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Toxicity and Repellency of Plant Extract and Termiticide against Fungus Growing Subterranean Termites (Blattodea: Termitidae)

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Cover Page Footnote

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TOXICITY AND REPELLENCY OF PLANT EXTRACT AND TERMITICIDE AGAINST FUNGUS GROWING SUBTERRANEAN TERMITES (BLATTODEA: TERMITIDAE)

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ABSTARCT

Different methods such as physical, biological and chemical are used to manage soil fungus increasing termites. Synthetic insecticide plays a vital part in the management of termites. The pesticide used in big quantities causes phytotoxicity, mammalian toxicity and resistance to pesticides in target pests and insect outbreaks. Intensive pesticides exert chronic effects on living organisms with annoyance for beneficial insects. It also accompanied with environmental hazards and developed resistance. Plant leaves extracts provide a distinct variety of biochemical compounds with diverse prospective uses. Resistance development requires the discovery of fresh biological compounds with a wide spectrum of action. Plant leaves extract and Chlorfenapyr solution in methanol and water with various concentrations (15 %, 10 %, 5 % and 0 %) were applied to the soil against termites to determine mortality and repellency. Posttreatment data was obtained and evaluated through statistical analysis. The result revealed that the extract of *Conocarpus lancifolius* with the solution of methanol and solution of water exhibited higher mortality of subterranean termites, whereas the solution of methanol had higher repellency and mortality than water solution of botanical extract. Water and methanol solution of insecticide chlorfenapyr used against the subterranean termites, both are found to be efficacious against termites, while insecticide with the solution of methanol revealed 100% mortality. Nonetheless, plant extract of *C. lancifolius* with water and methanol solution and chlorfenapyr with methanol solution can be applied as new biological control tools against subterranean termites.

Keywords: Botanical extracts, biopesticides; biological control, termiticide, repellency.

INTRODUCTION

Termite as social insect comprised of a king, queen, workers and soldiers in the colony. Termites can be found in wood, above the floor and underground. Currently, 2,500 species of termites known to world, out of 300 species are acknowledged as a pest. Fungus increasing soil termites belong to order blattodea, family termitidae and subfamily

macrotermitinae of insects (Bonachela et al., 2015).

There are three main groups of termites i.e. Subterranean, dry wood and damp wood termites, living in distinct communities and their nest appears like sprawling network of galleries and are constructed their nests in decaying wood and standing trees. The colonies of subterranean termites are constructed in soil, trees, in high moisture areas. However, in some cases, their nests can spread from the floor surface in the form of mounds (Horwood and Eldridge, 2005). A constant supply of humidity is needed for underground termites (Potter, 2004).

Subterranean termites are social insects and have an apportionment of labor in a colony (Hattori et al., 2013). They have distinct morphological characteristics in distinct castes of termites, which are easily distinguishable from all other significant classes of insects, and the termites are the most significant social insects (Krishna, 1992). The nuptial flight through male reproductive, in this way the insemination fertilizes together with the queen. The queen saves the semen in spermatheca after copulation and the role of the queen is to lay 300 eggs day^{-1} and lifespan up to 25 years (Thompson et al., 2000). Termites being to eusocial insects having a caste system and each individual's caste is determined through post-embryonic growth (Lainé and Wright, 2003). Termites fake employees are known as pseudergates, which do the work of employees such as collection of food and care of brood (Roisin and Korb, 2010).

Termites generally feed on lignocellulose and cellulose in tropical areas and are famous to practice 50-100 % of deceased biomass of decay and biomass of crop (Bignell and Eggleton, 2000). Termites feed on a multitude of foods, soil, cultivated fungi, grass and dead wood, making termites ecosystem engineers that can change soils chemical and physical properties (Dangerfield et al., 1998). For digestion of cellulose termite, they are entirely depending on intestinal symbionts for example protozoa (Ikeda ohtsubo and Brune, 2009), whereas greater termites including termitidae generate their enzyme for the digestion of cellulose (Li et al., 2013). Termites play a significant role in ecosystems such as impaction soil chemical and physical structure, decomposition of plants, recycling of nitrogen (N), carbon (C) and activity of microbes (Holt and Lepage, 2000).

