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Repellency and toxicity of essential oils of Mentha piperita and Mentha spicata on larvae and adult of Amblyomma hebraeum (Acari: Ixodidae)

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Abstract

The toxicity and repellency effects of essential oils of M. piperita and M. spicata at concentrations of 5%, 10% and 20% v/v were evaluated against adults of Amblyomma hebraeum using fumigant toxicity and glass plate repellency bioassays. The feeding deterrent of essential oils of M. piperita and M. spicata on larvae of Amblyomma hebraeum was also tested using feeding deterrent bioassay. High percentage repellency (range 90 -100) was observed at all concentrations of both essential oils of M. piperita and M. spicata. The repellency for 5%, 10% and 20% v/v concentrations of essential oil of M. piperita persisted for 60, 40 and 20 minutes respectively and eventually the ticks died during the experiment. While, the repellency of essential oil of M. spicata persisted for 80, 50 and 30 minutes at 5%, 10% and 20% v/v respectively and the ticks also died thereafter. The mean mortality effects of the essential oils of M. piperita and M. spicata at all concentrations was 100% at different time intervals. Larvae treated with both essential oils did not attach and engorge on rabbits. GC-MS analysis of both essential oils showed significant variations in the concentration of active compounds such as γ-terpenene, menthol and piperitone ($P<0.01$).

Key words: Mentha piperita, Mentha spicata, Essential oil and Amblyomma hebraeum

1. Introduction

The biology of ticks and the problems they cause to man and animals are well documented (Sonenshine, 1991). However, these ectoparasites continue to be second only to mosquitoes as vectors of disease causing agents (Balashov, 1972). This situation indicates that the presently used control measures, which rely largely on the use of synthetic acaricides, are less effective. In addition, over-reliance on synthetic chemical products for tick control had led to several problems including: the emergence of tick resistant strains to acaricides (Mekonnen et al., 2002) and the accumulation of toxic substances in the environment (Frisch, 1999).

As a result of the problems that come with the indiscriminate use of synthetic acaricides, the need for alternative tick control methods that are environmental friendly, target specific and inexpensive cannot be over emphasized. Most recently, plant-based products are being explored by many researchers (e.g. Nchu et al., 2005; Kaaya, 2000; Mkolo and Magano, 2007) for possible acaricidal activity. The recent

trend in the use of plants as possible sources of tick control agents is motivated by the understanding that, plant-based products are biodegradable and that traditionally used plant products, which shows acaricidal properties, may be cultivated by users themselves thus avoiding high monetary costs. Thus, in this study the repellency and toxicity of the essential oils of *Mentha piperita* and *Mentha spicata* on adult *Amblyomma hebreaum* were evaluated. These are mint plants belonging to the family Labiateae and native of the Mediterranean region, now grown all over the world (Scora and Chang, 1997).

2. Materials and Methods

2.1 Tick breeding and maintenance

Colonies of *A. hebraeum* used in this study were bred on Himalayan rabbits at Animal Production unit of the Department of Biology, Medunsa campus of the University of Limpopo. Off - host stages of this tick species were kept at 25 ± 1^{o}C and 75 ± 5 % RH (relative humidity) under natural day and night regimen.

2.2 Extraction of essential oils

Mentha piperita and *Mentha spicata* were planted and collected at Riebeek Valley in Western Cape, South Africa. The extraction of essential oils of *M. piperita* and *M. spicata* was done at Still Pure Essential Oil Company, using a hydrodistillation. The yield of essential oil was 0.6% v/w, 0.4% v/w of fresh leaves of *M. piperita* and *M. spicata*, respectively. No adulterations of the essential oils were made. The essential oils extracted were stored in dark blue glass bottles with tight fitting tops and stored in a cool dark location for not more than 2 days prior to it use. Since, the oil does not dissolve in water; the 100% essential oil of each plant was diluted in dichloromethane, to make 5%, 10% and 20% v/v concentrations. These dilutions were used in the repellency bioassay and fumigant toxicity bioassay.

