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Bioactivity of medicinal plants *Mentha arvensis* and *Peganum harmala* extracts against *Heterotermes indicola* (Wasmann) (Isoptera)

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Key words: Antitermitic Assay, *Heterotermes indicola, Mentha arvensis, Peganum harmala,* Repellency.

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Abstract

As termites are hazardous causing damages to wooden structures leading to huge economic loss, efforts are directed towards the study of plant based products for effective and eco-friendly termite control. Present study was performed to determine the chemical constituents of the seed extracts from two medicinal plant species of *Peganum harmala and Mentha arvensis* against *Heterotermes indicola*. An impregnated filter paper no-choice bioassay method was applied. In Gas Chromatography Mass Spectrometry (GC-MS) analysis of *M. arvensis,* eleven different compounds were identified. Compounds in seed extractives of *P. harmala* were Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans 5-Octadecenoic acid, methyl ester, Linoleic acid ethyl ester, Leptaflorine and Harmine. The biological activity of these seed extracts was investigated against *H. indicola* in laboratory bioassay. Results revealed that extracts of *P. harmala* exhibited anti-termitic activity in a dose-dependent manner and showed a significant activity after 40 hours of exposure while the extract of *M. arvensis* was toxic and also attractant.TheLT_{50} value of *M. arvensis* was 38.48, 67.54 and 83.19 hour respectively for 10%, 5% and 3% respectively. However, In case of *P. harmala* the LT_{50} was76.56,142.6 and 147.9 hours for 10%, 5% and 3% respectively. Gas Chromatography Mass Spectrometry (GC-MS) identified components could be further utilizes to explore antitermitic activity of each component against other pest termite species in Pakistan.

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Introduction

Termites cause seriousdamage to household materials, plants and agricultural crops such as sugar cane, millet, barley and rice (*Sattar et al.,* 2014). There are more than 2,800 described species of termites, but the nuisance species are about 185 (Lewis, 1997; Verma *et al.,* 2009). From Pakistan 50 species of termites have been described, and 11 have been recorded damaging man made wooden structures, in buildings (Akhtar, 1983). One of the most important termites in urban and rural areas is *Heterotermes indicola* (Wasmann).

In the past, the fight against termites was completely relied on synthetic insecticides (Hertel, 2000; Venkateswara *et al.,* 2005; Sattar *et al.,* 2014). These insecticides were carcinogenic, toxic to mammals and environmental pollutants. With the increasing spread of termite infestation, there is an increased need to find out human and environment safe treatments (Meepagala *et al.,* 2006). There is a growing interest in natural toxic substances from plants (Chang *et al.,* 2001; Elango *et al.,* 2012).

Substitution of synthetic by biological insecticides is an acceptable procedure and common practice worldwide (Logan *et al.,* 1990; Nisar *et al.,* 2012). Plant extracts with complex mixtures of these compounds were tested for their insecticide, repellent and devoured properties (Zhu *et al* 2001; Isman *et al.,* 2006; Nisar *et al.,* 2012). Many researchers tried plant extracts against termites (Sakasegawa *et al.,* 2003, Park and Shin, 2005, Jembere *et al.,* 2005, cheng *et al.,* 2007, Ding and Hu, 2010, Supriadi and Ismanto, 2010; Ahmed *et al.,* 2011). Extracts repellent to the termites include *Eucalyptus globules*, lemon grass, clove bud and vetiver grass (Zhu *et al.,* 2001a, b; Ahmed *et al.,* 2011). Extract of some other plants such as *Veliveria nigeitana, C. schoenanthus, Digitari sp, Pennicetum purpurerum, Sanseviera libercum, and Ocimum basilicum* have also been reported to have chemical substances that are repellent to termites (Oliver, 1960;Benner, 1993; Delat and Grace, 1995; Malaka, 1996; Ajayi *et al.,* 2012).

The present study was undertaken to assess the toxicant potential of the extracts from *P. harmala* (harmal) and *M. arvensis*(mint) against *H. indicola*. It includes: Ethanolic extraction of selected plant seeds using soxhlet extractor. Collection of *H. indicola* (Wasmann) to determine the feeding bioactivity in extracts under laboratory bioassays. Structural characterization of compounds in seeds extracts through GC-MS.