The utmost damaging subterranean termite *Copotermes gestori* is indigenous to the South Africa and distributed to the most omnipresent tropical areas, while *Copotermes formosan* is formed in an area where colonies were eliminated through treatments in China temperate and sub-tropical areas (Evans et al., 2013). Both species have separate ecological requirements (Grace, 2014), and the population is now developed in many non-native fields due to human activity termites (Hochmair and Scheffrahn, 2010). Termites are responsible for structural harm of over $1 billion across the Southern United States (Corn and Johnson, 2013).

Termites are one of agriculture's most significant insects, causing major losses to crop plants, trees and houses, infesting the various phases of plant development (Verma et al., 2009). The *Odontotermes obesi* is the economically significant species in Pakistan, *Heterotermes indicola* and *Microtermes obesi* are fungus developing termites and *Coptotermes heimi* exploits the soils to forage into tunnels and thus recycle nutrients (Ahmed et al., 2006). The use of intensive pesticide leads to chronic effects on living organisms and has side effects on beneficial insects (Abudula et al., 2001). One of the basic methods for protecting construction structures from underground termites is to use soil barrier control measures (Rust and Saran, 2006).

The use of chemical insecticides causes environmental and water pollution and, harmful effects on the non-target organisms. Fipronil, a termiticide is used as a non-repellant against subterranean

termites in the construction of the foundation and Chlorpyrifos is also used as repellent termiticide, like soil barrier around the globe (Gatti et al., 2002; Potter and Hillery, 2001). Fipronil and its metabolites disperse gradually in the deeper soil layer over time and the residues were recognized only up to 30 cm after 56 months (Sharma et al., 2008). Different plant extracts were screened against termites, resulting in higher mortality in lab bioassays and in field in different termite species at different formulations (Tellez et al., 2001). In crops comprising insecticidal features against termites, bioactive chemicals are in the form of metabolites (Siddiqui et al. 2004).

Bio-insecticide is acceptable worldwide, plant products have insect repellent and anti-feed properties (Maia and Moore, 2011) and plant materials are deemed excellent for controlling health issues and environmental pollution (Godlewska et al., 2021). Essential oils and plant extracts are the alternative sources for the management of termite, plants with chemicals in roots, flowers, leaves, seeds and stems have reported insecticidal characteristics (Sakasegawa et al., 2003). Plants are a valuable source of active chemicals such as glycosides, alkaloids, flavonoids, terpenoids and other compounds against multiple insect pests (Koul and Walia, 2009).

Plants are an inexpensive source of insecticide against the farm crop termite. This study aims to determine the efficiency of botanical extracts against the subterranean termite and to evaluate the impacts of termiticide solution and plant extracts on termites' repellence and mortality.

MATERIALS AND METHODS

Experimental Site

The experiments were performed at Entomological Research Laboratory, Department of Plant Protection, Faculty of Agricultural Sciences, AirPort Campus, Ghazi University, Dera Gazi Khan, Punjab, Pakistan to demonstrate the repellency and mortality of termiticide solution and native botanical extracts against soil subterranean termites.

Experimental design

The research was carried out in Completely Randomized Design (CRD) having two treatments plant leaf extracts and termiticide having four concentrations each i.e., 15 %, 10 %, 5 % and 0 % considered as control concentration. Each concentration consists of three 3 replications.

Termite Collection

The collection of workers and soldiers of the termites was done with corrugated cardboard installed in PVCs monitors at various locations in the Ghazi University, Dera Ghazi Khan, Punjab, Pakistan.

Treatments

T1: *Conocarpus lancifolius* (Plant Extract) (15 %, 10 %, 5 % and 0 %)

T2: Chlorfenapyr (Termiticide) (15 %, 10 %, 5 % and 0%)

Preparation for Insecticide solution

The dilution of 15 %, 10 %, 5 % and 0 % dilution was prepared in 2 solvents i.e., methanol and water from the active ingredient.

Method for extraction

The fresh leaves of *Conocarpus lancifolius* plant were collected from Ghazi University and then leaves samples were cleaned and washed with ddH_2O and dried under the shade having ensured adequate airflow to prevent damping. The leaves were then grinded in an electrical grinder to make the powder for five minutes.

