Asian Journal of Chemistry; Vol. 25, No. 5 (2013), 2405-2408

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13335

Chemical Composition of Essential Oils Derived from Eucalyptus and Lemongrass and Their Antitermitic Activities Angainst *Microtermes mycophagus* (Desneux).

FARKHANDA MANZOOR[1,*], NARJIS NAZ[2], SAADIYA A. MALIK[1], SAMRA ARSHAD[1] and BINA SIDDIQUI[3]

[1]Department of Zoology, Lahore College for Women University, Lahore, Pakistan
[2]Department of Chemistry, Lahore College for Women University, Lahore, Pakistan
[3]HEJ Research Institute, Karachi, Pakistan

*Corresponding author: E-mail: doc_farkhanda@yahoo.com

(*Received*: 27 December 2011; *Accepted*: 7 November 2012) AJC-12369

The essential oils of eucalyptus (*Eucalyptus citriodora*) and lemon grass (*Cymbopogon citratus*) were analyzed by GC-MS and tested against economically important termite species of Pakistan *i.e.*, *Microtermes mycophagus* (Desneux). Their physical parameters were also scanned. The major constituents identified in eucalyptus oil are: eucalyptus, limonene, terpinen-4-ol, gravenone, *n*-tetradecane, 2,2′6,6′-tetramethyl piperidin-4-ol, piperitone and the chemical components of lemon grass oil are: myrcene, citronellol, citral, nerol and neric acid. The repellency, toxicity, fumigation activities of test oils and tunneling behaviours of *M. mycophagus* were evaluated. At 25 µg/10µL oil concentration, lemon grass showed 63.33 % and eucalyptus oil showed 62.22 % mortality and at the same concentration, the repellency test revealed that both eucalyptus and lemon grass oils showed efficient repellency *i.e.*, 42.6 and 36.33 % respectively. Fumigation results revealed that lemon grass caused 100 % mortality at 5 ppm concentration on 9th day of treatment whereas eucalyptus gave same result in the end of test period (day 12). When tunneling behaviour was observed for *M. mycophagus*, the average time to cross the tube at 50 µg was 28 and 50 h in eucalyptus and lemon grass oil one-to-one.

Key Words: Anti-termitic activity, Eucalyptus, Lemongrass, *Microtermes mycophagus* (Desneux).

INTRODUCTION

Essential oils extracted from aromatic plants have been used since ancient times as safe condiment or spice, in flavour and fragrances in medicines and to combat against microbes and to prevent or keep stored agricultural products safe from insects[1-4]. Essential oils are successful alternatives to insecticides without causing any danger to the environment[5-7]. Biological compounds with improved insecticidal properties and being non-toxic or only mildly toxic to humans are needed on constant basis[2].

Microtermes mycophagus (Desneux) is a subterranean termite species that infests commercially important timber species like *Dalbergia sissoo, Cedrus deodara, Mangifera indica, Zyziphus spp, Morus* spp. and *Acacia* spp, attacking logs and stumps, causing a lot of damage to forests, wooden structures and buildings. This species has also been reported as agricultural pest of nurseries, vegetables and sugarcane field[8-11].

Essential oil obtained from the leaves of *Eucalyptus citriodora* belonging to the family Myrtaceae, is used as a pesticidal agent and as insect repellent[12]. Lemon grass (*Cymbopogon citratus*), member of family Poaceae (Gramineae), is a local grass native to India, Sri Lanka and Pakistan.

The present study is the continuation of our research work[13] to test the different essential oils as potential termiticides and to detect the constituent profile of these oils by GC-MS, so eucalyptus and lemon grass oils are assessed as insect repellent, toxicant and fumigant against *M. mycophagus* (Desneux).

EXPERIMENTAL

Test essential oils: Reverse Dean-Stark method was used for steam distillation of the essential oils from the leaves of *E. citriodora* and *C. citratus*. The percentage yield of each essential oil was determined.

Oils were also tested for physical parameters such as solubility, specific gravity, refractive index, acid value and ester value.

GC-MS analysis: GC-MS of Varian, Saturn model 2000, equipped with ion trap detector (ITD) was used for the detection of various components of essential oils. Sample was injected on a DB-5MS (30 m × 0.25 µm id, 0.25 µm film thickness) column. Helium (carrier gas) with a flow rate of 1.0 mL/min and split ratio 1:5 was used. The temperature of column was maintained at 50 ºC at start for 3 min with a 4 ºC rise/min to

180 ºC. Potential components of the test oils were recognized by their retention time and peak enhancement with standard samples in gas chromatographic mode and MS library search from the derived mass fragmentation pattern of various components of the essential oil.

