Fumigant Toxicity of *Mentha arviensis* Leaves Extracts on *Coptotermes heimi, Heterotermes indicola* and Their Gut fFlagellates

by

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ABSTRACT

The extract of *Mentha arviensis* (mint) has been found to have considerable application against microbial diseases. The present study is designed to check the insecticidal properties of *Mentha arviensis*. Crude extract of the leaves of *Mentha arviensis* was obtained in benzene-ethanol (2:1) solvent using soxhelt apparatus and dried extract was applied (25mg, 50mg and 100 mg) against termite workers, soldiers and their gut flagellates of *Coptotermes heimi* and *Heterotermes indicola*. A significant increase in mortality of termite workers, soldiers and their gut flagellates was observed depending upon a lethal dose over time, in both termite species. It, however, took a relatively longer time period to achieve 100% mortality of flagellates than their respective hosts. The dose dependent death of flagellates over time also indicates the mortality of flagellates was found to be associated with fumes originated from the extract of the plants as in starvation, termites and their associated flagellates can survive for three to four days and also their was no mortality of workers and their flagellates, in control. Thus *Mentha arviensis* extract can be safely used to control termites and other pests.

Key words: pest control, termites, gut flagellates, mint plant

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INTRODUCTION

Termites are small insects (Isoptera, Insecta) are worldwide and recorded firstly in Japan in the 9th century (JTCA 1998). Globally, termites are a big problem in both the agricultural and urban areas as they cause significant damages to crops, plants, buildings and woodwork and account for considerable economic damage. In order to make cellulose digested and ready for assimilation the termites live in strong mutualistic relationship with a variety of hindgut inhabiting endomicrobes (flagellates, bacteria and archea). These endomicrobes mainly flagellates produce the enzyme cellulase, which acts to render the plant celluloses suitable for assimilation (Breznak 2000). It is logical therefore to presume that the termites cannot live without their flagellate fauna. In other words, to control the termite infestation, effectively one should focus on the symbionts living within their gut (Stingl *et al.* 2004, 2005; Ikeda *et al.* 2007; Ohkuma 2008), the elimination of gut fauna will cause starvation and then ultimately casualty of termites (Honigberg 1970; Brune 2003).