Extraction Method

Methanol and Water Leaf Extract

In 200 ml of methanol and 200 ml of double distilled water, 100 g of leave powder were mixed in a 1:2 (w/v) ratio for the preparation of methanol leaf extract and water leaf extract. The plant material was soaked for 24 hours in solution of methanol and shaken with electrical shaker for 72 hours. After that 2 layers of Whatman filter paper No. 42 were used for filtering the solution. To attain maximum extractable, the procedure was repeated 3 times, previously described by (Truong et al., 2019) after slight modifications.

Bioassay via using Botanical Extracts and to Treat Soil

Soil for Bioassay

For lab bioassay, the soil was collected from the field where termites were present and its physical composition/properties were determined. The soil was sieved and soil moisture was also determined by a 30 mesh screen. The experiment was conducted in Petri dishes of 1.5 cm in height and 10 cm in diameter containing 10 grams of sterilized soil and strip of sugar cane (1.5 x 6 cm) to sustain the termites alive. Each 15 %, 10 % and 5 % extract and control (untreated 0 %) were repeated three times. The 100 grams of soil were wetted with plant leaf extracts concentration in a petri dish with a sugarcane strip under controlled environmental conditions at 28±2 °C and 70-80 % RH. The 5 soldiers and 25 active workers were released into the Petri dishes bearing treated and untreated (control) soil. Mortality data was recorded after 2 h intervals up to 12 h and then after every 12 h up till 5 soldiers and 25 active workers died.

Bioassay with Insecticide Solution Treatment of Soil

To determine the toxic effects of the termiticide solution, the experiment was conducted in 10 cm diameter and 1.5 cm high Petri dishes containing 100 grams of sterilized soil and strip of sugar cane (1.5 x 6 cm) to sustain the termites alive. Each 15 %, 10 % and 5 % solution and control (untreated 0 %) were repeated three times. The 100 grams of soil were wetted with termiticide solution concentrations in a petri dish with a sugarcane strip and kept under controlled environmental conditions at 28±2 °C and 70-80 % relative humidity. The 5 soldiers and 25 active workers were released into petri dishes bearing treated and untreated (control) soil. Mortality data was recorded after 2 h intervals up to 12 h and then after every 12 h up till 5 soldiers and 25 active workers died.

Repellency Bioassay on Filter Paper against Insecticide and Plant Extract for Underground Fungus Increasing Termites

Repellent action of botanical extract and termiticide against subterranean termites has been recorded. For this cutting of Whatman filter paper No. 1 into two parts depending on the petri plate size (70 x 10 cm) and then placed in the petri dish so that there is space between the two parts to enable distinct treatments. Half filter paper was wetted with a particular quantity of insecticide solution and plant extracts having concentrations i.e. 15 %, 10 % and 5 %. The distilled water was used for control (untreated 0 %) treatment. After that, the treated filter papers were air dried. There were three replications of treated dished and control without treatments. In each petri dish 1 soldier and 19 mature workers (ratio 1:19) of termites were introduced between the treated and untreated area and observations were done at every 15 minutes time intervals until the workers (sum of three replicates) moved towards the

untreated area against the corresponding concentrations.

ETHICS APPROVAL

Not applicable.

RESULTS

Effect of Cococarpus Lancifolius Leaf Aqueous Extract at Different Concentrations

The results demonstrated that the untreated (control 0%) concentration exhibited a great difference from all other concentrations with LT_{50} (310.000) and the upper and lower limits were 313.770 and 289.550, respectively (Table 1). The concentration (5 %) has expressed a great difference as compared to the concentration (15 %) with an upper and lower limits were 273.633 and 224.188, respectively. The LT_{50} value of concentration (5 %) was 248.911. At 10 % concentration, the LT_{50} value was 225.641 which show a difference from other treatments. The upper limit and lower limit of concentration (10 %) were 253.624 and 197.659, respectively. The LT_{50} values of 15 % concentration were 207.996 with an upper and lower limit were 238.329 and 177.664, respectively. The 15 % concentration has a significant difference between 0 % control and 5 % concentration but revealed less difference through 10 % concentration (Table 1).