2.3 Repellency bioassay

The glass plate repellency bioassay was used for this study. Whatman no. 1 filter papers were trimmed to fit a 10 cm diameter glass plate and divided into two equal halves (*Figure 1*). Half of each filter paper was applied with 10 ml of the plant extract concentration prepared while the other half was used as control using dichloromethane. The whole setup was covered with a transparent 8 cm diameter plastic petri-dish (with diminutive openings) to prevent the tick from escaping.
Before recording the data, the unsexed adults *A. hebraeum* ticks (n=10) were placed on the centre of the filter paper. The number of ticks repelled was determined at 10 minutes intervals. After 10 minutes interval, the ticks were moved back to the centre. Ticks found on the treatment were considered repelled while those found on the control were considered not repelled. Three replications were done for all the concentrations.

2.4 Fumigant toxicity Bioassay

The method used was a modification of the method described by Kéïta et al. (*2000*). However, the method was used to test for the bioactivity of essential oils *M. piperita* and *M. spicata* as fumigants. Glass vials (height of 7.2 cm and diameter of 2.3 cm) were used, with each vial having 10 unsexed adults with *A. hebraeum* ticks covered with a mesh. For each essential oil type, 20 µl were applied on a 7 cm X 2 cm filter paper. Each of these filter papers was included in new vials. The vials containing the ticks were then turned upside down over the vials containing the filter paper strips with essential oil. The fumes or volatile were expected to saturate the atmosphere of the glass vials. In the controls, the filter papers were treated with dichloromethane and they were placed into the separate glass vials. Both

control and test were replicated 5 times for each oil type and tick mortality was recorded at 10 minutes intervals.

2.5 Feeding deterrent bioassay

The bioassay was based on the feeding deterrent of unfed larvae of *A. hebraeum* from the hatched eggs batch of a single *A. hebraeum* female which was obtained from a pathogen free laboratory colony bred on Himalayan rabbit (3 – 4 kg) . The feeding deterrent bioassay used in this study was a modification of rearing and infection technique used by Heloise Heyne et al. 1987. However, the modification was substantial enough necessitating a full re-description. The fur on the back of the rabbits was shaved and the nails of the rabbits were clipped. The circular transparent plastic container with a lid was used to feed unfed larvae on the back of rabbits. The bottom of the container was cut off and the base of the container was shaped to fit over the rabbit's back. The container was attached to the back using a contact adhesive (Genkem) and it was left to dry for 24 hours. Perspex collars were placed around the necks of the rabbits to prevent them from grooming. Twelve rabbits were used and they were divided into group A, B and C: treated *with M. piperita* essential oil, group D, E and F: treated with *M. spicata* essential oil, Group G, H and I: untreated which served as control. After 5 minutes, the stopper and glass tubes (20mm inner diameter X 50 mm long) containing the larvae was placed inside the container and the lid (with large ventilation hole covered with a fine nylon mesh) was closed. The stopper and the glass tube were removed after 24 hours and the lid of the container was screwed on. The rabbits were fed with water and rabbit standard pellets diet (Epol-Primer Food Industries, SA) and they were placed individually in the cages (56 X 56 X 38 cm) for the whole experimental period at the production unit of the University of Limpopo, department of Biology, Medunsa campus. The cages had screened metal floors and stood on metal platforms to allow ventilation from underneath. These rabbits were kept at room temperature (21-25^{o} C) under natural day/night regime. The containers attached to rabbit's back were checked daily and the engorged larvae were collected and counted.

