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TOXIC POTENTIAL OF *ALSTONIA SCHOLARIS AND SALVADORA OLEIODES* LEAVES EXTRACT AGAINST *SUBTERRANEAN TERMITES, MICROTERMES OBESI* (ISOPTERA: TERMITIDAE)

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Keywords:

Microtermes obesi, *Dodonaea viscosa*, *Nerium oleander*, *Delphinium ajacis*

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ABSTRACT: Termites impart hazardous impacts on the ecosystem by causing severe damage to wooden components. They decrease crop yield, restrain the production of economically important trees, and destroy wooden buildings. The utilization of insecticidal plants provides a promising strategy for the control of insects. The undertaken study is intended to highlight the anti-termite potential of plant extracts as a promising strategy for termite control. Insecticidal potential of *Alstonia scholaris* and *Salvadora oleiodes* extract revealed by investigating their impact on mortality and tunneling activities of *Microtermes obesi*. Mortality of *Microtermes obesi* was investigated by treating the soil and filter paper with different concentrations of methanolic and aqueous extracts. Least LT_{50} value was shown by 40% methanolic extract of *S. oleiodes i.e.* 40.476 and 37.425 hours, in soil and filter paper assay, respectively. Termites showed least tunneling activity at 40% concentration of methanolic extract of *S. oleiodes* thus indicating that toxicity of phytochemicals might have apprehended the movement of termites. Methanolic extract of *S. oleiodes* showed grade 1 (v. v. strong) repellent activity, whereas varying concentrations of *A. scholaris* showed grade 2 (v. strong) repellent activity. Hence, it can be concluded that *S. Oleiodes* possess more promising anti-termite potential as compare to *A. scholaris*.

INTRODUCTION: Termites are referred to as tropical insects with a complicated communal organization.

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They are widely distributed in lower temperature regions associated with higher latitudes and altitudes [1].

Termites impart hazardous impacts on the ecosystem by causing severe damage to wooden components. They decrease crop yield, restrain the production of economically important trees, and destroy wooden buildings [2, 3]. It has been estimated that in 2010 approximately $ 40 billion was spent world widely to buy synthetic pesticides in order to manage termites and to repair wooden property [3, 4]. Various methods are being employed to cope with the hazardous impacts of termites. Synthetic pesticides are widely used for the protection of termites, but they cause severe human health and environmental dilemma.

Chlorpyrifos, fipronil, bifenthrin, imidacloprid, endosulfan, lindane, aldrin, coldrane, dieldrin, endrin, and methyl bromide are highly effective for the management of termites, but they cause depletion of the ozone layer that ultimately leads to skin cancers. Moreover, these agents also pollute aquatic system [5]. Cultural control is based on conventional knowledge that is not obtained from research findings. This method involves the use of faeces, decomposed animals, meat, fish viscera, and sugarcane husks to reduce termite attacks. This strategy is not always effective for the control of termites as the mechanisms involved behind the use of these agents are not justified [6].

The utilization of insecticidal plants provides a promising strategy for the control of insects. Phytoconstituents are decomposed upon exposure to sunlight, thus having short persistence to the environment. Plant-based pesticides provide an eco-friendly approach without imparting negative effects on non-targeted insects, human health, environment, and crops. The undertaken study is intended to highlight the anti-termite potential of plant extracts as a promising strategy for termite control [7]. Insecticidal potential of *Alstonia scholaris* and *Salvadora oleiodes* extract revealed by investigating their impact on mortality and tunneling activities of *Microtermes obesi*.

MATERIALS AND METHODS:

Assortment of Termites: *Microtermes obesi*, were assorted from effected sugarcane fields in the vicinity of Faisalabad, Pakistan.

Acquisition of Plants: Leaves of different plants were acquired *i.e*., *Alstonia scholaris* and *Salvadora oleiodes* from Vicinity of Faislabad. Leaves were washed and shade dried, followed by grinding to a fine powder.

Preparation of Methanolic Extract: One hundred gram of each plant was soaked in 80% methanol, and sonication aided maceration were adopted [8]. It was kept at room temperature for 72 h and filtered by a Whatman filter no. 42. The solvent was removed from filtrate followed by drying in vacuum desiccators, and the dried extract was obtained. The solution of crude extract was prepared by using 2% methanol.