Effect of Chlorfenapyr Aqueous Solution at Different Concentrations

According to results in Table 1 aqueous solution of Chlorfenapyr control (0 %) concentration showed a high difference towards all other concentrations with LT_{50} (136.000) and the upper and lower limits were 142.980 and 127.006, respectively. The concentration (5 %) has revealed a great difference in comparison to the concentration (15 %) with lower and upper limits were 54.009 and 102.504, respectively. The LT_{50} value of concentration (5 %) was 78.2563. At 10 % concentration, the LT_{50} value was 72.970 which show a difference from other treatments. The upper (95.590) and lower limits (47.351) of concentration (10 %) were observed. The LT_{50} value of concentration (15 %) was 68.304 with an upper and lower limit (91.874 and 43.734). The 15 % concentration has significant variation by the 0 % control and 5 % concentration but exhibited less difference through 10 % concentration (Table 1).

Effect of Cococarpus Lancifolius Methanol Leaf Extract at Different Concentrations

The results for *Cococarpus lancifolius* methanol leaf extract revealed that control (0 %) concentration exhibited significant difference towards all other concentrations with LT_{50} (294.000) as well as lower and upper limits were 277.084 and 310.915, respectively. The concentration (5 %) has revealed great variations towards concentration (15 %) with upper and lower limits were 268.668 and 217.598, respectively. The LT_{50} value of concentration (5 %) was 243.133. At concentration (10 %), the LT_{50} value was 216.185 exhibiting a difference among other concentrations. The upper limit (245.154) and lower limit (186.882) of 10 % concentration were recorded. The LT_{50} value of concentration (15 %) was 190.182 with a lower limit (157.710) and upper limit (222.806). The concentration (15 %) has significant variation by the 0 % control and 5 % concentration but revealed less difference through 10 % concentration (Table 2).

Effect of Chlorfenapyr Methanol Solution at Different Concentrations

According to results in Table 2 for Chlorfenapyr methanol solution, control replication at concentration (0 %) exhibited a high difference to all other concentrations with LT_{50} (130.000) and upper and lower

limits were 143.990 and 116.011, respectively. The concentration (5 %) has revealed a considerable difference in concentration (15 %) with lower and upper limits were 53.675 and 101.837 respectively. The LT_{50} value of concentration (5 %) was 76.923. At concentration (10 %), the LT_{50} value (70.970) shows a difference among other concentrations. The upper limit (97.564) and lower limit (48.217) of concentration (10 %) were observed. The LT_{50} values of concentration (15 %) were 66.304 with a lower limit (44.734) and upper limit (92.874). The concentration (15 %) has great difference to the control (0 %) and concentration (5 %) but revealed less variation through concentration (10 %).

Effect of Conocarpus Lancifolius Methanol Extracts on the Termite Repellency

The results for *Conocarpus lancifolius* methanol extracts on the termite repellency represented non-significant differences among all concentrations (15 %, 10 %, 5 % and 0 %) at 105 minutes to 180 minutes time intervals. At 15 % concentration, all-time intervals except 15 minutes represented non-significant variation. In the case of 10 % concentration, 45-120 minutes (time intervals) showed the same results but 15 and 30 minutes of time intervals have differed as compared to all other time intervals. A significant difference was observed at 5 % concentration from 15-75 minutes of time intervals. Control (0 %) concentration exhibited statistically dissimilar results from 15 to 105 minutes (time intervals) (Table 3).

Effect of Chlorfenapyr Methanol Solution on the Termite Repellency

The results of Chlorfenapyr methanol solution on the termite repellency (Table 3) expressed that a non-significant difference was recorded at 15 % concentration from 45-180 minutes (time interval) and 15 and 30 minutes of time interval exhibited significant difference. All-time intervals showed the same results except for 15, 30 and 45 minutes (time intervals) which exhibited significant variations at 10 % concentration. At 5 % concentration, 15 to 75 minutes of time intervals represented dissimilar repellency and the remaining time intervals exhibited similar repellency. In case of control, 15 minutes (time interval) represented significant variation towards other observing time intervals. Statistically same results were observed among 30-45, 60-90, 105-120 and 135-180 minutes (time intervals).

