Influence of some plant oils *Eugenia caryophyllata*, *Elettaria cardamomum* and *Citrus sinensis* on the termite species *Coptotermes heimi*

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> **Abstract** Termites are one of the most troublesome pest of plants, trees, wooden infrastructure and agricultural crops. The aim of the present study is to evaluate the population of some plant oils using *Eugenia caryophyllata, Elettaria cardamomum* and *Citrus sinensis* against *Coptotermes heimi* (Wasmann) at Punjab University, New Campus Lahore, Pakistan. All plants were effectively toxic against *C. heimi* as 100 % mortality was observed at 62.5, 125 and 250 µl/ml doses within three to four days of exposure. All plant oils extracts proved to be highly repellent to the termites even in the low concentrations. These plant oils can be used for effective termite control being less expensive and biodegradable, hence environmentally suitable.

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Keywords: Termites, Plant extracts, Repellency

Introduction

Received: December 23, 2017

Accepted:

Published.

March 26, 2018

September 30, 2018

Termites are the most abundant pests of plants, trees and wooden structure. They severely damage agricultural crops and urban infrastructure. Although termites feed on dead wood and cellulose rich sources, they only become problematic when they attack on trees, crops and logs (Pearce, 1997; Manzoor et al., 2011; Balachander et al., 2013). They cause serious damage to different crops causing to unhealthy growth of crops (Sheikh, 2009), even almost 2800 species of the termites were found with approximately 185 possible pest's species (Lewis, 1997; Balachander et al., 2013). More than one hundred species belong to the group of the subterranean termites and just 28 species are of economic importance (Lewis, 1997). In Pakistan, there are almost 50 species which cause damage (Akhtar, 1974) but eleven species have been found destructive and need serious attention (Akhtar, 1983). As termites have become a major threat to human economy, economical and ecofriendly alternative biological control strategies such as the use of seeds extracts, essential oils, wood extracts, fruits, leaves and barks oil (Adams et al., 1998; Sakasegawa et al., 2003; Cheng et al., 2004; Jembere et al., 2005; Park and Shin, 2005; Verma et al., 2009; Ding and Hu, 2010; Rasib and Aihetasham, 2016). Their mode of action is upon causing interference in gut flora in order to repel or kill the termites (Adams et al., 1998).

Many Plant's oil extracts showed their activity even as an anti-termite activities or has a repellent effect. They were such as *Pinus roxburghii*, Eucalvptus camaldulensis and Morus alba (Rasib and 2016), Aihetasham, lemongrass, Eucalyptus citrodora, cedar wood, vetivergrass and clove bud (Zhu et al., 2001), Taiwania cryptomerioides (Chang et al., 2001) purple hop brush; Dodonaea viscose (Anonymous, 2001). *Cymbopogon winterianus*, Ocimum basilicum, Rosmarinus officinalis and Cinammomum campho (Sbeghen et al., 2002) and Coleus ambionicus (Singh et al., 2004) Plants oils and powder have been used extensively in the control of insect pests because they are believed to contain myriads of natural chemical that could be insecticides (Adeyemo et al., 2015).

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The present research work aimed to present the antitermitic and antifeedant activities of clove (Eugenia caryophyllata), Elachi (Elettaria cardamomum) and Citrus (Citrus sinensis) plants essential oils against a subterranean termites Coptotermes heimi (Wasmann).

Material and Methods

Termite collection

Coptotermes heimi (workers and soldiers) were collected from Populus euramericana (populous) trees of University of the Punjab, Lahore, Pakistan. Termites were separated from the tree barks and debris in the laboratory and were kept at $\pm 27^{\circ}$ C only healthy individuals were taken for experiments. The apparatus used in the experiment was autoclave. Moist filter paper were placed in Petri plates for termite's survival.

Extracts of plants oil

The essential oil samples were extracted from the collected plant material like peel of oranges (C. sinensis), fruits of Clove (E. caryophyllata) and seeds of Elachi (E. cardamomum) by hydro distillation for 5 hours using the process of Guenther (1949). After that these extracts were kept in glass vials and held air tight at 4°C.

Experimental soil preparation

Soil was collected from lawn of Department of Zoology, University of the Punjab, Lahore. It was free of contamination by insecticides as there has been no known application of any chemical. The soil was used to maintain termites like natural environment. Soil was sieved through a 10- mesh oven dried at 72°C for 24 h. Petri plates were washed and sterilized at 180°C for 2h for accuracy of experiment and for avoidance of contaminants growth.

Mortality test

For mortality test Petri dishes, filter paper and soil were sterilized. Twenty grams of sterilized soil were taken in each Petri plate and sprayed with distilled water. In each Petri plate 300 termite workers and 5 soldiers were released in arena with treated filter paper with respective dilutions. Then Petri plates were covered with black cloth. Dead termites were removed and counted after 24 hour interval to avoid fungal growth. Each experiment was replicated three times.

Repellency test

Repellency test was performed following the method of Aihetasham et al., 2018. Filter papers cut according to the size of Petri plate and were divided into two equal halves in such a way that there was space between two halves. One half treated with oils dilutions (250, 125 and 62.5 µl/ml) and other half was served as a control using distilled water. Thirty workers were released in between treated and untreated filter paper. Numbers of termites were counted in treated and untreated zone after 60 minutes time interval

Data analysis

Statistical analysis and General Linear Model (GLM) Tukey's comparison analysis were conducted using software Minitab 16.