In practice termite infestations have been controlled by using synthetic insecticides such as; dieldrin, chlordane and heptachlor (Ward *et al.* 2009; Jamil *et al.* 2005; Logan *et al.*1990). It was estimated that an about US$ 0.8-1.0 billion per year is used to prevent and control termite infestations, (Fushiki 1998; Tsunoda 2003). These insecticides cause serious environmental hazards, if there are any leakages after heavy rain and have strong pungent odors (Logan *et al.* 1990; Martius 1998). These are a source of many disorders e.g., chlordane cause cancer, chlorpyriphos, dimethoate, and endosulfan cause genotoxicity (Jamil *et al.* 2005). Polychlorinated biphenyls and organochlorine pesticides (e.g. α- and γ-chlordane, p, p′ -DDT (dichlorodiphenyltrichloroethane), p, p′ -DDE (dichloro diphenyl dichloro ethylene), methoxychlor, and pentachlorophenol, persist in carpets and cause leukemia and neuroses in children. Alongside these drugs are also fatal for reproductive and immune system e.g. one popular termiticides chlordane proved to be causing cancer and has been banned in some countries. Therefore, these are not recommended for indoor applications (Ward *et al.* 2009; Hatlelid *et al.* 2004; Ramos & Rojas 2005; Gautam & Henderson 2008). The use of many pesticides has therefore been banned in many countries (Robinson 2005). Considering the toxigenic nature of synthetic pesticides, termiticides that are plant-derived organic chemicals are being explored which would be environmentally safe to use e.g. the plants like *Polygonal hydropiper*, *Progesterone parviflorus*, Alaska yellow, red ceder, tung tree, pine resins red wood etc. The extracts of the Tung tree and pine resin, has been reported to have anti-termite properties (Bläske & Hertel 2001; Nunes *et al.* 2004). *Polygonal hydropiper*, *Progesterone parviflorus*, Alaska yellow, red ceder and red wood strongly show anti feedant and toxic activities against termites (Tellez *et al.* 2001; Hennon *et al*. 2007). Solvent and aqueous extract of plants such as *Diospyros*, *Polygonal, Eucalyptus, Dalbergia, Morus*, Neem etc. have termiticidal activities. In this reference, monoterpenoides isolated from *Flourensia cernua* have proven to be strongly effective for termite control. Matias (2005) explained insect pest repellent activities of menthol propylene glycol carbonate and its analogs also exhibit anti-inflammatory and anti-antigenic effects on human. Similarly chemicals like menthol, α-terpineol, *p*-menthone, menthol acetate and others were isolated from *Mentha arvensis* (Deschamps *et al.* 2006; Brito *et al.* 2007). Menthol, the main component of *Mentha arviensis,* has been used for centuries in medicines, proven to have anti algeric, anti-ulcer anti-spasmodic and antimicrobial activities (Nascimento *et al*. 2009). Menthol shows competitive inhibition for acetylcholine esterase produced by insects (Lee *et al*. 2001). Besides, it has been used as insecticides, a cooling agent, as food and in cosmetics. Menthone, another constituent of *Mentha arviensis* has been found to have antibacterial (Kurita and Koike 1982), anti-allergic (Arakawa *et al.* 1992) and spasmolytic properties (Gamez *et al.* 1990). The extract of *Mentha arviensis* has fumigant toxicity and proven affective against *Sitophilus oryzae* L, a rice weevil (Lee *et al.* 2001). Matias (2006) compared the structure of menthol and menthol propylene glycol carbonate (MR-8), the latter found to be more effective for pest control. The joint FAO/WHO Expert Committee on Food Additives (JECFA) reconfirmed the safety of menthol and its derivatives for use as an ingredient in food and cosmetics assessment conducted in 1999 (Matias 2006).

Owing to the advantageous properties of *Mentha arviensis,* the present research work is designed to investigate the toxigenic nature of mint plant (*Mentha arviensis*) extract against termites and their entozoic flagellate populations.

MATERIALS AND METHODS

Extraction Process

Dry leaves of *Mentha arviensis* were ground and the extract was prepared using benzene- ethanol solution (2:1) as a solvent following the method of Vogel (1964). Dry leaves (20 gm) were taken in a soxhelt extraction flask containing 300 ml of solvent. It was then connected with the extraction apparatus and the distilled water was allowed to flow into the condenser. Heating was adjusted to boiling temperature. The extraction cycle was carried out at least 6 times per hour. After 4-5 hours extraction, the flask were then removed and allowed to cool.

Insect procurement and maintenance

Termite workers and soldiers of species *Coptotermes heimi* (Wasman) and *Heterotermes indicola* (Wasman) were collected from old trees of Government College University gardens, Lahore. They were kept for at least 1 week in glass jars containing water soaked filter papers at 30°C in an oven to clear their intestines from debris and other wood particles following the method of Ramos and Rojas (2005) with some modifications.

Dosage

The solid extract in three different dosage concentrations (25 mg, 50 mg and 100 mg) was sprinkled at the base of glass jars of 15 cm height and 7cm diameter.It was partitioned from rest of the space in the jars by a narrow iron net placed at 10cm height from base so that fumes can pass through the net. 100 termite workers of *C. heimi* were placed on the net so that they were not in direct contect with the crystals of *Mentha arviensis* leaves extract. A small piece of filter paper for food source and water soaked cotton plug to keep humidity and water supplied to the termites were placed in the jar, on the net along with termite workers and tightened the jar with a lid. Same sets of experiments were desinged for soliders of *C. heimi* and also for workers and soldiers of *H. indicola* (n =100 each). The fumigation effect of the extract on termite workers, soldiers and their intestinal flagellates was studied over 1to 6 hours of exposure. Three replicates of each concentration were run. Control preparations were run in parallel without the application of the extract.