Effect of Conocarpus lancifolius Aqueous Extracts on the Termite Repellency

The results for *Conocarpus lancifolius* aqueous extracts on the termite repellency represented non-significant differences were observed in all examined time intervals except 15, 30 and 45 minutes (time intervals) at 15 % concentration. While 10 % concentration showed the same results from 75-180 minutes of time intervals except for 15-60 minutes which exhibited dissimilar outcomes.

In case of 5 % concentration, the same repellency was observed among 15-45 minutes, 60-75 minutes, 90-105 and 120-180 minutes (time intervals). In control (0 %) concentration, statistically similar repellency was noticed among 15-60 minutes, 75-105 minutes, 120-135 minutes and 150-180 minutes (Table 4).

Effect of Chlorfenapyr Aqueous Solution on the Termite Repellency

The results of Chlorfenapyr aqueous solution on the termite repellency (Table 4) revealed that a non-significant difference was recorded at 15 % concentration from 45-180 minutes (time interval) and 15 and 30 minutes of time interval showed significant difference.

Table 1: Comparison of LT_{50} values against termites in different concentrations of aqueous leaves extracts of *Conocarpus lancifolius* and Chlorfenapyr aqueous solution.

Treatment	Concentration	LT_{50} (Hours) ± SE	95 % Confidence Interval	
			Lower Limit	Upper Limit
Conocarpus lancifolius	15 %	207.996± 15.475	177.664	238.329
	10 %	225.641 ± 14.61	197.659	253.624
	5 %	248.911± 13.280	224.188	273.633
	0 %	310.000 ± 4.828	289.550	313.770
Chlorfenapyr	15 %	68.304 ± 12.536	43.734	91.874
	10 %	72.970 ± 12.561	47.351	95.590
	5 %	78.256 ± 11.545	54.009	102.504
	0 %	136.000 ± 4.582	127.006	142.980

Table 2: Comparison of LT_{50} values against termites in different concentrations of Methanol leaves extracts of *Cococarpus lancifolius* and Chlorfenapyr.

Treatment	Concentration	LT_{50} (Hours) ± SE	95 % Confidence Interval	
			Lower Limit	Upper Limit
Conocarpus lancifolius	15 %	190.182 ± 15.939	157.710	222.806
	10 %	216.185 ± 14.283	186.882	245.154
	5 %	243.133 ± 12.694	217.598	268.668
	0 %	294.000 ± 8.630	277.084	310.915
Chlorfenapyr	15 %	66.304 ± 11.536	44.734	92.874
	10 %	70.970 ± 11.208	48.217	97.564
	5 %	76.923 ± 12.371	53.675	101.837
	0 %	130.000 ± 7.137	116.011	143.990

Table 3: Comparison of Means ± SE at different concentrations of methanol leaf extract of *Conocarpus lancifolius* and Chlorfenapyr.

Treatments	Time Intervals	Concentrations			
		15 %	10 %	5 %	0 %
Conocarpus lancifolius	15 minutes	09.50 ± 1.94 c	05.25 ± 0.85 d	03.25 ± 0.75 f	09.50 ± 0.65 c
	30 minutes	14.00 ± 2.94 ab	13.00 ± 1.08 c	05.75 ± 1.31 e	14.00 ± 0.65 g
	45 minutes	19.00 ± 0.48 a	18.25 ± 0.85 b	9.50 ± 0.65 d	04.50 ± 0.65 f
	60 minutes	19.25 ± 0.58 a	19.25 ± 0.48 ab	14.50 ± 0.65 c	05.50 ± 0.65 e
	75 minutes	19.50 ± 0.50 a	19.25 ± 0.48 ab	17.50 ± 0.65 b	08.50 ± 0.65 d
	90 minutes	20.00 ± 0.00 a	19.75 ± 0.25 ab	19.00 ± 0.41 a	12.75 ± 0.85 c
	105 minutes	20.00 ± 0.00 a	19.75 ± 0.25 ab	19.50 ± 0.50 a	16.25 ± 0.63 ab
	120 minutes	20.00 ± 0.00 a	19.75 ± 0.25 ab	20.00 ± 0.00 a	18.75 ± 0.48 a
	135	20.00 ±	20.00 ±	20.00 ± 0.00 a	19.75 ± 0.25 a