Preparation of Aqueous Extract: The leaves of medicinal plants were rinsed in distilled water and left to dry in a shady place. After drying, the leaves were chopped in pestle and mortar and soaked in distilled water for 72 h. These chopped leaves were separated out from the solvent through cheesecloth, and solvent was filtered by a Whatman filter paper no. 42. The filtrate was stored at a low temperature.

Soil Preparation for Bioassay: Sandy clay loam soil (52.6% sand, 24.8% silt, and 20.6% clay) was sieved, and moisture content was determined by a moisture meter. Moisture content was further increased in order to avert the dehydration in termites.

Execution of Bioassays: The following bioassays were conducted to investigate the impact of test extracts on mortality and tunneling activity of termite species.

Bioassays by Treating the Soil with Test Extracts: Antiemetic sugarcane strip bioassay (ASSB) was carried out by adopting the previously revealed protocol [9]. The assay was carried out in petri dishes containing 20 g sifted sterilized soil and strips of sugarcane. Mortality was investigated at varying concentration of test extracts *i.e*. 0% (control), 10%, 20% and 40% of extracts. Petri dishes were placed in a growth chamber having 28 ± 2 °C temperature and 80% ± 5 humidity and 30 termites were released in each petri dish having treated and untreated soil. The assay was carried out in triplicate, and observations were recorded as mean of replications with standard deviation. Probit analysis was used for toxicological inferences.

Filter Paper Bioassay: Filter paper bioassay was carried out by following the previously revealed protocol [9]. Whatman filter paper no. 42, 9 cm in diameter was treated with 10, 20, and 40% concentration of plant leaves extract and put in Petri dishes of 3½ inches in diameter × 1.5 cm in height at the rate of 31 µl/ cm^2. 30 workers of each species were released in the petri dishes having treated and untreated filter paper and were placed in a growth chamber under controlled conditions of 28 + 2 °C and 80% + 5 humidity. Data for mortality were recorded 100% mortality occurred. Each treatment was repeated three times, and mortality data was recorded as mean of replications with standard deviationprobit analysis.

Determination of Galleries Formation (FG): The activity of termites in the soil can be estimated by observing their potential of galleries formation during foraging. Termite's potential of galleries formation was investigated at different concentrations of test extracts, *i.e.*, 0% (control), 10%, 20%, and 40%. The activity was determined at 5, 10, and 15 h by plotting the tunnels on the cellophane paper, and the length was measured in mm^2 by using a planimeter. The assay was run in triplicate, and mortality data were recorded as mean of replications with standard deviation. Activities were correlated with the concentration of test extracts, and tunneling activities were analyzed by Factorial Analysis (CRD). Probit analysis was used for toxicological inferences.

Statistical Analyses: Biological parameters were the result of three independent replications (n = 3), and final data was expressed as mean ± standard deviation (SD). Mortality data were subjected to Abbott's formula [10]. All statistical computations/analyses were undertaken using IBM SPSS version 21.0 statistical software (SPSS Inc., Chicago, Illinois, USA). Statistical differences between biological parameters of treatment and control groups were determined by the analysis of variance (ANOVA) at $p < 0.05$. A posthoc Tukey's test for multiple comparisons was employed to discriminate statistically significant treatments when ANOVA indicated significant differences among treatments. In all statistical tests, values of $P < 0.05$ were accepted as indicating significance. The results of mortality and survival bioassays were subjected to probit analysis [11,] and median lethal times (LT_{50}) of the plant extracts against termites were determined by probit analysis.

RESULTS AND DISCUSSION:

Estimation of Termites Mortality: Mortality of *Microtermes obesi* was investigated by treating the soil and filter paper with different concentrations of methanolic and aqueous extracts of *Alstonia scholaris* and *Salvadora oleiodes*. It was noted that the mean mortality of *Microtermes obesi* increased in a concentration as well as time-dependent manner, as shown in **Fig. 1**.