2.6 Gas Chromatography-Mass Spectrometry analysis

The Identification and qualitative analysis of the chemical components of the *M. piperita* and *M. spicata* was made using QP 20-10 Shimadzu Gas Chromatography-Mass Spectrometry (GC-MS).The column temperature was programmed to rise from 50ºC to 300ºC at 10ºC/minute. The injector temperature was 250ºC. The total flow rate was 24 ml/minute and column flow rate was 1ml/ minute. Supelco equity 1 column with a film thickness of 30m X 0.25 microns was used. One micro liter of each of the sample (essential oil of *M. piperita* and *M. spicata*) was used. Ultra high purity Helium was used as the carrier gas with injector split ratio of 20:1. The ion source cut time of 4 minutes and detector gain was conducted on major peaks of each sample in order to identify the components of the sample. The relative percentage of each compound was determined by the area normalization methods, whereby the area under the peak was calculated as width at ½ height X height (Houghton and Raman, 1998).

2.7 Statistical analysis

2.7.1 Repellency bioassay

The repellent effect was calculated as percentage repellency, according to the formula: Percentage repellency = 100 – [mean number of ticks on test]/[Mean number of ticks on control] X 100. Student's t-test was used to calculate the significance of the differences between the repellent effects of *M. spicata* and *M. piperita.* The effective concentration to repel 50% (EC_{50}) of the ticks was calculated using Probit analysis a free software package (US Environmental Protection Agency; http:// www.epa.gov/nerleerd/nerleerd/sta2.htm).

2.7.2 Fumigant toxicity bioassay

Data are presented as percentage mortality in

fumigant toxicity bioassay. Probit analysis a free software package (US Environmental Protection Agency; http://www.epa.gov/nerleerd/nerleerd/sta2.htm) was used to determine lethal concentration to kill 50% of ticks (LC_{50}). The t-test was used to determine significant difference between the toxic effects of *M. spicata* and *M. piperita*.

2.7.3 Feeding deterrent bioassay

The number of larvae that successfully fed to engorgement was recorded, their feeding period was recorded. Significant differences between larvae that fed on rabbits in the treatment groups and control groups were determined using the student's t-test.

3. Results

3.1 Repellency bioassay

Essential oil of *M. piperita* showed a positive repellency effects throughout the duration of the experiment when all ticks avoided filter paper treated with essential oil of *M. piperita*. Similar, observations were made for essential oil of *M. spicata*. One hundred percent repellency was recorded against *A. hebraeum* in all concentrations (5%, 10% and 20% v/v) of *M. piperita* when diluted in dichloromethane. However, one hundred percent repellency was recorded only at 10% and 20% v/v concentrations when essential oil of *M. spicata* was used in this study. The percentage repellency of 5% v/v concentration of *M. spicata* ranged from 90% to 100%. The repellency for 5%, 10% and 20% v/v concentrations of essential oil of *M. piperita* persisted for 60 minutes, 40 minutes and 20 minutes respectively, eventually the ticks died during the repellency experiment. On the other hand, the repellency of essential oil of *M. spicata* persisted for 80 minutes, 50 minutes and 30 minutes at 5%, 10% and 20% v/v respectively, and the ticks also died eventually. In general, the repellent strength of essential oils of *M. spicata* against adults of *A. hebraeum* was similar ($P > 0.05$) to that of *M. piperita*. The EC_{50} could not be determined in all the time intervals may be because the data did not contain different repellency effects when the different concentrations are used in study for both essential oils of *M. spicata* and *M. piperita.*

3.2 Fumigant toxicity bioassay

The fumigant toxicity results obtained in this study are illustrated in Figure 2 and 3. Generally, the essential oils of both *M. spicata* and *M. piperita* were effective in killing the adults of *A. hebraeum*. However, at 10 minutes intervals the mortality effects of essential oil of *M. piperita* in all 3 concentrations were significantly high ($P < 0.05$) to that obtained with the essential oil of *M. spicata*. Moreover, ticks did not die when dichloromethane was used as control during the experiment. This was as a result of the significant difference between the treatment and control of both essential oils used in this study. The mortality effects of both essential oils increased significantly with an increased concentration against *A. hebraeum*. The LC_{50} `generally decreased with increasing time for both essential oils of *M. spicata* and *M. piperita* (*Table 1*).

