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Anti-termite activity of essential oil and its components from *Myristica fragrans* against *Microcerotermes beesoni*

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ABSTRACT: The essential oil obtained by hydrodistillation of the fruits of *Myristica fragrans* was analyzed by GC and GC-MS. Twenty eight compounds were identified representing 95.9% of the oil. The major constituents of the oil were α-pinene (6.4%), Sabinene (37.7%), β-pinene (7.3%), myrcene (3.5%),Limonene (4.7%),Terpine-4-ol (5.8%), safrole (3.4%) and myristicin (6.8%).The essential oil and its major constituents were evaluated at different dilution against *Microcerotermes beesoni,* test termite. The LC_{50} value of fruit essential oil is 28.6 mg/g. Furthermore, exposure to myristicin caused 100% mortality at a dosage of 5 mg/g after 14d. @JASEM

Termite are known to cause to tremendous losses to finished and unfinished wooden structures in buildings, besides loss in agriculture, forestry crops and stored products (Sen-Sharma *et al*. 1975).It is also known that termites cause damage to a variety of materials ranging from paper fabrics to even non cellulosic materials such as asbestos, asphalt bitumen, lead, and metal foils (Bultman *et al*.1979). Damage to wooden structures and other cellulosic materials by termites has been estimated to exceed $ 3 billion annually worldwide (Su *et al*.1990). The use of inorganic compounds or synthetic pesticides like DDT, BHC, aldrin, dieldrin and chlorinated pesticides to preserve the woods and prolong their application life great hazard to environment. Therefore to avoid environmental pollution and even carcinogenic caused by the use of synthetic pesticides, there is increasing interest in naturally occurring pesticides from plants. Many plant extracts and essential oils (Kinjo *et al*. 1986; Kondo *et al.* ,1986; Asada *et al*.1989; Yoneyama *et al*. 1990; Nabeta *et al*.1992; Morita *et al*.1997; Chang *et al,* ,2002) may be alternative sources of termite control. World over, research is going on for an effective natural formulation, which can reduce the damage by termites in soil as well as crop treatment , at the same time being environmentally acceptable. Therefore, the purpose of this study is to determine the bioactivity of *M. fragrans* oil and its major components against the test termite, *Microcerotermes beesoni*.

MATERIAL AND METHODS

The fruits of *M. fragrans* were obtained from south India.Hundred grams of dry fruits were hydrodistilled in Clevenger type apparatus for 6 h. The distilled oil was further coloumn chromatography and isolated major components like *α*-pinene, *β*-pinene, myrcene, limonene, *α*-thujene, sabinene, safrole and myristicin. GC and GC-MS analysis was carried out and compounds of essential oil were identified by comparison of the fragmentation patterns of the mass spectra available on (Adams, 1995; Jennings *et al*. 1980; Joulain *et al*. 1998).The test termite species, *M. beesoni*, was collected from forest of Dehradun. The colony was reared in an incubator at 26.5^0C and 80% relative humidity (RH) for more than 2 year. Water and newspapers were used as food source. The anti-termite activity test followed the method of (Kang *et al*. 1990). Samples of 10, 25, and 50 mg of fruit essential oil as well as 1 and 5 mg of each individual compound dissolved in 600 μl of acetone were applied to 1 g filter paper samples (What man #3, 8.5 cm in diam). A piece of filter paper treated with solvent only was used as control. After air-drying at room temperature, 50 active termites (45 workers and 5 soldiers) above the third in star were placed on to each filter paper impregnated with the test materials in a Petri dish (9 cm diam × 1.5 cm height). The test dishes with covers were then placed into an incubator maintained at 26.5^0C and 80% RH.A few drops of water were periodically added to the bottom edge of each Petri dish. Three replicates were prepared for each test sample, and the mortality of the termites was counted daily for 14 d. All results were obtained from three independent experiments and expressed as mean ± SD. Significant differences (P <0.05) were determined by using the Student's t- test.