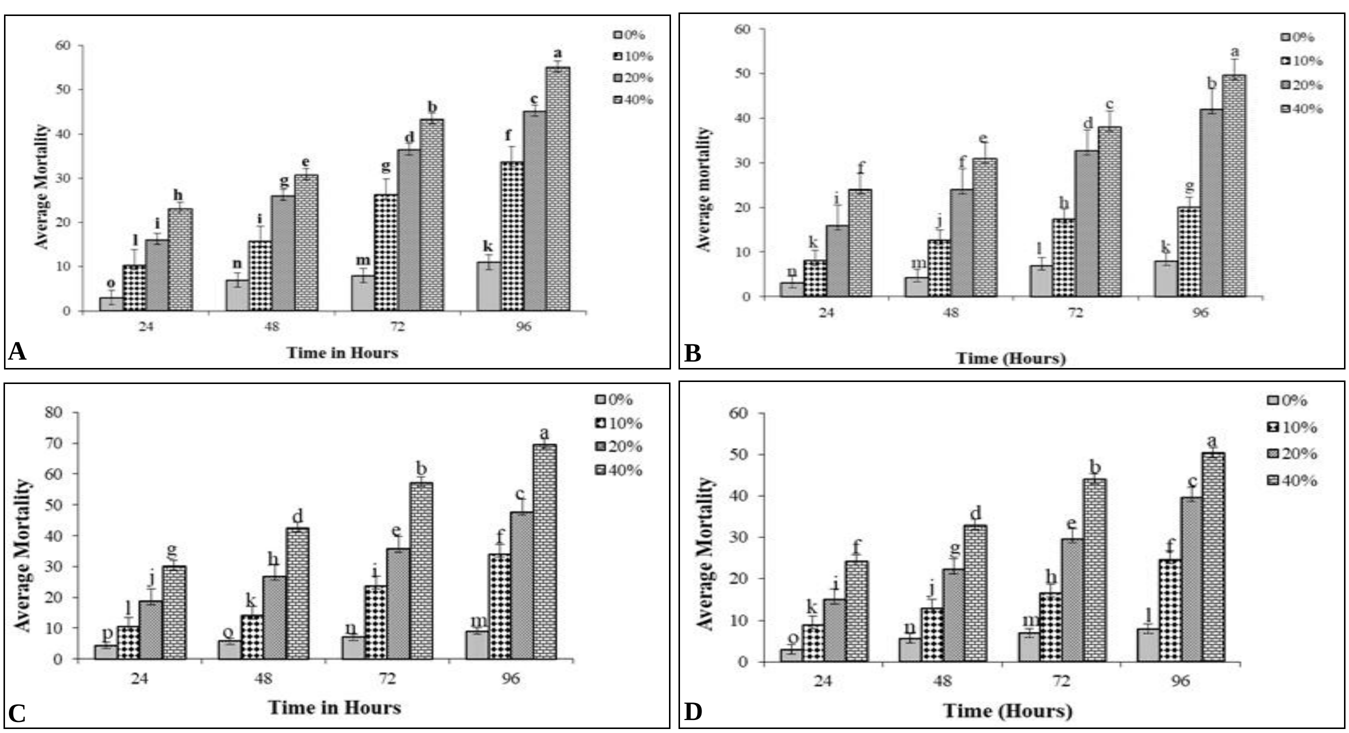

FIG. 1: MEAN MORTALITY OF THE *MICROTERMES OBESI* AT DIFFERENT TIMES AND CONCENTRATIONS WITH METHANOLIC AND AQUEOUS EXTRACT OF *ALSTONIA SCHOLARIS* (A, B) AND *SALVADORA OLEIODES* (C, D) TREATED WITH SOIL

Methanolic extracts of selected plants were found to be more potent as compared to aqueous extracts.

LT_{50} value of crude extracts was also investigated at different concentrations against *M. obesi* in petri plates having treated soil as shown in **Table 1** is significantly less than control values. Mortality of *Microtermes obesi* was further investigated by treating the filter paper with different concentrations of test extracts, as shown in **Fig. 2**. Results were found in accordance with those revealed by soil assay.

LT_{50} values of crude extracts were also calculated at different concentrations against *M. obesi* in Petri plates having treated filter paper, as shown in **Table 2**.