Materials and methods

Collection of termites

Termite workers and soldiers of species *H. indicola* were collected from old trees of Lahore. The termites were maintained for at least 1 week by placing water soaked filter papers and 5 gram oven dried soil in each petri-plate.

Seeds Collection

Seeds of locally used medicinal plants, *Mentha arvensis* (Podina) and *Peganum harmala* (Harmal) were purchased from local market.

Extract preparation

The seeds of the medicinal plants were ground into fine powder using a grinder. Twenty grams of each seed powder was taken separately for extraction in Soxhhlet extractor with 200ml of ethanol. Rotary evaporator was used to obtain dried residues and stored in refrigerator for making stock solution. Stock solution was prepared for each plant extract by taking 1 g dried extract in 10ml of absolute ethanol to get a solution of 10% concentration. 10%, 5% and 3% concentrations were prepared from it.

Gas chromatography mass spectrometry conditions

All seed samples were analyzed by gas chromatography coupled with mass spectrometry. The gas chromatography conditions include a temperature range of 50 to 250°C with 4°C/min, with a solvent delay of 5 minutes. The temperature of injector was maintained at 250°C. Helium was used as an inert gas with a flow rate of 1.0 mL/min. and the volume of injected sample in the splitless mode was 2µL. The MS conditions were the following:

ionization energy, 70 eV; quadrupole temperature 100°C; scanning velocity, 1.6 scans /s; weight range, 40-500 amu.

The percent composition of the samples was calculated. The qualitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each each compound was compared with the mass spectrum from the spectra library NIST 98 (USA National Institute of Science and Technology software).

Anti termitic assay

Tests were conducted following the procedure adopted by Abbas *et al.,* 2013. Circular filter papers were cut and the bottom of each sterilized glass petri dish was provided with one. Each filter paper was soaked with 0.5 ml of the 10%, 5% and 3% extracts concentrations. The filter papers were dried at ambient temperature and placed in petri dish separately. Then population of 50 workers and five solider of *H. indicola* was added to each petri plate. Initially after every 2 hours observations were taken up to 12 hours then Data for the mortality of the termite was recorded after an interval of 12 hour up to 96 hours.

$$\text{Mortality Rate (\%)} = \frac{\text{Number of dead termites after test}}{\text{Number of initial termites used in test}} \times 100$$

Repellency assay

For the estimation of repellency filter papers of 9cm in diameter were cut into two equal halves. One half of each filter paper was treated with 10%, 5% and 3% concentration of both extracts served as treated and second half was served as untreated. The two halves were placed into the petri dishes with a cut space in the middle. 10 termites were released into the middle space. Repellency was noted after every 15 minutes by counting the number of termites on treated (T) and untreated (UT) filter paper discs and experiment was conducted for 2 hours. Three replicates were prepared for each concentration of all four plant extracts. A treatment concentration was considered repellent when 21 (sum of three replicates) of 30 termites were present on untreated area against respective % treatment.

Statistical analysis

Percentage mortality of termites was calculated and analyzed by using one way ANOVA at P= 0.05 were considered statistically significant ($P<0.05$). LT_{50} was calculated by Probit analysis (Finney, 1971; Qureshi *et al.,* 2015).

Results and discussion

Gc-Ms analysis

In GCMS analysis of *M. arvensis*11 different compounds were identified i.e N-ethyl-hexahydro-1H-azepine, Isoborneol, Tricyclo[4.2.1.1(2,5)] deca-3,7-dien-9-one, 10-hydroxy-10-methyl-, stereoisomer, 7-Oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl), Phenol, 2-methyl-5-(1-methylethyl), 3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl), 2-Cyclopenten-1-one,2-(2-butenyl)-4-hydroxy-3-methyl, 5,5,6-Trimethyl-2-phenylethynyl-bicyclo[2.2.1]heptan-2-ol,1-(1-chloro-2,3 dimethylcyclopropyl)-3, 3-dimethyl-1-butyne,5,8,11,14-Eicosatetraenoic acid, methyl ester and 2-Methyl-E-E-3,13-octadecadien-1-ol as shown in table 1 and Fig. 1.

