

## Evaluation of the toxicity of the essential oils of certain mints grown in the region of Settât (Morocco): *Mentha piperita*, *Mentha pulegium* and *Mentha spicata* against, *Sitophilus Granarius*, *Sitophilus Oryzae* and *Sitophilus Zeamais*

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### ABSTRACT

Essential oils are secondary metabolites produced by plants as a defense against phytophagous pests. These extracts contain complex compounds. The essential oils present many previous works, especially in Morocco. The present work is a continuation of chemical study of the essential oils of some mints grown in the Settât Morocco region: *Mentha piperita*, *Mentha pulegium* and *Mentha spicata*, against insects of cereals: *Sitophilus Granarius*, *Sitophilus Oryzae* and *Sitophilus Zeamais*.

Experiments were conducted in the laboratory using different doses of these oils to evaluate the mortality they cause on the adults of the insects tested.

The results of the sensitivity showed that the three essential oils of mint had important insecticidal properties against the pests studied. And as a result, they were able to destroy all the insects at a concentration of 2 µl/cm<sup>3</sup> for a duration of 24 hours of treatment.

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## 1. Introduction:

The development of chemistry has given birth to synthetic methods for insect protection of stocks have the use of chemical insecticides. However, the search for alternatives is essential. It is in this context that it is the use of essential oils in the protection of stocks as bio insecticides with properties that respect the environment. The beetle's pests of mostly beetle commodities can cause the total loss of a stock. as well the beetles are the most damaging pests of the grain stocks that for many developing countries represent the bulk of the diet that supports more than 70% of the population [1].

## 2. Material and methods:

### 2.1. harvesting, extraction and analysis by gas chromatography (GC) / mass spectrometry (MS):

The methods of harvesting the plants studied and the extraction of essential oils and the analysis of the oils by gas phase chromatography coupled with mass spectrometry are indicated in the first worked (see back articles) [2].

### 2.2. The insects studied:

#### 2.2.1. *Sitophilus granarius*:

*Sitophilus granarius*, also known as wheat weevil, is a species of beetle's insects of the family of Curculionidae with an almost cosmopolitan distribution. [3]. Adults are small, uniformly dark brown beetles about 5 mm in length, unable to fly. These larvae, white and legless, develop inside the grains.

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### 2.2.2 *Sitophilus oryzae*:

The rice weevil (*Sitophilus oryza*) is a species of beetle's insects of the family of Curculionidae, cosmopolitan distribution. Adults are brown with small sizes (2.5 to 4 mm) [4]. The elytra are marked with four characteristic reddish yellow spots. In adulthood, the rice weevil is identical to the corn weevil (*Sitophilus zeamais*), and a dissection is needed to distinguish the two species. These larvae, white and legless, develop inside the grains.

### 2.2.3 *Sitophilus zeamais*:

*Sitophilus zeamais* (corn weevil) is a species of beetle's insects of the family Curculionidae, pantropically distributed [5]. In adult stages, corn weevils are identical to rice weevils (*Sitophilus oryza*), and dissection is required to separate the two species. Adults are reddish-brown with four yellow to red spots on elytra and have a characteristic long rostrum.

### 2.3. Insecticide tests:

The insects were reared on wheat, rice and maize, tender in 1-liter plastic boxes, transparent and screened. The set is placed in speakers whose temperature is 30 ° C and the relative humidity is 70 %. The insecticidal tests used in this study are as follows: in experimental chambers containing ten insects of each species (*Sitophilus granarius*, *Sitophilus oryza* and *Sitophilus zeamais*), the sensitivity of each essential oil (*Mentha piperita*, *Mentha pulegium* and *Mentha spicata*) is tested in increasing concentrations of 1 µl / cm<sup>3</sup>, 1.5 µl / cm<sup>3</sup> and 2 µl / cm<sup>3</sup> respectively on Whatman paper number one. The assembly is introduced into a fumigation chamber included in the experimental chamber (semi-ventilated). Three repetitions were performed for each concentration and each species. The number of dead insects is recorded as a function of time after 1 day. Corrected mortality in treated insects is expressed as Abbot's formula.