TABLE 1: LT_{50} VALUES OF CRUDE EXTRACTS AT DIFFERENT CONCENTRATIONS AGAINST *M. OBESI* IN PETRI PLATES HAVING TREATED SOIL

Extract name	Concentration (%)	LT_{50}	L. Limit	U. Limit	Slope (b)	Intercept (a)
	0	257. 3230	248. 5782	266.4719	2.98504	-7.1954
MeOH extract of	10	107.4989	100.3555	115.2180	1.90412	-3.8680
A. scholaris	20	74.1595	68.1063	80.7824	1.58777	-2.9694
	40	55.7952	50.3681	61.8898	1.37872	-2.4081
Aqs extract of *A.*	0	308. 6222	297. 2739	320. 9317	2.86167	-7.1239
scholaris	10	136.3747	128.5326	144.8091	2.11981	-4.5252
	20	84.9881	78.1882	92.4631	1.58187	-3.0520
	40	62.6463	56.7365	56.7365	1.39180	-2.5009
MeOH extract of	0	314. 766	302. 416	328. 226	2.621	-6.548
S.oleiodes	10	98.064	91.605	105.078	2.099	-4.181
	20	74.490	67.580	82.4166	1.489	-2.788
	40	40.476	36.174	45.422	1.404	-2.257
Aqs extract of *S.*	0	351. 3629	336. 2104	368. 2969	2.51388	-6.3997
oleiodes	10	139.0901	130.9975	147.7806	2.03855	-4.3692
	20	98.2669	90.8452	106.3744	1.61597	-3.2197
	40	63.2791	57.2457	70.0526	1.37278	-2.4727