Physical observations of termites: Termites workers and soldiers in each set of experiment were observed with magnifying glass for six hours. Mortality percentage was calculated after each hour.

Gut flagellates:

Ten termites from each treatment group workers , soldiers and controls of both termite species were dissected to study the effect of the extract on gut flagellate population. For the study of flagellate population workers and soldiers of termites were decapitated and their entire guts were pulled out using a dissecting needle. The guts were dissected and the contents were squeezed out in a drop of 0.2 % saline solution on cover glasses. The contents were allowed to spread on the cover glass. The procedure was carried out under a stereo zoom microscope (Olympus). The cover glasses were then inverted on improving Neubaur's chambers and the flagellates were counted under in four W squares (1 mm2) of the chamber at low magnification. The arithmetic mean of flagellates was calculated by adding the number of flagellates in all the four areas and dividing it by 4. The diameter of the spread smear was also measured. Area of the smear was calculated according to the formula πr^2. The total number of flagellates in a smear was calculated by using the following formula. Total number of flagellates = average number of flagellates in 1 W area or 1mm^2 × total area of the smear in mm.

Dead termites were also disected to study the survival of their flagellates.

Data analysis

Data were analyzed using two-way analysis of variance (ANOVA) and means were separated using Tukey's HSD quantile function, using SPSS 10.0. All tests were conducted at á = 0.05 levels.

RESULTS AND DISCUSSION

Presently, the effect of mint extract was investigated to control termite infestation.The two castes of termites i.e. workers and soldiers were forced to live in an environment of 25mg, 50 mg and 100 mg dosage concentrations for a period of 6 hours. Hundred percent mortalities of workers and soldiers were observed in a period of 3hours and there was also a significant decrease in flagellates population residing in the gut of both workers and soldiers but the hundered percent mortality in flagellates population was achieved within

6 hours as was observed in the gut of dead termites . Maximum mortality (100%) of workers and soldiers was recorded at 3, 2 and 1 hours subsequently on exposure to 25 mg, 50 mg and 100 mg of extract respectively($P < 0.05$).