	minutes	0.00 a		0.00 a				
	150 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	19.75 ± 0.25 a	
	165 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	20.00 ± 0.00 a	
	180 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	20.00 ± 0.00 a	
Chlorfenapyr	15 minutes	11.00 ± 0.91 c	±	6.50 ± 0.65 d	±	03.50 ± 0.65 f	01.00 ±1.65 k	
	30 minutes	15.25 ± 1.11 b	±	12.50 ± 0.65 c	±	09.50 ± 0.65 e	02.00 ± 1.08 ij	
	45 minutes	18.75 ± 0.75 a	±	17.25 ± 0.85 b	±	12.50 ± 0.65 d	03.50 ± 1.04 ih	
	60 minutes	19.75 ± 0.25 a	±	19.25 ± 0.48 a	±	15.75 ± 0.85 c	5.50 ± 1.85 hgf	
	75 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	18.50 ± 0.65 b	07.50 ± 2.02 gfe	
	90 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	19.75 ± 0.25 ab	09.25 ± 2.17 fed	
	105 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	10.50 ± 1.66 ed	
	120 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	12.75 ± 1.25 dc	
	135 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	15.50 ± 1.04 cb	
	150 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	16.00 ± 1.47 abc	
	165 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	18.25 ± 1.18 ab	
	180 minutes	20.00 ± 0.00 a	±	20.00 ± 0.00 a	±	20.00 ± 0.00 a	20.00 ± 0.00 a	

Table 4. Comparison of Means ± SE at different concentrations of aqueous leaf extract of *Conocarpus lancifolius* and Chlorfenapyr.

Treatments	Time Intervals	Concentrations			
		15 %	10 %	5 %	0 %
Conocarpus lancifolius	15 minutes	07.25 ± 1.11 d	03.75 ± 0.85 e	02.50 ± 0.65 f	01.75 ± 0.85 g
	30 minutes	11.50 ± 1.32 c	07.50 ± 0.65 d	02.75 ± 0.85 f	01.75 ± 0.85 g
	45 minutes	15.75 ± 1.55 b	10.75 ± 0.85 c	04.25 ± 1.11 f	03.25 ± 0.85 g
	60 minutes	18.75 ± 0.75 a	15.50 ± 0.65 b	07.75 ± 0.85 e	03.50 ± 0.65 g
	75 minutes	19.25 ± 0.75 a	18.50 ± 0.65 a	10.50 ± 0.65 e	06.75 ± 1.11 f
	90 minutes	20.00 ± 0.00 a	19.00 ± 0.58 a	13.50 ± 0.65 c	07.75 ± 1.31 fe
	105 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	14.25 ± 1.31 c	10.00 ± 1.47 e

	120 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	18.75 ± 0.75 ab	12.75 ± 1.31 d
	135 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	19.75 ± 0.25 a	14.50 ± 0.65 d
	150 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a	15.50 ± 1.32 cb
	165 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a	17.50 ± 0.65 ab
	180 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a	19.25 ± 0.75 a
Chlorfenapyr	15 minutes	09.75 ± 1.93 c	06.75 ± 1.38 f	03.50 ± 0.65 e	02.50 ± 0.65 f
	30 minutes	13.75 ± 1.75 b	9.50 ± 2.02 ed	06.00 ± 1.08d c	02.50 ± 0.65 f
	45 minutes	17.00 ± 1.47 a	12.25 ± 1.80 dc	08.25 ± 1.30 c	04.50 ± 0.65 e
	60 minutes	18.75 ± 0.95 a	15.00 ± 1.96 cb	12.00 ± 2.04 b	05.50 ± o.65 e
	75 minutes	19.50 ± 0.50 a	17.75 ± 1.31 ab	14.75 ± 1.80 b	08.50 ± 0.65 d
	90 minutes	20.00 ± 0.00 a	19.50 ± 0.00 a	18.25 ± 1.03 a	12.75 ± 0.85 c
	105 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	19.50 ± 0.50 a	16.25 ± 0.63 b
	120 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a	18.75 ± 0.48 a
	135 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a	19.75 ± 0.25 a
	150 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a	19.75 ± 0.25 a
	165 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a
	180 minutes	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a	20.00 ± 0.00 a