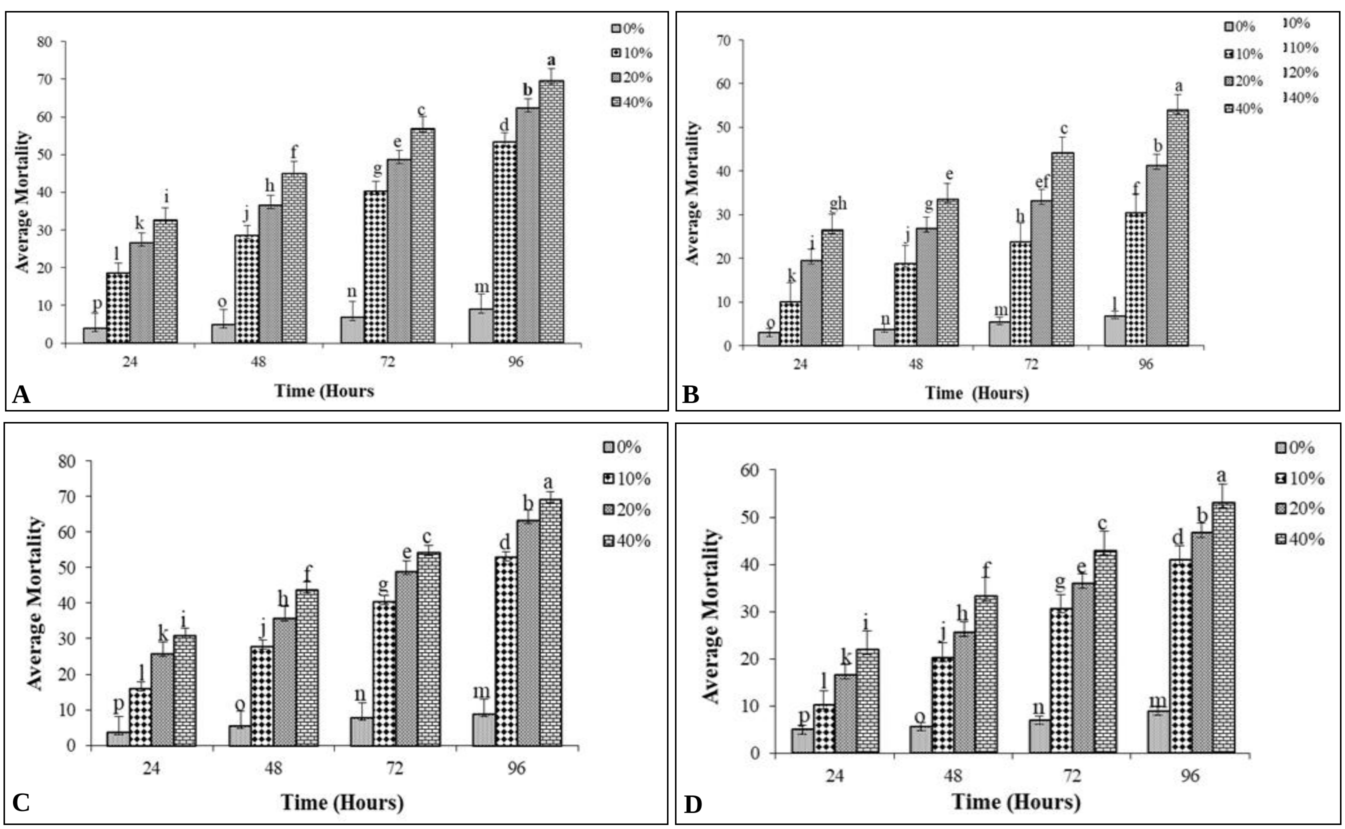

FIG. 2: MEAN MORTALITY OF THE *MICROTERMES OBESI* AT DIFFERENT TIMES AND CONCENTRATIONS WITH METHANOLIC AND AQUEOUS EXTRACT OF *ALSTONIA SCHOLARIS* (A, B) AND *SALVADORA OLEIODES* (C, D) TREATED WITH FILTER PAPER

TABLE 2: LT_{50} VALUES OF CRUDE EXTRACTS AT DIFFERENT CONCENTRATIONS AGAINST *M. OBESI* IN PETRI PLATES HAVING TREATED FILTER PAPER

Extract name	Concentration (%)	LT_{50}	L. Limit	U. Limit	Slope (b)	Intercept (a)
	0	269. 2367	259. 9396	279. 0127	2.90220	-7.0527
MeOH extract of	10	64.2978	59.4363	69.5187	1.87993	-3.3993
A. scholaris	20	47.5149	43.1603	52.3036	1.54339	-2.5880
	40	39.4588	33.4740	41.9411	1.35342	-2.1297
Aqs extract of *A.*	0	320. 5559	310. 1704	331. 6788	3.2069	-8.0361
scholaris	10	111.2063	104.3946	118.4534	2.06195	-4.2190
	20	78.7102	72.0546	86.0434	1.47411	-2.7950
	40	56.6086	50.7696	63.2447	1.27847	-2.2410
MeOH extract of	0	262. 943	253. 690	272. 688	2.878	-6.964
S. oleiodes	10	64.784	60.035	69.855	1.967	-3.563
	20	48.167	43.889	52.850	1.611	-2.711
	40	37.425	35.104	44.409	1.348	-2.151
Aqs extract of *S.*	0	293. 5937	282.7564	305. 2105	2.73795	-6.7566
oleiodes	10	89.1949	83.8127	94.8198	2.33066	-4.5456
	20	69.7963	64.2808	75.7924	1.70738	-3.1481
	40	59.3049	53.4783	65.9610	1.41076	-2.5014

Tunneling Activities of *Microtermes Obesi*: Tunnelling activity of *M. obesi* was also investigated at different times with varying concentrations of test extracts, as shown in **Fig. 3**. The maximum time for observing tunnel formation was 15 h; after that, profuse tunneling in control treatment made it difficult to determine the area of tunnels. Means of the tunneling activities of *M. obesi* in methanolic extract treated soil were less than the means of the tunneling activities in aqueous extract treated soil.