Collection of termites: The workers and soldiers of *M. mycophagus* (Desneux) were collected using the tissue paper traps with sugarcane piece as bait attractant. Termites (workers and soldiers) were isolated and kept in plastic boxes with moist filter paper. Before treatment with oil, healthy termites were maintained in the laboratory at 26 ± 2 ºC, 80 % relative humidity in constant darkness, which were used for the experimentation.

Experimental protocols: Repellency, toxicity, tunneling bioassays fumigation test with slight modification were conducted as reported in literature[14,15].

Experimental conditions: All the experimental units for each type of bioassay were kept in climatic room maintained at 28 ºC and 80 % relative humidity at constant darkness.

Repellency Test: A Petri dish (5 cm in diameter and 1 cm high) with 2 filter paper disks (1 cm dia, 0.78 cm^2 area) attached at opposite ends of the dish with 1 µL dichloromethane was used. One of the 2 disks was treated with 0.3 mL of 1, 5, or 25 µg/10 µL concentrations of essential oils and evaporated for 0.5 h to remove the solvents. Control disks were treated with 10 µL of ethanol only and evaporated. Each treatment dose was replicated 3 times. Ten workers were released in the centre of each Petri plate. These plates were placed in a climatic room. The distribution of termites was recorded every 15 min for 5 h. Twenty observations were taken. The numbers of termites on the untreated and treated sides were calculated. The recorded data was analyzed.

Toxicity test: Filter paper disks (40 mm dia) were oven-dried and weighed prior to the treatment application. 1, 5, 10, 15 and 25 µg/cm^2 of essential oil treatments on the disks was made. The dilutions were topically applied on Whatmann filter paper No.1. Solvent treated filter papers were used as control. Allowed the complete evaporation of solvent at room temperature (65 % relative humidity, 20 ºC); then the filter papers were placed in a 50 × 9 mm Petri plates over a thin layer of moist sand. Thirty active termites were released into the Petri plate containing filter paper disks treated with the test material. The covered Petri dishes were then kept in climatic room. Dead termites were removed by forceps after every counting. After 8 days the non-consumed filter papers were removed, cleaned of debris, oven dried and weighed to determine the consumption. Live termites would be counted to determine mortality. The rates of mortality were examined daily up to 8 days. Three replicates for each concentration of oils including control were prepared and the data was recorded for further statistic procedures.

Fumigation test: The reported method[15] with some modifications was used to evaluate the vapour action of essential oils against species of termites *H. indicola*. Tall cylindrical glass containers (inside volume 880 cm^3) were used. The size of filter paper used was according to the dimension of the Petri dishes. It was moistened with distilled water and placed in the Petri dish at the bottom of each container. Twenty five termite workers were released in this Petri dish. A 4.25 cm diameter filter paper was treated with test oil to produce a concentration 5 ppm. This filter paper was suspended from the lid of the container with the help of a thread in order to avoid contact with the termites after allowing the ethanol to evaporate completely. For control, the suspended filter paper was treated with ethanol alone evaporated and then suspended in the control set. The mortality was checked regularly. Each oil treatment was replicated for 3 times. Based on the mortality rate, the oils were classified as toxic without contact or not.

Tunnelling bioassay: Three compartment tunneling test apparatus was designed for this bioassay. The first compartment was a plastic vial containing moistened but untreated soil. The second compartment was a 10 cm long tygon tube, containing oil treated soil linked the two plastic vials and third one was another vial containing untreated sand. About 5 g sand moistened with 1 mL of distilled water was placed in each of the two 30 mL plastic vials with lids. The vials were connected with a tygon tube filled with sand treated with 0, 1, 5 and 25 µg/10 µL concentrations of test compounds. The solvent was evaporated from the treated sand. Each treatment dose was replicated 3 times and in each controls no treatment. Thirty workers were released into one of the vials allowing them to travel to treated zone and through the whole apparatus as well. Tunneling activity was noted daily. A test was terminated once termites tunnelled all the way through the tube.

RESULTS AND DISCUSSION

The physical characteristics such as colour, odour, specific gravity, refractive index, yield, solubility, acid value and ester value of both essential oils *i.e.*, Eucalyptus oil (*Eucalyptus citriodora*) and lemon grass oil (*Cymbopogon citratus*) were determined as shown in Table-1. The percentage yield of essential oil was determined *i.e.*, 0.24 %. Literature reported[16] that fresh lemon grass contain 0.26-0.525 % essential oil. The yield of essential oil of *C. citratus* was found very close to the % yield reported.