At 10 % concentration, the same repellency was noticed among time intervals (90-180 minutes) and the first time interval (15 minutes) exhibited a significant difference from other time intervals. Similarly, in 5 % concentration, the first-time interval (15 minutes) showed significant variation towards other examined time intervals and the same repellency was recorded between 30 and 45 minutes, 60 and 75 minutes and from 90-180 minutes (time intervals). Control (0 %) concentration attained non-significant repellency from 120 to 180 minutes (time intervals). Statistically same results were recorded among 15 and 30 minutes, 45 and 60 minutes while 75, 90 and 105 minutes of time intervals depicted statistically dissimilar repellency.

DISCUSSION

The mortality of subterranean (fungus growing) termites through the plant leaves extract and termiticide were found to be noteworthy for the management of subterranean termites. Our results overlap with the results of (Iqbal and Saeed, 2013) who observed the insecticides of several groups such as

indoxacarb, fipronil, imidacloprid, thiamethoxam, spinosad and chlorfenapyr were utmost destructive against *Macrotermes mycophagus.* Two places, an agricultural farm and one tree plantation were used to collect termites in Multan. The outcome exhibited that chlorfenapyr insecticide caused higher mortality in comparison with imidacloprid, fipronil, indoxacarb, thiametoxam and spinosad. The population of termite gathered from tree plantations posed further resistance against the insecticide tested than agriculture farm termite.

The toxic effect of crude leaf hexane, ethyl acetate acetone and methane extracts of *Andrographis lineata* were used against the termites. In this experiment impregnated filter paper no choice bioassay method was used. The anti-hermitic activity of all these crude extracts is dose-dependent and showed significant activity after 24-48 hours of exposure. After 24 hours the maximum mortality of termite was found in leaf hexane extract of *Andrographis bracteolate*, ethyl acetate extract of *Andrographis paniculata*, *Datura metel*, *Eclipta prostrata*, methanol extract of *A. lineata* (LD_{50} = 363, 371, 298, 292, 358, and 317 ppm; LD_{90} = 1433, 1659, 1308, 1538, 1703, and 1460 ppm). After 48 hours the hexane extract of *Tagetes erecta*, acetone extract of *Alfaroa mexicana*, methanol extract of *Sesbania grandiflora* and *T. erecta* exhibited activity (LD_{50} = 245, 253, 289, 409 ppm; LD_{90} = 1378, 1511, 1508, and 2425 ppm).

Our results showed that the efficiency of *C. lancifolius* botanical extract was assessed with methanol at 15 % (190.182), 10 % (216.185), 5 % (243.133) and 0 % control (294.000) and the efficiency of aqueous leaves extract of *C. lancifolius* at 15 % (207.996), 10 % (225.241), 5 % (248.000) and control (310.000) were assessed against the subterranean termites. Whereas efficiency of chlorfenapyr through methanol was assessed at 15 % (66.304), 10 % (70.970), 5 % (76.923) and 0 % control (130.000) and the efficiency of water solution through chlorfenapyr at 15 % (68.304), 10 % (72.970), 5 % (78.256) and 0 % control (136.000) were assessed against the subterranean termites. The results of the current research showed that *C. lancifolius* plant extract of water and methanol at concentration (15 %) exhibited higher mortality and chlorfenapyr solution with water and methanol at concentration (15 %) showed 98 % mortality against fungus growing subterranean termites. While chlorfenapyr at concentration (15 %) exhibited 100 % mortality of fungus growing subterranean termites which were less in plant leaves extracts. The effectiveness of various concentrations of neonicotinoids insecticide, thiamethoxam (ACTARA® 25WG) was checked in the lab against three species of Philippines termites including *Nasutitermes luzonicus*, *Macrotermes gilvus*, and *Macrotermesilos banosensis*i. Our results are in line with (Acda, 2007).

