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## Autecology and Insecticidal Potential of *Mentha longifolia* from Madinah el Monawara

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### Abstract

Mentha plants are grown in abundance in Al-Madina. Their potentials in the medicine and aromatic industry are widely studied. Soil physicochemical studies were carried out to determine the best condition for the growth of *Mentha longifolia* L, while GC-MS analysis was carried out to isolate and identify the essential oils in the leaves and then further tested on the *Tribolium castaneum* to investigate repellency properties. *M. longifolia* were found to grow well in neutral soil that contains high percentage of organic matter (OM). The plants also grew on soil that recorded very low amounts of heavy metals. Some secondary metabolites (e.g., cathechin, epicathechin, gallochatechin and myricetin-3-glucoside) were isolated and identified from the leaves of *Mentha longifolia* L. and the extracted phytochemicals were then assessed for their insecticidal activity. Only cathechin, epicathechin and gallochatechin successfully repelled the *T. castaneum* which showed more than 70% repellency activity. Further investigation is necessary to assess the suitability of *M. longifolia* in repelling stored product insect pests.

**Keywords:** *Mentha longifolia*; Physicochemical; Secondary metabolites; Repellant; *Tribolium castaneum* 

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## Introduction

Given their medicinal and aromatic properties, a lot of interest has been placed in plants [1,2] stated that among all plants, those that have garnered much interest in the field of research include mint plants owing to their medicinal and aromatic characteristics. Historically, mint or Mentha plants have been cultivated for medicinal and culinary use [3] and a lot of research has been performed to identify secondary metabolites in these plants [4]. They are rich in phenolic compounds and other substances with antioxidant activity [5]. Several terpenoids and phenolic compounds [6] have been identified in mint plants and the antioxidant activity of these plants has also been researched [7]. In Saudi Arabia, Mentha longifolia is locally called Al-Madina hasawy mint, which is typically used to make herbal teas for their particular aroma and flavour; it is traditionally referred to as Habaq [8]. Mint plants have also been found to possess antimicrobial characteristics [9]. However, researchers have not yet reported on how M. longifolia, which grows wildly in Madinah El- Monowara, interacts with its environment [10]. There is also a paucity of data on the biological activities of extracts from this species of mint.

The use of insecticide is the most common practice to reduce the amount of damages that insects causes. This is especially for tropical and subtropical areas as it is effective, of reasonable cost, easy to apply and there are less available choices of control methods where the places are warmer [11]. Nevertheless, detrimental consequences of uncontrolled insecticide application can reduce the number of beneficial insects and cause outbreak of pest population as well as the development of resistance in pest populations. According to Carlini [4,12] and Montesdeoca [13], the overuse of insecticide has caused concerns and stated that there is an absolute need to develop other alternatives which are safer and numerous researchers [14-17] have suggested the use of secondary metabolites with insecticidal activities from plants. According to Kumar [18] in Mentha species, most of the insecticidal properties are found in the essential oils or plant extracts. This study was performed to determine the physicochemical properties of the soil where the studied plants grow. It is also aimed to investigate the phonelic compounds in the extract of *Mentha longifolia* plants. The potential of some extracted active metabolites of *M. longifolia* L. in repelling *T. castaneum* was also determined.

## **Materials and Methods**

### **Collection of soil and plant samples**

This experiment was conducted using fresh *M. longifolia* plants, which were collected from a mint farm in AlMadina, Saudi Arabia. The leaves were picked and washed prior to air drying. Three samples of soil were randomly collected from fields where mint plants were grown and analyzed for their physico-chemical properties.