TABLE-1
PHYSICO-CHEMICAL INVESTIGATION OF ESSENTIAL OIL OF EUCALYPTUS (*Eucalyptus citriodora)* AND LEMONGRASS (*Cymbopogon citratus)*

S. No.	Physical Properties	*Eucalyptus citriodora*	*Cymbopogon citratus*
1	Colour	Greenish (light)	Pale sherry
2	Odour	Lemon scented	Pungent
3	Yield	0.74 %	0.24 %
4	Solubility in alcohol (70 %)	1.3 to 1.5 vol.	Soluble in 70 % alcohol
5	Specific gravity	0.8670 at 20 ºC	0.846 at 20 ºC
6	Refractive Index	1.4550	1.474
7	Acid value	-	3.116
8	Ester value	12 to 45 %	2.244 to 12.1 %

GC-MS analysis of eucalyptus oil and lemon grass oil revealed the presence of different components reported in Table-2 and also established by the literature[17-19]. These components individually or in combination with others, may be the potential termiticidal agents.

The number of termites, *M. mycophagus* exposed to different concentrations of eucalyptus oil (*E. citriodora*) and

TABLE-2
GC-MS ANALYSIS OF ESSENTIAL OILS OF EUCALYPTUS (*Eucalyptus citriodora*) AND LEMONGRASS (*Cymbopogon citratus*)

S. No.	Components identified in eucalyptus oil	Components identified in lemon grass oil
1	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane(eucalyptus)	7-Methyl-3-methylene-1,6-octadien(myrcene)
2	1-Methyl-4-(1-methylethenyl)-cyclohexene(limonene)	3,7-Dimethyloct-6-en-1-ol (citronellol)
3	3-Cyclohexen-1-ol(terpinen-4-ol)	3,7-Dimethylocta-2,6-dienal (citral)
4	3-Methyl-5-propylcyclohex-2-en-1-one (gravenone)	(*Z*)-3,7-dimethyl-2,6-octadien-1-ol(nerol)
5	*n*-Tetradecane	(2*Z*)-3,7-Dimethyl-2,6-octadienoic acid(neric acid)
6	2,2`6,6`-Tetramethyl piperidin-4-ol	
7	6-Isopropyl-3-methyl-1-cyclohex-2-enone(piperitone)	

lemon grass oil (*C. citratus*) in ethanol solvent were recorded to test the repellent effect of oils. Graphical presentation of the results depict that both essential oils tested had excellent repellent activity against the workers of *M. mycophagus* (Desneux) at concentration of 25 µg/10 µL in 5 h treatment while a significant repellency was marked in two other treatments of the both test oils as compared to the untreated disks (Fig. 1).

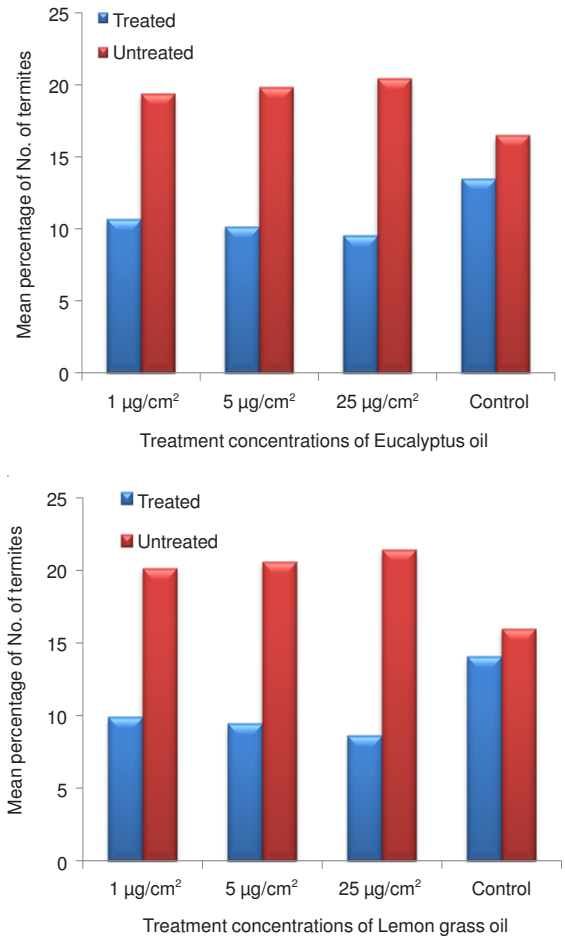

Fig. 1. Bar charts showing the repellency test results of different treatments of eucalyptus oil (*Eucalyptus citriodora*) and lemon grass oil (*Cymbopogon citratus*) against workers of *M. mycophagus* (Desneux)