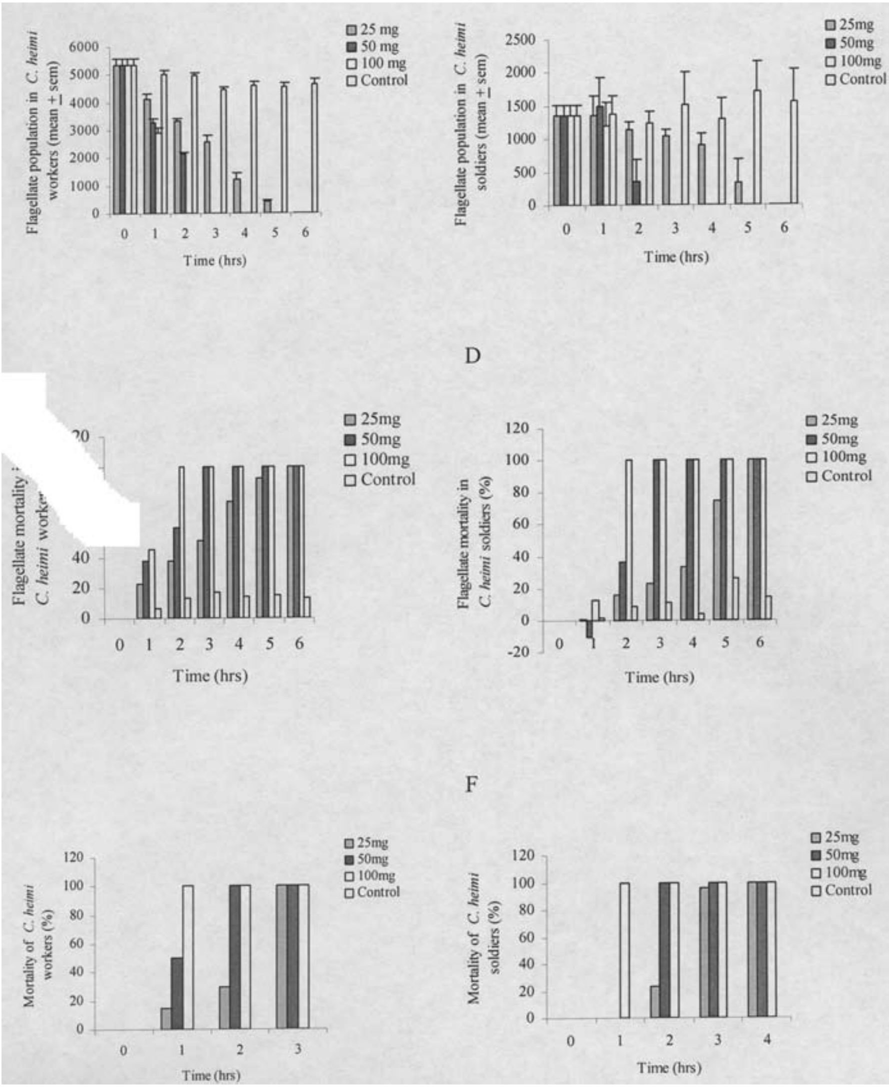

Fig. 1 (A-F) Effect of different concentrations of Mentha arviensis leaves extracts on Coptotermes heimi and its entozoic flagellates.

whereas no mortality of workers or soldiers occurred in the control groups(Fig 1(E & F)). The flagellates population in the guts of soldiers and workers were also recorded and their 100% mortality was obtained in 6, 3, and 2 hours