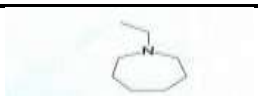
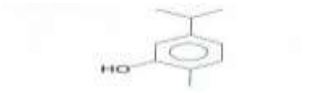
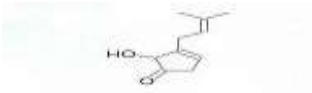
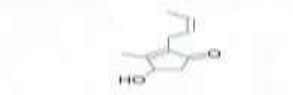
The GC-MS analysis of *Mentha arviensis* revealed that it mainly composed of menthol (63.2%), menthone (13.1%), limonene (1.5%), β pinene (0.7%), α pinene (0.6 %), and linalool (0.2%). Among these menthone showed 8.1 times more insecticidal properties and has an inhibitory effect on acetylcholineterase in rice weevil (Lee *et al.,* 2001; Qureshi *et al.,* 2012).

GC-MS analysis identified many compounds in extract of seed of *P. harmala* were Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans 5-Octadecenoic acid, methyl ester, Linoleic acid ethyl ester, Leptaflorine and Harmine as shown in Figure b2 and table 2.

Anti termitic assay

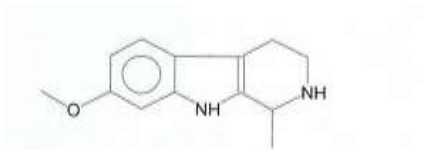
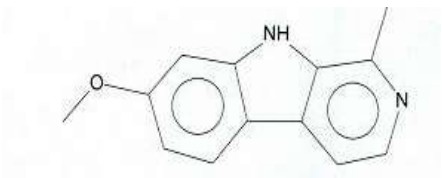
The ethanolic seed extracts of *M. arvensis and P. harmala* were used to determine their efficacy against subterranean termite *H. indicola*. Greater mortality was observed in 10% concentration it decreased in 5% and low in 3% concentration. The result showed that ethanolic extract of *M. arvensis* caused 100% mortality in workers of termite *H. indicola*. The rate of mortality at 10%, 5% and 3% concentration was 100%, 72% and 60% respectively after 96 hour with LT_{50} value 38.48, 67.54 and 83.19 hour respectively. The % mortality of *P. harmala* at 10%, 5% and 3% was 64%, 33%, 24% with LT_{50} 76.56, 142.6 and 147.9 hours respectively.

Table 1. Compound obtained from GC-MS analysis of *Mentha arvensis*.

Compounds	R_t[a*] (min.)	R(%)[b*]	Structural formula
N-ethyl-hexahydro-1-H-azepine	5.200	7.31	
Isoborneol	7.001	8.23	
Tricyclo[4.2.1.1(2,5)]deca-3,7-diene-9-one10-hydroxy-10-methyl	7.918	8.64	
7-oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl)-	8.131	10.57	
Phenol, 2-methyl-5-(1-methylethyl)	8.887	8.31	
3-cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl)	9.031	10.07	
2-cyclopenten-1-one, 2-(2-butenyl)-4-hydroxy-3-methyl	9.685	100	
5,5,6-Trimethyl-2-phenylethynyl-bicyclo[2.2.1]heptan-2-ol	10.170	35.47	
1-(1-chloro-2,3-dimethylcyclopropyl)-3, 3-dimethyl-1-butyne	11.130	9.58	
5,8,11,14-Eicosatetraenoic acid, methyl ester	12.429	57.35	
2-Methyl-E,E-3,13-octadecadien-1-ol	17.629	12.89	

a*=Retention time in minutes, b*= Relative percentage composition.

Table 2. Compound obtained from GC-MS analysis of *P. harmala*.

Compounds	R_t (min.)	R (%)	Structure
Cyclopropanepentanoic acid, 2-undecyl-, Methyl ester, trans	15.90	0.18	
5-octadecenoic acid, methyl ester	17.60	1.15	
Linoleic acid ethyl ester	18.17	0.46	
Leptaflorine	19.30	3.51	
Harmine	20.41	100	

Repellency assay

When termites workers were exposed to treated with 10%, 5% and 3% concentrations of *M. arvensis* along with untreated filter papers. Result showed that all the three concentrations were non repellent to *H. indicola* as less than 21 termites were present on untreated filter paper, However all concentrations of *P. harmala* i.e. 10%, 5% and 3% were repellent against *H. indicola*.