### Soil physiochemical

The soil pH was studied by mixing distilled water with soil and filtered. And the pH level was determined by using a pH meter (Mettler Toledo AG) [19]. Organic matter was determined by drying the soil in an oven for two hours at 110°C and allowed to cool. The cooled soil was heated at 500°C for three hours before being allowed to cool [20] the dried soil was weighed and the quantity of organic matter was determined using a formula. To determine soil moisture, 5 g of soil were dried at a temperature of 105°C for 24 hours. The soil was weighed and oven-dried several times until a constant weight was attained [21-25]. A formula was used to calculate the quantity of water in the soil sample. The concentration of heavy metals in the soil was investigated by mixing 10 g of soil sample with nitric acid and hydrofluoric acid [26]. The sample was allowed to reach near dryness, and the residual soil was dissolved again in 2 M hydrochloric acid. The solution was repeatedly washed with distilled water [27] and the soil heavy metal content was determined by placing the sample in an atomic absorption spectrophotometer/ 5100 (Perkin Elmer).

### **GC-MS** analysis

## GAS chromatography-mass investigation of secondary metabolites

The GC-Ms analysis of the Essential oil samples was carried out using gas chromatography– mass spectrometry with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5MS column (30 m × 0.32 mm i.d., 0.25  $\mu$ m film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 mL/min at a split ratio of 1:10 and the following temperature program: 80°C for 1 min; rising at 4°C/min to 300°C and held for 1 min. The injector and detector were held at 220 and 200°C, respectively. Diluted samples (1:10 hexane, v/v) of 1  $\mu$ L of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450.

**Qualitative analyses:** Most of the compounds were identified using two different analytical methods: (a) KI, Kovats indices in reference to n-alkanes ( $C_q$ - $C_{22}$ ); and (b) mass spectra (authentic

chemicals, Wiley spectral library collection and NSIT library). The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology (NIST) published.

**Test insect:** *T. castaneum* were collected from laboratory cultures which have been fed with wheat flour. The insects were reared in round plastic containers in a covered area and exposed to equal periods of light and darkness. They were kept at a constant temperature of  $28.0 \pm 0.5^{\circ}$ C and relative humidity ( $65.0 \pm 2.0\%$ ) throughout their rearing and experimental period.

### **Repellent/attractant activities**

Repellent or attractant activities were carried out by diluting the ethanol extracts of (Catechin, Epicatechin, Myricetin-3-glucoside and Gallo catechin) in ethanol. These extracts were chosen based on their references to their potential insecticidal properties. The concentration of the ethanol extracts used was 0.05 gml<sup>-1</sup> and 4 ml of each extract was evenly applied onto (9 cm in diameter) filter papers (Whatman No. 1). Prior to the experiment, fluon was applied in thin layers to the border of the Petri dishes. This helps to prevent test insects from crawling out of the dishes. Ten adult insects (T. castaneum) were placed onto the Petri dish in between the treated and untreated areas. The total amount of insects present on each side of the Petri dish was counted and recorded after 1 hour. Data recording was again repeated at the  $3^{\mbox{\tiny rd}}$  and  $6^{\mbox{\tiny th}}$ h. Each extracted material was tested and replicated five times. The repellency proportion (RP) was calculated as  $RP=[(N_c-N_{T})/$  $(N_c+N_{\tau})$  × 100, where N<sub>c</sub>=absolute value of insects in the control area and  $N_{\tau}$ =absolute value of insects in the treatment area.

### **Statistics**

The data were analyzed using the Statistical Package for the Social Sciences (SPSS, IBM Inc., New York, USA), version 16.0. A paired t-test was used to determine the distribution of insects in the treated and control areas. Analyses were conducted using  $\alpha$ =0.05.

## **Results and Discussion**

### **Soil physiochemical**

These analyses revealed that the pH of soil was 7.36, which falls within the neutral range. The soil contained  $4.68 \pm 0.03\%$  of OM, whereas the average water content was  $4.32 \pm 0.12\%$  (Table 1). Contrary to the finding in this study, the effect of OM in soil samples in the study conducted by other authors was negligible. In the current study, traces of heavy metal were detected in the soil, with the highest concentration being manganese (7.1317 ± 0.0129) and the least being cadmium (0.0006 ± 0.0000;) (Table 2).

**Table 1** Some physical properties of representative soil samples in the*M. longifolia.* 

Characteristics	Mean (± S.E.)			
рН	7.36			
OM (%)	4.68 ± 0.03			
Water content (%)	4.32 ± 0.12			

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Table 2 Mean ± standard error analysis of available heavy metals in soil.