3.3 Feeding deterrent bioassay

The engorgement success was significantly reduced ($P < 0.05$) in larval *A. hebraeum* ticks exposed to rabbits treated with either the essential oil of *M. piperita* and *M. spicata.* Larvae on Groups G,H,I rabbits (control rabbits) attached and fed after ± 24 hours of infestation. While, the larvae on Groups A,B,C rabbits (treated with essential oil of *M. piperita*) and Group D,E, F rabbits (treated with essential oil of *M. spicata* did not attached to the rabbits and they settled on the lid (ventilation fine nylon mesh) for the whole experiment. Moreover, they did not respond to human breath and they were dead after 1 hour of exposure. The highest mean number (524.5.) of ticks that dropped off from rabbits in the control groups were recorded on day 14.

3.4 Gas Chromatography-Mass Spectrometry analysis

Some of the main compounds identified in the

essential oils of *M. piperita* and *M. spicata* are presented in *Table 2*.

4. Discussion

In general, both essential oils of *M. piperita* and *M. spicata* were found to possess fumigant toxicity. Lee et al. (*2001*) found the compound 1,8-cineole, a consitituent of essential oils of Rosemary and *Eucalyptus* to be the most toxic fumigant against *Sitophilus oryzae* (L.). They also found other compounds such as pinene to possess fumigant toxicity. These compounds were detected in the essential oils of *M. spicata* and *M. piperita* and may account for their toxicity on adults and larvae of *A. hebraeum* found in this study. The essential oil of *Cunilia* species was found to be toxic to *Rhipicephalus (Boophilus) microplus* and its constituents included alpha-pinene and 1.8 cineole (*Apel et al., 2009*).

These compounds were also identified in the essential oils of *M. spicata* and *M. piperita* which were repellent and toxic to adults of *A. hebraeum* in the present study. The acaricidal activity of volatile essential oil of *Satureja thymbra* consisting of γ-terpenene which was evaluated against unfed adults *Hyalomma marginatum* showed approximately 90% knockdown at 105 minutes (*Cetin et al., 2010*). This compound was identified in the essential oils of *M. piperita*, which may explain the effectiveness of this plant over *M. spicata* (fumigant toxicity bioassay) especially when time is taken into consideration.

It is already clear that the current tick control methods are partially effective and loaded with many problems. In order to have effective tick control and alleviate the problems associated with the current methods of tick control, it is important to consider alternative tick control methods that are effective and user friendly even by small scale live stock keepers. Data reviewed and presented in this study undoubtedly suggests that plants are a possible source of anti-tick agents. Both the essential oils of *M. piperita* and *M. spicata* were found to be effective against *A. hebraeum* ticks.

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Figure legends

Figure 1 "The glass plate repellency bioassay. A: Petri-dish B: Filter paper treated with organic solvent C: Filter paper treated with plant extract".

Figure 2 "Mortality effect of essential oil of *M. piperita* against adults of *A. hebraeum* recorded at 10 minutes intervals during fumigant toxicity bioassay".

Figure 3 " Mortality effect of essential oil of *M. spicata* against adults of *A. hebraeum* recorded at 10 minutes intervals during fumigant toxicity bioassay".

Figure 1: The glass plate repellency bioassay. A: Petri-dish B: Filter paper treated with organic solvent C: Filter paper treated with plant extract.

