	8 (1.60)*	26 (5.19)*	14 (1.74)*	32 (3.17)*
Cow urine T3	% Wt. loss	140 (44.45) *	222(42.94)*	268(48.37)*	206(36.72)*	266(38.27)*	288 (38.14)*
	%infestation	12 (4.90) *	0 (00.00)	0 (00.00)	13 (2.60)*	40 (3.95)*	50 (4.94)*
Melathion	% Wt. loss	95 (30.16) *	226(43.72)*	402(72.57)*	450(80.22)*	480(30.94)*	564 (25.30)*
	%infestation	80 (32.66) *	120(26.61)*	67 (1.34)*	670(15.95)*	129(15.95)*	58 (5.74)*
Fipronil	% Wt. loss	232 (73.66) *	220(42.55)*	328(59.20)*	344(61.32)*	368(52.94)*	488 (64.63)*
	%infestation	10 (4.09)*	20 (4.44)*	29 (5.80)*	101(20.16)*	70 (8.66)*	30 (2.97)*
Thiomethoxane	% Wt. loss	224 (71.12) *	370(71.57)*	370(66.79)*	414(73.80)*	445(64.03)*	512 (67.81)*
	%infestation	20 (8.16)*	33 (7.31)	42 (8.40)*	109 19.42)*	35 (4.32)*	51 (5.03)*

* significant at P< 0.05 and 0.01 level. Separate wood sticks were seasoned and planted in the garden soil. The infestation was calculated at one moth duration for which both test and control sticks were dug out after one month. Combinatorial mixtures C-ST1 *C. decidua* stem powder 90 gm, Coconut oil 50ml, Terpene oil 50ml, Glycerol (50 ml) + Sulphur (11) dissolved in 15 liters of water. *C*-ST2 135 gm C. deciduas stem powder, CST3 C deciduas 180 gm powder. Rest of the ingradeints were similar in amount. *C*-BT mixture contained borate (11 gm) at the place of sulphur while C-CoT mixtures contained 11 gm copper in each. PCU denotes photoactivated cow urine, *C*-CuT1, T2 and T3 mixtures contained 90, 135, 180 gm stem C. decidua powder dissolved in 15 liters of cow urine, Malathion, Fipronil and Thiamethoxam mixture contained 75 gm/liter

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