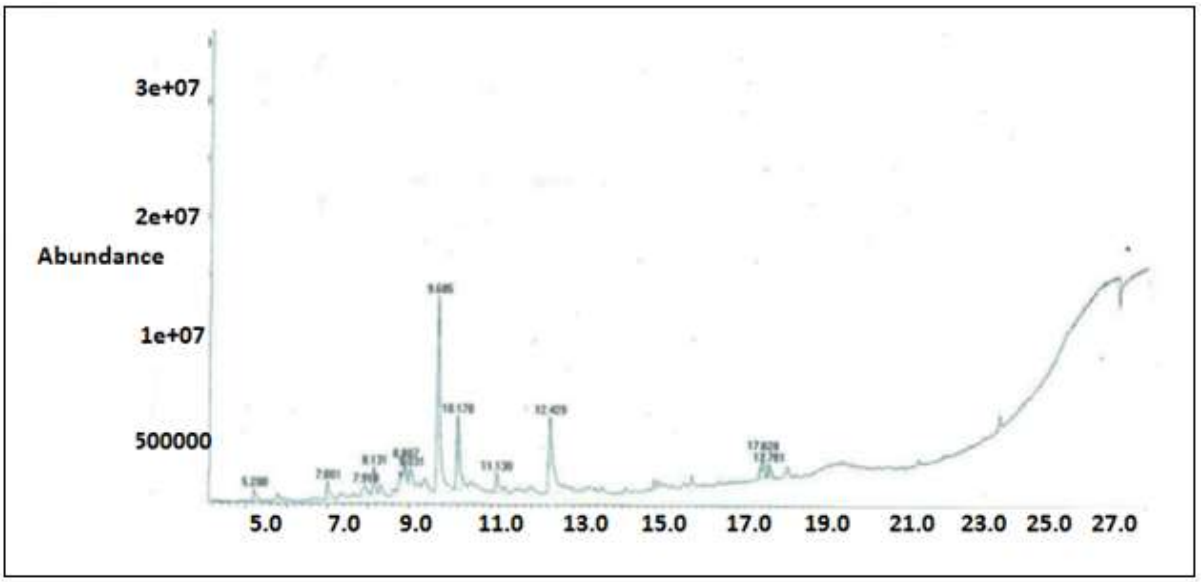


Fig. 1. GC-MS chromatogram of essential oils obtained from*Menthaarviensis*.

Discussion

The termites (Isoptera) are social and highly organized insects. They live in colonies in soil or woods and damage valuable products of humans (Alawy and Mehsin, 1987; Thamer, 2008). The plants used in this study to control termites are *M. arvensis and P. harmala* and contained biological active compounds.*M. arvensis* showed highest mortality against *H. indicola*. Many components have been recognized by GC-MS analysis. The major components in *M. arvensis* were 3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl), 2-Cyclopenten-1-one, 2-(2-butenyl)-4-hydroxy-3-methyl and 1-(1-Chloro-2,3-dimethylcyclopropyl)-3, 3-dimethyl-1-butyne at retention time of 9.685, 10.170 and 12.429 min. respectively. Several species of *Mentha,* belonging to the Lamiaceae family, have been investigated for essential oils produced by their leaves (Sartoratto *et al.,* 2004; Bertini *et al.,* 2005; Gende *et al.,* 2014). The aqueous extract of *M. piperita*has considerable antibacterial activity against *Helicobacter pylori*, the main etiological agent of chronic gastritis and peptic ulcer disease (Castillo-Juarez *et al.,* 2009;Pramila *et al.,* 2012). Matias 2005; Qureshi *et al.,* in 2012 explained insect pest repellent activities of menthol propylene glycol carbonate and its analogs. They 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 *M.arvensis*(Deschamps*et al.* 2006; Brito*et al.* 2007; Qureshi *et al.,* in 2012). Menthol, the main component of *M.arviensis,* has been used for centuries in medicines, proven to have anti allergic, anti-ulcer antispasmodic and antimicrobial activities (Nasciment *et al.* 2009; Qureshi *et al.,* in 2012). The extract of *M.arviensis*has fumigant toxicity and proven effective against *Sitophilusoryzae* L, a rice weevil.