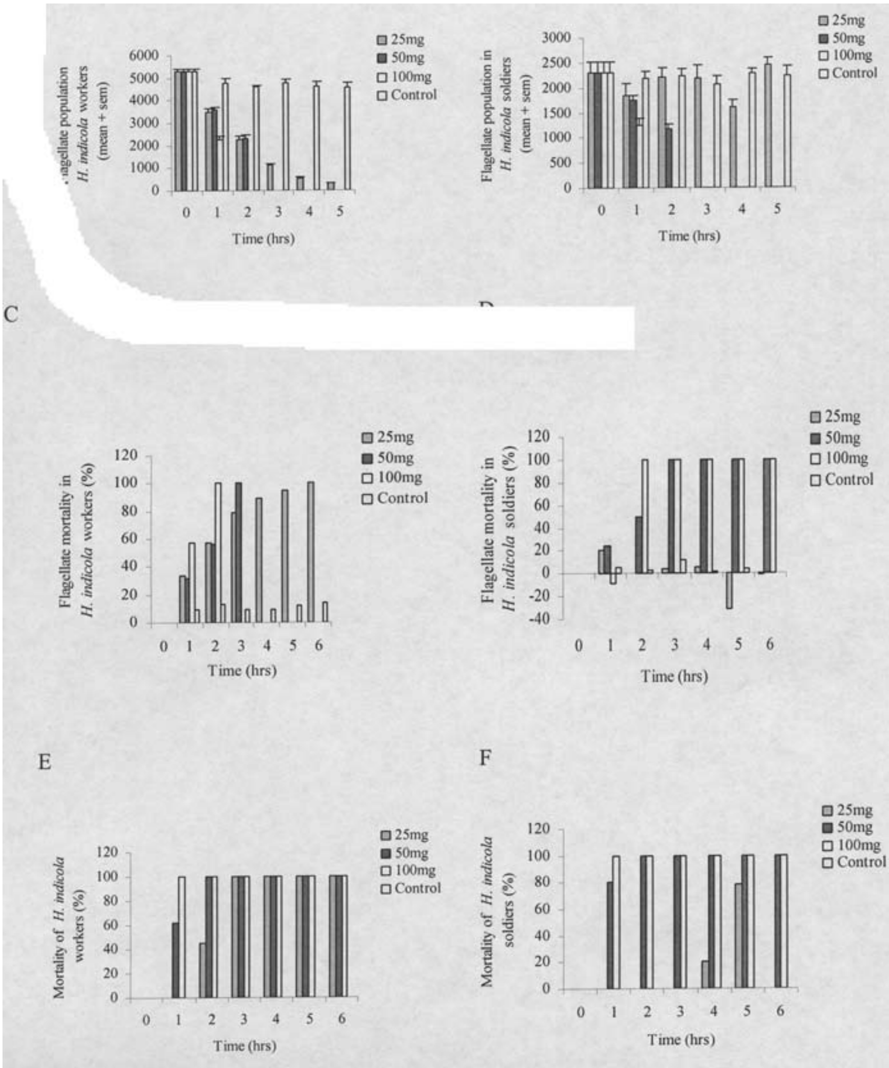

Fig. 2 (A-F) Effect of different concentrations of Mentha arviensis leaves extracts on Heterotermes indicola and its entozoic flagellates.

on exposure to 25 mg, 50 mg and 100 mg of extract respectively ($P < 0.05$) . After three hours although the termites had died but their gut flagellallates were still surviving upto six hours. An insignificant decrease in the flagellate population was observed in the control groups (Fig. 1(A to D)).

H. indicola also behave in a same manner as *C.heimi* on expoure to different concentrations of *Mentha arviensis* extract and underwent 100% mortality at 25 mg, 50 mg and 100 mg of extract respectively at 3, 2 and 1 hours ($P < 0.05$). Control non-exposed workers, soldiers were not affected (Fig. 2 (A & B)) and their gut flagellates showed 100% mortality at 6, 3 and 2 hours after exposure to increasing concentrations of dose ($P < 0.05$) in the control groups no sinificant mortality was observed (Fig. 2 (C to D)).

The mean population of flagellates in both termite species, decreased dose-dependently in a very similar dose-dependent pattern, the mortality percentages of termite workers and soldiers increased. On the whole, the effect of the extract on termites and their flagellate fauna was quite possibly due to the vapors of its volatile components. The termites rapidly became less active and senseless on exposure to the mint extract indicating the vapors of the extract was to be toxic to the termites. The low percentage of extracts affects the flagellates earlier than their host termites. Thus the termites at low concentration of doze underwent death as a consequence of the mortality of their flagellate protozoans. This appears to be true because termites and flagellates have developed a mutualistic type of association with each other and therefore in a stravation or defaunated worker termites can survive for more than two days and subsequently die of starvation, without flagellates (Honigberg 1970).

The GC-MS analysis of *Mentha arviensis* revealed that it mainly composed of menthol (63.2%), menthone (13.1%), limonene (1.5%), β pinene (0.7%), α pinene (0.6 %), and linalool (0.2%). Among these menthone showed 8.1 times more insecticidal properties and has an inhibitory effect on acetylcholineterase in rice weevil (Lee *at al.* 2001). In the present investigations the fumigant toxicity of *Mentha arviensis* leaves extracts found to have anti-termitic and antiprotozoal activity and since it has been reported by US FDA as GRAS (Generally Recognized AS Safe) chemical agent (Nan-Yao Su and Scheffrahn, 1998) that is why can be a good substitute for the synthetic termitcidal and the antiproozoan drugs.

The present study shows that the extract of, *Mentha arviensis* can be an effective natural anti-termite drug and its use would lessen the risk of environmental contamination as compared to the synthetic insecticides.

ACKNOWLEDGMENTS

The authors deeply acknowledge Government College University, Lahore and Quaid-i-Azam University, Islamabad for providing necessary research facilities.

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