Results and Discussion

Termiticidal activity of different plants oils was tested against the workers of C. heimi. Many plants extracts have been used against termites to replace used insecticides (Ahmed et al., 2011; Addisu et al., 2014; Rasib et al., 2017). Oils of both E. caryophyllata and C. sinensis on 250 and 125 ul/ml doze killed all termites (100% mortality) in two days while the same results was achieved at 62.5 µl/ml three days posttreatment. Oils of orange peel was used in USA to termite species (Coptotermes overcome formosanus Shiraki) in past (Raina et al., 2007). Many different compounds such as d-limonene [(R)-4isopropenyl-1-methylcyclohexene] which was extracted from citrus peel, caused 98% mortality (Hink and Fee, 1986) of termite population (Zhu et al., 2001; Osipitan and Oseyemi, 2012). General Linear Model was used to estimate the difference between plants, days and concentrations. All parameters showed significant difference i.e plants (d.f= 2, 74; p= 0.000); days (d.f= 2, 74; p= 0.000)and concentration (d.f= 2, 74; p=0.003). In control no mortality was recorded during experiment (Table 1). Seven species of insects had been reported toxic to orange peel (Raina et al., 2007). The Limonene oil was used to exterminate house flies (Musca domestica) and red imported ants (Solenopsis invicta). According to Hollingsworth (2005) citrus extract was used to control mealy bug.



The d-limonene vapors were used to exterminate cat flea all life Ctenocephalides felis (Hink and Fee, 1986), German cockroach, (Blattella germanica) and rice weevils, (Sitophilus oryzae) (Karr and Coats, 1988). The Eugenia caryophyllata has been used as fever reduction and mosquito repellent (Medline Plus, 2014). The clove oil has mainly composition of Eugenol (Koul et al., 2008), that attached to benzene ring by hydroxyl and ether linkage. Within the compound the aldehyde group shows high level of termite's resistant activity (Chang and Cheng, 2002). The clove oil was excellent repellent to termites, fungus and nematodes (Gupta et al., 2011). The oil of E. cardamomum mainly consist of 1,8-cineol (29.7%) and α -terpineol acetate (26.1%) (Korikontimath et al., 1999; Olivero-Verbel et al., 2010) but basic cardamom aroma was produced by mixing of these two major component (Lawrence, 1979). According to Abbasipour et al. (2011), the E. cardamomum was use to eradicate the Callosobruchus maculatus (bruchid beetle), Tribolium castaneum (red flour beetle) and Ephestia kuehniella (flour moth). This oil was used to fumigant toxicity against S. granarious (Mahmoudvand et al., 2011). E. cardamomum also showed different responses on egg stages and active stages of stored product insects (Huang et al., 2000).

In the repellency test, the E. cardamonum was repellent (28.667) to termites than the other two E. caryophyllata and C. sinensis (28 and 27) at the dilution of 250 ug/ml. While at 125 ug/ml E. cardamomum (27.33) is more repellent than other two plants oils (26). E. cardamomum show equal termites repellent at even at low concentrations (d.f: 2, 6; F: 1.04; P: 0.410). In E. caryophyllata (d.f: 2, 6; F: 6.68; P: 0.030) and C. sinensis (d.f: 2, 6; F: 11.38; P: 009) repellency fades with decreases in concentration (Table. 2). The oil of E. cardamomum was most the effective oil extract with respect to other extracts and repellency remain constant even at low concentration. Many plants extracts was used to prevent the pest population and reported the extracts under laboratory conditions (Talukder et al., 2006; Aihetasham et al., 2016). This study shows that E. cardamomum was most effective once and after that E. caryophyllata was effective and in the end C. sinensis was least effective but these all plant oils can be used to suppress the termite population (Coptotermes heimi).

Plant Essential oils	Dilutions	No. of days							
	µl/ml	1 ^A	2 ^B	3 ^C	4				
C.sinensis ^A	250 ^A	59.33±5.54	213.33±10.36	300.00±0.00					
	125 ^A	117.33 ± 21.32	204.00 ± 2.64	300.00±0.00					
	62.5 ^в	99.33±11.83	224.00 ± 19.52	300.00±0.00					
E. caryophyllata ^B	250 ^A	76.66±11.56	166.00 ± 18.58	300.00±0.0					
	125 ^A	78.00±12.16	172.00 ± 11.37	300.00±0.00					
	62.5 ^B	53.33±13.96	130.66 ± 16.01	242.00±16.23	300.00±0.00				
E. cardamomum ^C	250 ^A	87.12±1.91	96.01±1.27	300.00 ± 0.00					
	125 ^A	68.23±2.27	99.00±2.21	300.00±0.00					
	62.5 ^в	59.23±2.13	98.01±2.31	187.67±1.23	300.00±0.00				
Control		0.00 ± 0.00	0.33 ± 0.33	1.66 ± 0.33	2.66±0.33				

 Table 1. Average mortality of C. heimi (soldiers) caused by different concentrations of different plant oils

	E. caryophyllata		Plants			
Dozes			E. cardamomum		C. sinensis	
	Т	U	Т	U	Т	U
250µl/ml	27.33 ^A (0.667)	2.667	28.667 ^A (0.882)	1.33	26.00 ^A (0.577)	4.00
125µl/ml	25.0 ^{AB} (1.15)	5.00	26.667 ^A (0.882)	3.33	24 ^{AB} (0.577)	6.00
62.5µl/ml	23 ^B (0.557)	7.00	27.33 ^A (1.20)	2.67	21.33 ^B (0.882)	8.667

Abbreviations: T: treated and U: untreated and (---): standard error

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