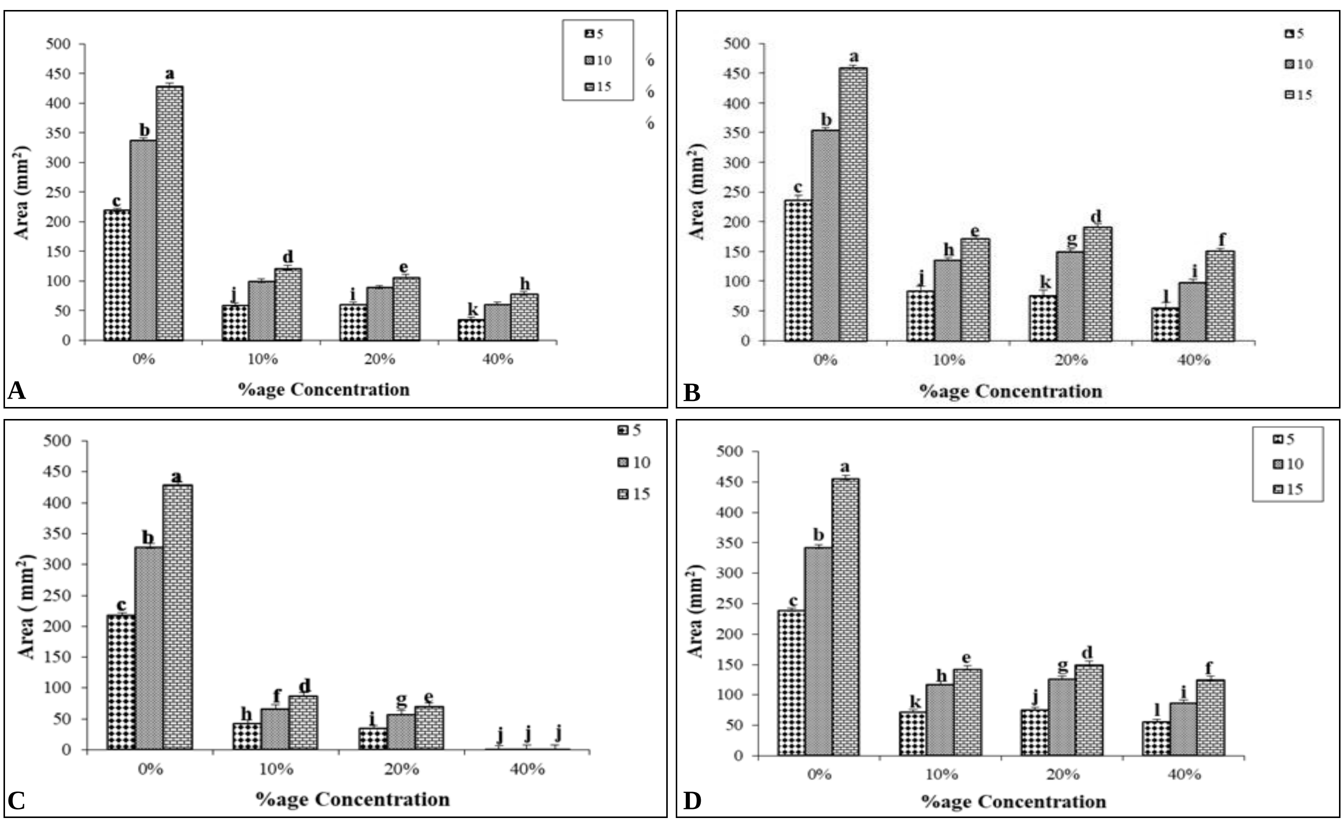

FIG. 3: TUNNELLING ACTIVITY OF *M. OBESI* AT DIFFERENT TIMES AND CONCENTRATIONS WITH METHANOLIC AND AQUEOUS EXTRACTS OF *ALSTONIA SCHOLARIS* (A, B) AND *SALVADORA OLEIODES* (C, D

Repellency Grading of Tunnelling Activities of *Microtermes Obesi*: Repellency grading of tunneling activities of *M. obesi* at different concentrations of Methanolic extracts was also checked as these were found to be highly active as compared to aqueous extracts. It was noted that repellent activity decreased with a decrease in the concentration of crude extract, as shown in **Table 5**. Methanolic extract of *S. oleiodes* showed grade 1 (v.v. strong) repellent activity, whereas varying concentrations of *A. scholaris* showed grade 2 (v. strong) repellent activity.

TABLE 3: ANOVA TABLE OF TUNNELLING DATA OF *M. OBESI* AND COMPARISON OF MEAN VALUES AT DIFFERENT TIMES & CONCENTRATIONS OF CRUDE EXTRACTS

Extract name	SOV	D_f	SS	MS	F-Value	P-value
MeOH extract of *A. scholaris*	Time	2	54495.500	27247.750	991.8291**	0.0000
	Concentration	3	407023.778	135674.593	4938.6100 **	0.0000
	TxC	6	27861.389	4643.565	169.0276**	0.0000
	Error	24	659.333	27.472		
	Total	**35**	**490040.000**			
Aqs extract of *A. scholaris*	Time	2	114960.667	57480.333	1461.3644 **	0.0000
	Concentration	3	327365.111	109121.704	2774.2806 **	0.0000
	T*C	6	15366.222	2561.037	65.1111**	0.0000
	Error	24	944.000	39.333		
	Total	**35**	**458636.000**			
MeOH extract of *S.oleiodes*	Time	2	34572.222	17286.111	653.6765 **	0.0000
	Concentration	3	554865.222	184955.074	6994.0994**	0.0000
	T*C	6	38777.111	6462.852	244.3936 **	0.0000
	Error	24	634.667	26.444		
	Total	**35**	**628849.222**			
Aqs extract of *S. oleiodes*	Time	2	76725.056	38362.528	1810.0275 **	0.0000
	Concentration	3	364204.306	121401.435	5727.9838 **	0.0000
	T*C	6	22474.944	3745.824	176.7361 **	0.0000
	Error	24	508.667	21.194		
	Total	**35**	**463912.972**			