Plant leaves extracts of two tropical plant species Clove *(Syzygium aromaticum)* and Cajuput *(Melaleuca leucadendra)* against subterranean termite *Coptotermes formosanus Shiraki*, with 70 % ethanol solvent. Termicidal performance of two different plant extracts due to eugenol, after 4 weeks of the exposure to extracts, determines losses of mass and mortality of termite. Results showed that crude of different plant species was applied at the similar eugenol based concentration that shows different reactions against subterranean termites, consumption rate and termites mortality is 0.3 % and 100 % respectively. The crude extract of *Syzygium aromaticum* at concentration 0.1 % as compared to crude extract of *Melaleuca leucadendra* showed low mortality of termites (36.8 %) and high consumption rate (9.1 %). Results exhibited that crude extracts of diverse plant species have a different influence on subterranean termites although they were applied at the same eugenol based concentration (Indrayani et al., 2016). The

repellency of plant leaves extracts and termiticides against termites were observed and showed that in termiticides repellent action was more as compared to plant leaves extracts.

The results of our study are in agreement with (Thorne and Breisch, 2001) who revealed the repellency and behavior of termites that had earlier been exposed to the insecticide, imidacloprid. The sub-lethal dosages of insecticide imidacloprid were prepared for the assessment of population and then left for 7 days to recover. The sand was treated through 100 and 10 ppm of imidacloprid the termites were again exposed for 4 hours and showed no behavioral response against termites. The result revealed that soil treated with imidacloprid can efficiently used against the termites to protect the buildings.

Fipronil, indoxacarb, imidacloprid and chlorfenapyr are midst the utmost illustrious insecticides (Gahlhoff Jr and Koehler, 2001). Bifenthrin and chlorfenapyr have been recorded for more residual influences in treated soil than imidacloprid, fipronil, and chlorpyrifos (Horwood, 2007). Excess amount of chlorfenapyr shows more toxicity to *Reticulitermes flavipes* when they come into touch, but when employees have been handled with up to 50, 100, 250 and 500 ppm, they demonstrate 100 % mortality (Shelton et al., 2006).

Zhu et al., (2001) studied the effects of Vetiver grass on the behavior of *Formosan subterranean* termites, as insect repellents. The result exhibited that Nootkatone, a sesquiterpene ketone extracted from the vetiver grass was virulent to *Formosan subterranean* termite and strong repellent at lowest concentration 10 ug g^{-1}.

Bläske and Hertel (2001) reported the effect of four plant extract formulations treated with filter paper on the orientation and survival of subterranean termites. In choice, experiments extract-treated filter paper has a significantly repellent effect on groups of *Reticulitermes santonensis, Reticulitermes virginicus, Coptotermes formosanus* and *Schedorhinotermes intermedius* and showed no specific species difference in avoidance behavior towards tested concentrations. In these experiments, the toxic properties of extracts by contact or airborne are not shown in *Reticulitermes santonensis*. However, the forced indirect or direct exposure to plant material showed maximum mortality of termites, the feeding deterrence could not be determined in these experiments. Extract treated barriers prohibited termites to enter in the treated soil without affecting mortality.

CONCLUSION

It is concluded that the botanical extract of *C. lancifolius* with water and methanol and, chlorfenapyr aqueous with methanol solution demonstrated maximum repellency and lethal against fungus growing subterranean termites.

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CONFLICTS OF INTEREST

The authors declare no competing interests regarding the publication of this work.

AUTHOR CONTRIBUTIONS

Conceptualization, M.S.N., M.A. and T.N.; data curation, M.A. and T.N.; formal analysis, H.M.U.A., S.Z. and T.N.; funding acquisition, M.S.N. and S.Z.; investigation, W.N. and M.A.; methodology, M.A. and T.N.; project administration, M.S.N. and T.N.; resources, M.S.N. and W.N.; software, S.I.H. and

N.A.K.; supervision, M.S.N. and T.N.; visualization, M.A., W.N. and T.N.; writing—original draft, S.I.H., N.A.K. and W.N.; writing—review and editing, M.S.N., H.M.U.A. and T.N. All authors have read and agreed to the published version of the manuscript.

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