In toxicity test, two parameters *i.e.*, feeding and mortality were assessed for test essential oils against *M. mycophagus* (Desneux). The weight loss provided a measurement of termite consumption rate. As can be seen from Table-3 essential oils tested were highly toxic against workers of *M. mycophagus* (Desneux) at dosage of 25 µg/10 µL, thus mean weight loss of filter paper after 9 days of treatment was very low in eucalyptus and lemon grass oil treatments. However, the filter paper consumption by the workers of *M. mycophagus* seems to decrease with the increase in oil concentration. The analysis of variance revealed significant difference in percentage mortality of *M. mycophagus* (Desneux) among different concentrations of both test oils ($F = 1.061$, $df = 2, 15$; $P = 0.0001$). LC_{50} values (calculated through EPA probit analysis program) for *M. mycophagus* (Desneux) against eucalyptus and lemon grass after nine days of treatment are also presented in Table-3.

The fumigation test results manifest that Eucalyptus oil and Lemon grass oils substantiated the repellency and toxicity against *M. mycophagus*, as can be perceived from Table-3 both oils tested were capable of killing all workers of *M. mycophagus* (Desneux) at 5 ppm dosage after 9 days. It was revealed from analysis of variance that there were significant differences between percentage mortality of *M. mycophagus* (Desneux) among both essential oils ($F = 0.00914$, $df = 2, 12$; $P = 0.9909$).

When tunneling behaviour of *M. mycophagus* (Desneux) was observed through the apparatus designed, the results revealed that the tested oils *i.e.* eucalyptus oil (*E. citriodora*) or lemon grass oil (*C. citratus*) did not affect tunneling activity at 0, 5, 25 and 50 µg/g and the termites tunneled through the tubes in all cases. In case of highest concentration treatment *i.e.* 50 µg/g lemon grass oil, for those termites that completely crossed the tubes, it took them an average of 50 h. However, for eucalyptus oil the average time to cross the tube was 28 h at the same concentration. In both oil treatments the termites became sluggish to moribund after crossing the tunnel. In control no termite mortality was observed and tunneling into the tubes was evident (Table-3).

In a comparative outlook, through results obtained from different tests applied for both oils, *C. citratus* oil treatments exhibited a significant higher efficacy against *M. mycophagus* (Desneux) in our studies. Literature provides a lot of research work for the use of different plant essential oils and natural monoterpenoids against several other species of subterranean termites but there was no previous evidence for the particular use of *E. citriodora* and *C. citratus* essential oils against *M. mycophagus* (Desneux) as potential termiticidal. However both of these oils along with others have been successfully evaluated as possessing toxicant and repellent components against Formosan subterranean termites[20]. The tunneling and fumigation test results of our study recommend the different concentrations of these oils as competent termite control measure in a closed and limited treatment area as the open air may reduce the efficacy volatility of these oils being the limiting factor.

TABLE-3
RESULTS OF DIFFERENT TESTS OF ESSENTIAL OILS OF EUCALYPTUS (*Eucalyptus citriodora)* AND LEMONGRASS (*Cymbopogon citratus*) APPLIED AGAINST WORKERS OF *M. mycophagus* (Desneux)