RESULTS AND DISCUSSION

The fruit essential oil of *M. fragrans* was obtained in a yield of 6.25% on dry weight basis. The oil was examined by GC and GC-MS. Twenty eight compounds were identified representing 95.9% of the oil. The major constituents of the oil were α-pinene (6.4%), Sabinene (37.7%), β-pinene (7.3%), myrcene (3.5%), Limonene (4.7%), Terpine-4-ol (5.8%), safrole (3.4%) and myristicin (6 .8 %).The oil has shown encouraging results against the test termite, *Microcerotermes beesoni* in (Fig.1). At a dosage of 10 mg/g, the fruit essential oil killed 25.7% of the termites after 14 d. Termite mortality increased to

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81.7% when dosage was increased to 50 mg/g. The LC_{50} value of fruit essential oil against *M. beesoni* was 28.6 mg/g. The eight constituents were tested for anti-termite activity at a dose of 1 mg/g. myristicin (19.3%), and safrole (15.0%) caused the highest termite mortality after 7 d, followed by myrcene (8.3%), *β*-pinene(4.7%), Sabinene(3.3%), *α*-pinene (2.8%), *α*-thujene (2.3%) and limonene (1.5%).When the test was extended to 14 d, termite mortality increased slightly Table-1.

Table-1 Anti-termite activity of eight major essential oil constituents from *M. fragrans* fruits at 1 mg/g dosage.

Compounds	Termite mortality (%)	
	7 d	**14 d**
Limonene	1.5 ± 2.5	1.5 ± 2.5
α-Thujene	2.3 ±0.0	2.3 ±0.0
α-Pinene	2.8 ± 1.2	6.3 ± 2.3
β-Pinene	4.7 ± 2.3	7.5 ± 3.2
Myrcene	8.3 ±2.1	10.3 ± 4.5
Sabinene	3.3 ±2.6	5.3 ± 1.2
Safrole	15.0 ±4.5	18.0 ±2.3
Myristicin	19.3 ± 3.1	22.0 ± 4.6
Control	0.0 ± 0.0	0.0 ± 0.0

Means (N = 3) using 50 termites per replicate; P <0.05 according to the Student's t- test.

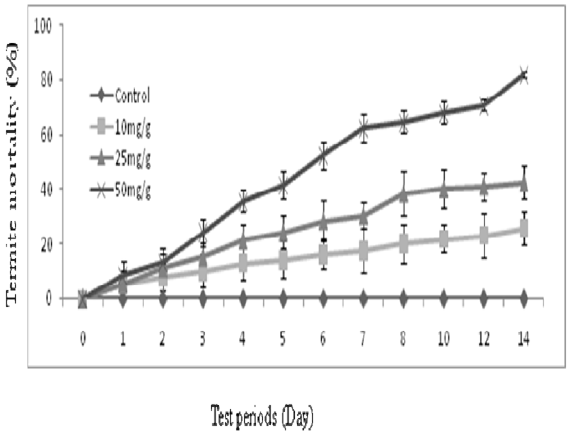

Fig- 1 Anti-termite activity of fruit essential oil from *M. fragrans against Microcerotermes beesoni*. Means (*N* = 3) using 50 termites per replicate.

The present study indicated that the fruit essential oil of *M. fragrans* possessed remarkable anti-termite activities. The order of anti-termite activity was myristicin, followed by safrole , myrcene, *β*-pinene, sabinene, *α*-pinene, *α*-thujene and then limonene. The respective termite mortalities (at 5 mg/g dosage after 14 d) were 100.0%, 48.0%, 38.5%, 28.7 %,20.5%,15.0%, 12.6 %, and10.0%.(Fig.2). The LC_{50} value of fruit essential oil was 28.6 mg/g and compound myristicin showed 100% mortality at 5 mg/g after 14 d. These results show that the Safrole and Myristicin are potential compounds for the development of termiticides in the near future.

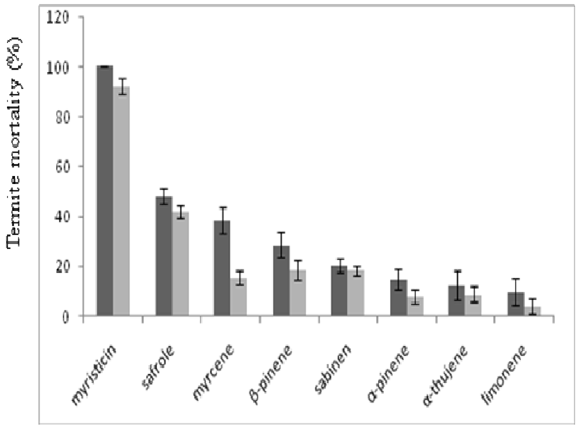

Fig-2 Anti-termite activity of eight major constituents from *M . fragrans fruits* essential oil at 5 mg/g dosages against *Microcerotermes beesoni* (white bar, after 7 d; black bar, after 14d). Means (*N*=3) using 50 termites per replicate at the level of *P* <0.05 according to the Student's t-test.

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