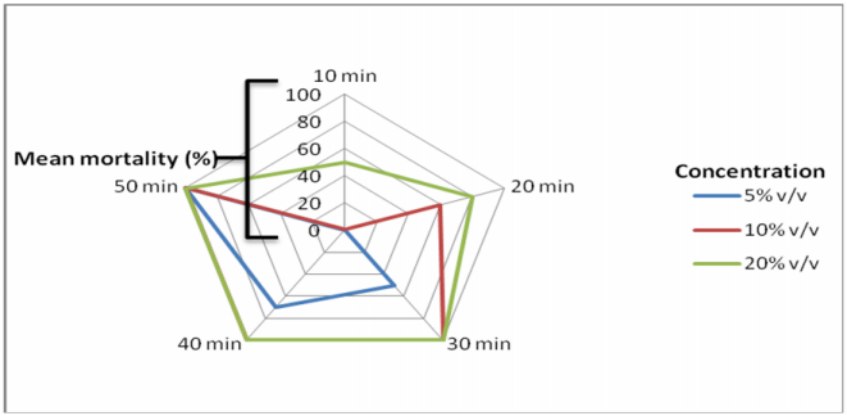

Figure 2: Mortality effect of essential oil of *M. piperita* against adults of *A. hebraeum* recorded at 10 minutes intervals during fumigant toxicity bioassay.

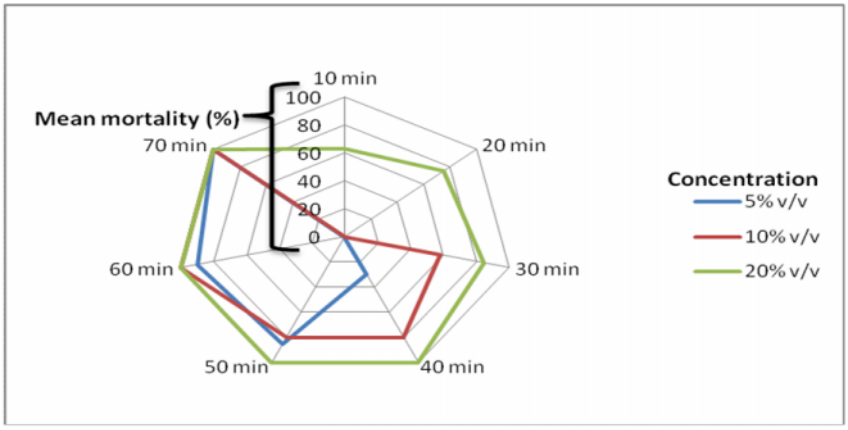

Figure 3: Mortality effect of essential oil of M. spicata against adults of A. hebraeum recorded at 10 minutes intervals during fumigant toxicity bioassay.

Tables legends

Table 1 "Lethal concentration to kill 50 percents (LC_{50}) adults of *A. hebraeum* using essential oils of *M. piperita* and *M. spicata"*.

Table 2 "The main compounds identified in essential oils of *M. spicata* and *M. piperita"*.

Table 1: Lethal concentration to kill 50 percents (LC50) adults of A. hebraeum using essential oils of M. piperita and M. spicata

		Duration					
		10 MIN	**20 MIN**	**30 MIN**	**40 MIN**	**50 MIN**	**60 MIN**
LC_{50}	*M. piperita*	-	8.333	5.001	3.571	-	-
	M. spicata	-	-	8.505	6.252	2.940	2.778
Not determined							

Table 2: The main compounds identified in essential oils of M. spicata and M. piperita

RT	**COMPOUNDS**	**% COMPOSITION**	
		M. spicata	***M. piperita***
13.19	a-Pinene	1.56	1.12
14.49	B-Pinene	3.38	3.04
15.41	a- Terpinene	0.33	0.33
16.07	1,8-Cineole + Limonene	25.27	11.87
16.63	γ-terpenene	-	0.61
16.99	Cis-sabinene hydrate	-	0.73
17.96	Linalool	2.13	0.33
19.79	P-menthone	0.30	17.66
20.13	Cyclohexanone	1.87	6.91
20.69	Menthol	-	35.68
22.27	Pulegone	-	0.85
22.71	Piperitone	-	0.41
22.96	L-carvone	55.17	-
23.02	Bicyclo (4.1.0) heptanes, 3.7,7-trimethyl	-	0.39
25.37	Neryl acetate	1.39	-
26.45	β-bourbonene	0.92	0.70
27.26	β-caryophyllene	-	1.84
27.66	Trans – β- farnesene	-	0.30
28.79	Germacrene- D	-	1.41
29.16	Bicyclo germacrene	-	0.31
31.61	Viridifol	-	0.97