** = Highly significant (P<0.01) * = Significant (P<0.05) NS = Non-significant (P>0.05)

TABLE 4: COMPARISON OF MEAN VALUES (MM2) OF TUNNELLING ACTIVITY

Extract name	Time (H)	Concentrations (% age)				Means
		0%	10%	20%	40%	
MeOH extract of *A. scholaris*	5	218.66c	76.66h	60.33i	35.00j	97.66c
	10	337.00b	130.00e	88.33g	60.33i	153.91b
	15	428.00a	158.66d	105.66f	77.33h	192.41a
	Means	**327.88 a**	**121.77 b**	**84.77 c**	**57.55 d**	
Aqs extract of *A. scholaris*	5	236.66c	108.66g	76.66h	56.00i	119.50 c
	10	355.00b	180.00e	149.66f	149.66f	195.83b
	15	459.66a	228.00c	192.00d	151.00f	257.66 a
	Means	**350.44 a**	**172.22 b**	**139.44 c**	**101.88 d**	
MeOH extract of *S. oleiodes*	5	217.33c	55.33g	34.66h	0.000i	76.83c
	10	327.66b	86.00e	57.00g	0.000i	117.66b
	15	427.66a	113.33d	69.66f	0.000i	152.66a
	Means	**324.22 a**	**84.88 b**	**53.77 c**	**0.00 d**	
Aqs extract of *S. oleiodes*	5	238.66c	94.00g	76.00h	56.33i	116.25 c
	10	342.33b	156.00e	126.66f	87.00g	178.00 b
	15	454.66a	187.66d	149.33e	125.00f	229.16a
	Means	**345.22 a**	**145.88 b**	**117.33 c**	**89.44 d**	

TABLE 5: REPELLENCY GRADING OF TUNNELING ACTIVITIES OF *MICROTERMES OBESI* AT DIFFERENT CONCENTRATIONS OF METHANOLIC EXTRACTS

S. no.	Name of plant	10%	20%	40%
1	*Alstonia scholaris*	2	2	2
2	*Salvadora oleiodes*	4	3	1

1 = V.V. STRONGLY REPELLENT = 0, 2 = V.STRONGLY REPELLENT = 1-15(mm^2), 3 = STRONGLY REPELLENT = 16-30 (mm^2), 4 = REPELLENT = 31-45 (mm^2), 5 = LESS REPELLENT = 46-60 (mm^2), 6 = VERY LESS REPELLENT = 61-75 (mm^2), 7 = V.V.LESS REPELLENT = 76-90 (mm^2)

A. scholaris possess a wide range of therapeutic activities *i.e.* febrifuge, tonic, anti-diabetic, anthelmintic, antiepileptic, anti-hypertensive and anticancer [12-15]. *S. oleoides* is conventionally used for the treatment of cough, enlarged spleen, and fever. It has also been to possess anti-inflammatory, analgesic, and antiulcer activity. Hypoglycemic, hypolipidemic, and anti-microbial activity of this plant has been reported [16-18]. Best of our knowledge, the anti-termite activity of these plants have not been investigated yet. Among tested extracts, the least LT_{50} value was shown by the methanolic extract of *S. oleiodes* in the case of substrate soil and filter paper assay. Tunneling activity data further supported the anti-termite activity of the afore-mentioned extract. Methanolic extracts of selected plants showed promising anti-termite activity; however, *S. oleoides* was found to have more anti-termite potential as compared to *A. scholaris*.

CONCLUSION: To our knowledge, this is the first report regarding the detrimental impact of *A. Scholaris* and *S. oleoideson* on the mortality and tunneling activities of *M. Obesi*. It is concluded that methanolic extracts of selected plants may act as promising arrested, and toxic to assorted workers of *M. Obesi*. Hence, these extracts could be employed as an alternative for synthetic insecticides to control termite overrun.

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CONFLICTS OF INTEREST: Authors report no. conflicts of interest to declare with the contents of this article.

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