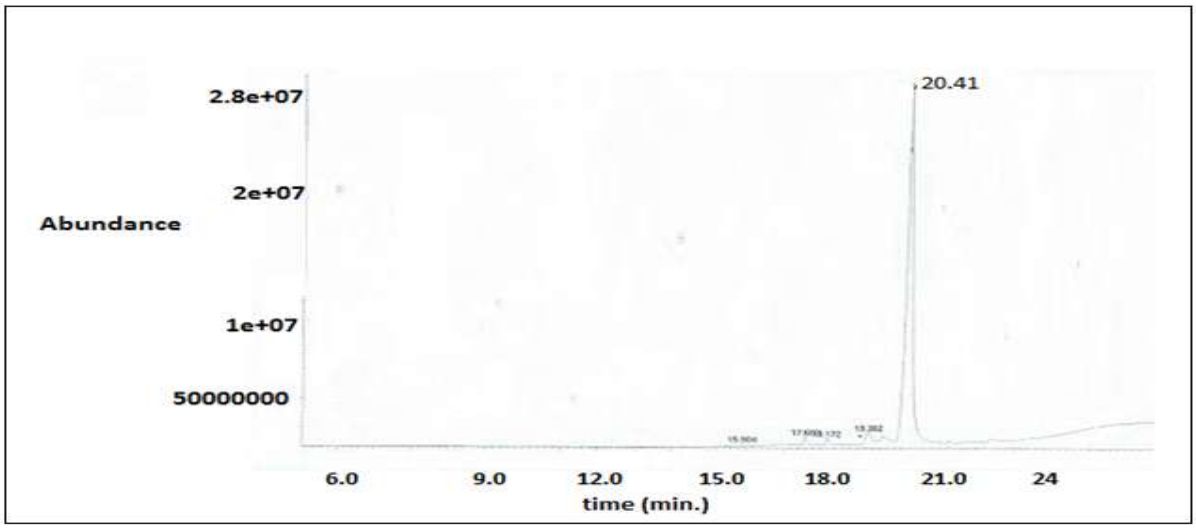


Fig. 2. GC-MS chromatogram of essential oils obtained from *P. harmala*.

The major essentail oil constituents of mint 1,8-cineole, carvone, limonene, linalool, linalyl acetate, menthol, menthone, menthyl acetate, and piperitenone oxide, were detected in GCMS analysis by Gracindo *et al.,* 2006. The study was designed by Qureshi *et al.,* in 2012 to check the insecticidal properties of *M.arviensis*. 25mg, 50mg and 100 mg concentration of extracts applied against termite workers, soldiers and gut flagellates of *Coptotermes heimi* and *H. 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 Thus *M.arvensis* extract can be safely used to control termites and other pests.

The chemical compostion of *P. harmala* was revealed by GCMS was cyclopropanepentanoic acid, octadecenoic acid, linoleic acid, leptaflorine and harmine. The leaf extract and its fractions of *P.*

harmala L.have shown pronounced mortal effect, decreased percent pupation and adult emergence of the cotton leaf worm, *Spodoptera littoralis* Boisd. The third instar larvae fed for two days on treated leaves were more susceptible to plant extract and its ethyl acetate and chloroform fractions. The active lowest concentration (5%) of the leaf fractions of *P. harmala* showed significant effect on the percentage of emerged adult parasitoids, *Micropliti srufiventris* Kok. GC/MS analysis showed the major constituent in ethyl acetate fraction was (23S) ethylcholest-5-en-3 beta-ol (28.04%) while those of chloroform fraction were hydroxyfuranocoumarin (Bergaptol) (15.68%), piperidinone (12.08%), thymol (11.82%), phosphoric acid, tributylester (9.80%) and trimethyl-nonenol (9.66%). The medicinal plant *P. harmala*could be carefully applied in integrated pest management due to its strong effect on cotton leaf worm pest (Shonouda *et al.,* 2008).*M. arvensis* has also the potential for use against control of termites because its 10% concentration was toxic and at the same time attractive to *H. indicola*.