	Soil Weight (mg/l)										
Elements	Cadmium	Cobalt	Chromium	Copper	Iron	Manganese	Molybdenum	Nickel	Lead	Vanadium	Zinc
	0.001 ± 0.00	0.07 ± 0.001	$0.01 \pm 0.00$	0.79 ± 0.02	4.73 ± 0.02	7.13 ± 0.01	$0.01 \pm 0.00$	0.26 ± 0.00	0.11 ± 0.00	0.39 ± 0.01	1.1± 0.01

### Extraction from Mentha longifolia

Several secondary metabolites where identified in the extracts, including catechin (2 mg), epicatechin (3 mg), gallocatechin (2 mg), and myricetin-3-glucoside (2 mg). These compounds have shown to have antioxidant properties and can therefore have a beneficial physiological effect [28]. Given the biological properties exhibited by these compounds, mint plants are considered an important source of food [29]. According to Donald, a high concentration of phenolic compounds has been detected in mint plants; this is especially the case of phenolic acids and flavonoids [30]. On the other hand, flavonols and dihydro flavonols have been infrequently reported in these plants. Nevertheless, some authors Stanisavljevic [31-35] identified kaempferol (a flavonol) and its glucosides, in mint plants [36]. Other flavanols that have been detected in Mentha plants include catechin [37] and epicatechin [38], which were identified in the current study. Findings from a previous report showed that rosmarinic acid was the most common flavonol in Mentha plants [39]. Other metabolites that were detected in relatively high concentrations include 12- hydroxyjasmonate sulphate and salvianolic acid B. and flavone luteolin. In addition to rutinoside, eriodictyol was also identified. Common flavonoids that have been described in mint plants include diosmin and myricetin-O-glucoside. Compounds such as organic acids were detected in negligible amounts in the extract of mint plants (Table 3).

# Repellency properties of *M. longifolia* extracted materials

The presence of cathechin, epicathechin and gallochatechin on the filter papers significantly repelled *T. castaneum*. More than 70% repellency was recorded and the effect of the repellency was able to sustain up to 6 hours. Peres stated that catechin and gallocatechin found in the methanolic extracts of *Croton urucurana* Baillon has insecticidal potential. Catechin and gallocatechin are phenolic compounds that give fruits its astringent properties and **Table 3** Percentage repellency of *T. castaneum* towards extractedmaterials of *M. longifolia*.

	Percentage repellency (PR)					
Extracted materials	Time (h)					
	1	3	6			
Catechin	72.0 ± 8.0*	76.0 ± 9.8*	88.0 ± 4.9*			
Epicatechin	80.0 ± 8.9*	88.0 ± 8.0*	88.0 ± 8.0*			
Myricetin-3- glucoside	44.0 ± 16.0	32.0 ± 18.5	32.0 ± 18.5			
Gallocatechin	76.0 ± 9.8*	72.0 ± 13.6*	84.0 ± 7.5*			

\*Significant difference between treated and untreated areas (Paired t-test) at  $\alpha{=}0.05.$ 

play an important role in plant defense [39]. According to Oliveira these compounds will bind to the digestive proteins in insects. This study requires further investigation as more information regarding the nature of catechin, particularly towards non-target organisms is needed. Moreover, it is vital to develop safe and suitable formulations of this potential phyto insecticide [40]. The analyses revealed that leaves extracted materials from *M. longifolia* were effective against *T. castaneum* with regards to insect-repellency. The results in this report suggested that insecticidal extracts of *M. longifolia* were a potential component of an eco-friendly approach to crop production and protection, involving methods that minimize the use of pesticides.

## Conclusion

Mint plants from Al-Madina thrive in neutral soil, which contain relatively large amounts of OM compared with findings from local studies. Only traces of heavy metal were identified in the soil sample. It is important to have more information regarding the physico-chemical properties of the soil as well as the characteristics of the plants to improve product development. Moreover, the secondary metabolites, namely cathechin, epicathechin and gallochatechin were found to show repellent properties against *T. castaneum*.

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