Test applied	Concentrations	Test oils					
		Eucalyptus citriodora essential Oil			*Cympobogon citratus* essential Oil		
		Mean mortality (%)	Filter paper consumption) (Ave. ± SEM, N = 3)	LC_{50}	Mean mortality (%)	Filter paper consumption) (Ave. ± SEM, N = 3)	LC50
Toxicity test / mortality test	1 µg/10 µL	23.33 ± 0.57	0.010 ± 0.000	14.94	24.44 ± 0.33	0.008 ± 0.002	13.35
	5 µg/10 µL	32.22 ± 0.67	0.008 ± 0.002		33.33 ± 0.57	0.007 ± 0.002	
	10 µg/10 µL	40.00 ± 1.15	0.008 ± 0.002		42.22 ± 0.67	0.005 ± 0.000	
	15 µg/10 µL	50.00 ± 0.57	0.007 ± 0.002		51.11 ± 0.88	0.005 ± 0.000	
	25 µg/10 µL	62.22 ± 0.67	0.003 ± 0.002		63.33 ± 0.57	0.002 ± 0.002	
	Control	0.00 ± 0.00	0.017 ± 0.003		0.00 ± 0.00	0.017 ± 0.003	
Tunneling bioassay		Percentage distance crossed at different test conc. (Ave. ± SEM, N= 3)	Average hours to cross tubes (X ± SE)		Percentage distance crossed at different test conc. (Ave. ± SEM, N = 3)	Average hours to cross tubes (X ± SE)	
	0 µg/g	100	24 ± 0.00		100	24 ± 0.00	
	5 µg/g	100	24 ± 0.00		100	24 ± 0.00	
	25 µg/g	100	26 ± 2.00		100	28 ± 2.00	
	50 µg/g	100	28 ± 2.00		100	50 ± 2.00	
Fumigation test at a concentration of 5 ppm (Ave. ± SEM, N= 3)		Reading day	Mean no. of dead termites		Mean no. of dead termites		
		1d	8.33 ± 0.57		9.33 ± 0.57		
		3d	13.33 ± 0.57		13.33 ± 0.57		
		5d	17.33 ± 0.57		17.33 ± 1.15		
		7d	20.00 ± 1.00		21.00 ± 1.00		
		9d	22.00 ± 1.00		25.000 ± 0.00		
		Control	0.00 ± 0.00		0.00 ± 0.00		

These essential oils with antitermitic properties are the impending natural sources for the development of novel termiticidal with least mammalian toxicity and most environment protection hence should be given priority to isolate the tangible antitermitic constituents and to determine their mode of action against termites.

REFERENCES

1. H.J.D. Dorman and S.G. Deans, *J. Appl. Microbiol.*, **88**, 308 (2000).
2. M.B. Isman, *Crop. Protect*, **19**, 603 (2000).
3. G. Rosell, C. Quero, J. Coll and A. Guerrero, *J. Pestic. Sci.*, **33**, 103 (2008).
4. F. Bakkali, S. Averbeck, D. Averbeck and M. Idaomar, *Food Chem. Toxicol.*, **46**, 446 (2008).
5. S.V. Phatak and M.R. Heble, *Fitoterapia*, **73**, 32 (2002).
6. I.K. Park and S.C. Shin, *J. Agric. Food Chem.,* **53**, 438 (2005).
7. S.T. Chang, S.S. Cheng and S.Y. Wang, *J. Chem. Ecol.,* **27**, 717 (2001).
8. M.I. Chaudhry and M. Ahmed, Termites of Pakistan. Identifying, Distributing and Ecological Relationships (Final Technical Report). Pakistan Forest Institute of Peshawar, pp. 13-19 (1972).
9. M.S. Akhtar, *Pak. J. Zool.*, **6**, 85 (1974).
10. M.S. Akhtar, *Materia. Organ.*, **18**, 277 (1983).
11. F. Manzoor, S.M.A. Rahim, B.M.A. Shiday, S.A. Malik, B. Habibpour, J.R.J. French and F. Jabeen, *Afri. J. Environ. Sci. Tech.*, **4**, 11 (2010).
12. A.F.M. Barton, *J. Ind. Ecol*., **3**, 161 (2000).
13. F. Manzoor, N. Naz, S.A. Malik, B.S. Siddiqui, A. Syed and S. Perwaiz, *Asian J. Chem.*, **24**, 2069 (2012).
14. K.R. Chauhan and A.K. Raina, in ed.: A.M. Rimando and S.O. Duke, Modified Vetiver Oil: Economic Biopesticide. Natural Products for Pest Management, ACS Symposium Series, **27**, 210 (2006).
15. A. Raina, J. Bland, M. Doolittle, A. Lax, R. Boopathy and M. Folkins, *J. Econ. Entomol.*, **100**, 880 (2007).
16. R.N. Chopra, Indeginous Drugs of India, Academic Publishers, Calcutta, India, edn. 2 (1994).
17. K. Jaimand, M.H. Assareh and M.B. Rezaee, *Iran. J. Pharm. Res.*, **1**, 73 (2006).
18. S.S. Basak and F. Candan, *J. Iran. Chem. Soc.*, **7**, 216 (2010).
19. E.E. Aziz and M.H. Abbass, *Am.-Eur. J. Agric. Environ. Sci.*, **8**, 411 (2010).
20. B.C.R. Zhu, G. Henderson, F. Chen, H. Fei and R.A. Laine, *J. Chem. Ecol.*, **27**, 1617 (2001).