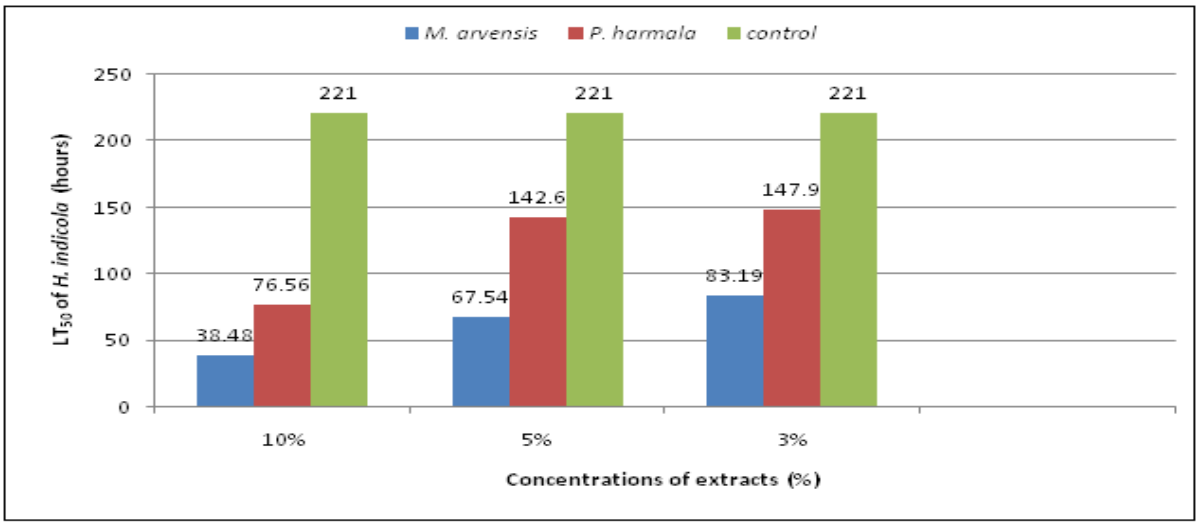


Fig. 3. LT_{50} of all concentrations of*M. arvensis* and *P. harmala* extracts against *H. indicola*.

GC-MS identified components could be used to further explore antitermitic activity of each component of these seed extracts, against other pest termite species in different ecological zones of Pakistan.

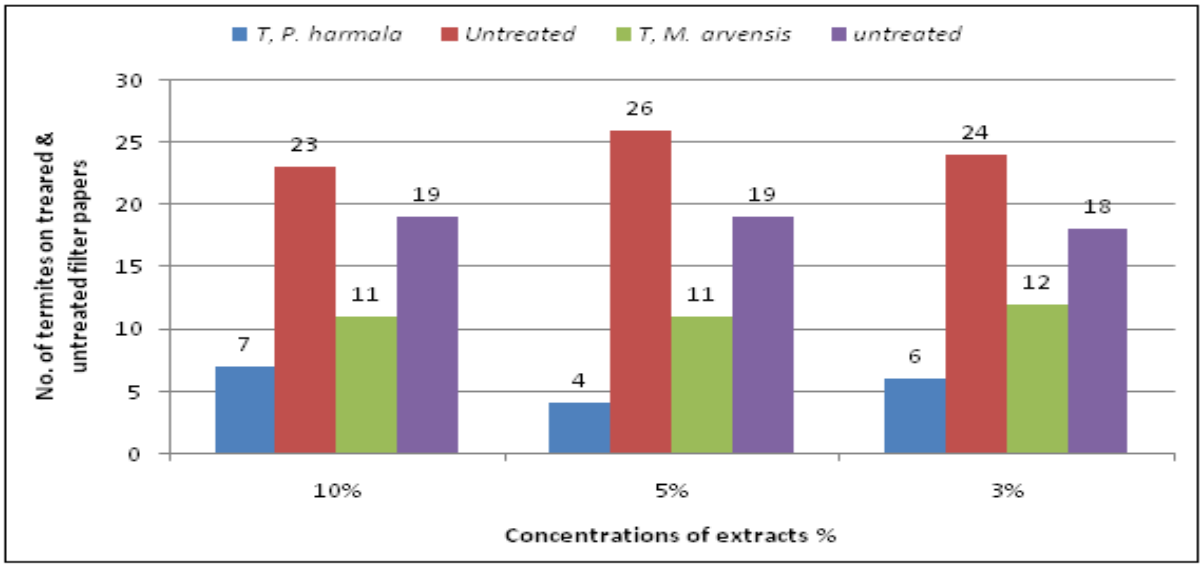


Fig. 4. Repellency test of M. arvensis and P. harmalaagainst H. indicola.

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