## 3. Results and Discussion:

### 3.1. Yield of essential oils & Analyzes of Chemical Compositions by GC-MC.

The results of the yield of essential oils and the results of oil analyzes by gas phase chromatography coupled with mass spectrometry are indicated in the first worked (see back articles) [2].

### 3.2 Biological tests:

The insecticidal activity of the essential oils of *Mentha piperita*, *Mentha pulegium* and *Mentha spicata* studied against *sitophilus granarius* *sitophilus oryza* and *sitophilus zeamaisa* was evaluated on Whatman paper number one, the results of the insecticide tests of three essential oils showed a very important insecticidal activity.

### 3.3 Discussion:

The results of the insecticide tests show the effect of each essential oil (*Mentha piperita*, *Mentha pulegium* and *Mentha spicata*) on the mortality of *Sitophilus Granarius*, *Sitophilus Oryzae* and *Sitophilus Zeamais*. In fact, at the lowest concentration (1µl/cm<sup>3</sup>) and after 24 hours of contact, the essential oil of *Mentha pulegium* causes a mortality of 46.66% of adults of *Sitophilus granarius* followed by *Mentha spicata* 40% and *Mentha pulegium* 36.66 %. For *Sitophilus Oryzae*, the low concentration (1µl/cm<sup>3</sup>) to show that 40% mortality caused by oil and *Mentha pulegium* after 24 hours of contact, while at this same concentration, the essential oil of *Mentha piperita* causes only 36.66% mortality and *Mentha spicata* oil also causes only 33.33% mortality. For *Sitophilus zeamais* the low concentration (1µl/cm<sup>3</sup>) to show 30% mortality in the presence of *Mentha pulegium* followed by *Mentha piperita* 26.66% and *Mentha spicata* 23.33%.

At the mean concentration (1.5µl/cm<sup>3</sup>) the parentages of mortalities recorded after 24 hours of contact with *Sitophilus Granarius* and as follows: *Mentha spicata* causes a mortality of 83.33 %%, *Mentha piperita* 76.66% and *Mentha pulegium* 70%. For *Sitophilus Oryzae* the percentages of the mortalities recorded at the average concentration (1.5µl /cm<sup>3</sup>) are: *Mentha piperita* and *Mentha pulegium* 56.66% and 53.33% for *Mentha spicata*. in *Sitophilus zeamais* the average concentration (1.5µl/cm<sup>3</sup>) to show 50% mortality either in the presence of *Mentha pulegium* or *Mentha piperita* then 23.33% for *Mentha spicata*.

At the highest concentration (2µl/cm<sup>3</sup>) and after 24 hours of contact, the essential oils have a significantly higher action compared to other concentrations (low and medium). The percentages of mortalities recorded in *Sitophilus granarius* are: 100% for *Mentha spicata*, 96.66% for *Mentha piperita* and 86.66% for *Mentha pulegium*. for *Sitophilus Oryzae* the percentages of mortalities recorded are: 100% for *Mentha spicata*, 96.66% for *Mentha pulegium* and 90% for *Mentha piperita* and for *Sitophilus zeamais* the mortalities cause are 100% for *Mentha piperita* and *Mentha spicata* then 86.66% for *Mentha pulegium*.

Table 1. The results witness for *Sitophilus granarius*.

Specie	Dose of oils	0 µl/cm <sup>3</sup>																			
	Time (hours)	0h			1h			3h			6h			24h							
		Mortality (%)																			
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	
	Averages	0			Averages			0			Averages			0			Averages			0	
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	
	Averages	0			Averages			0			Averages			0			Averages			0	
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	
	Averages	0			Averages			0			Averages			0			Averages			0	

Table 2. The results activity of three essential oils on *Sitophilus granarius* for concentration 1µl/cm<sup>3</sup>.

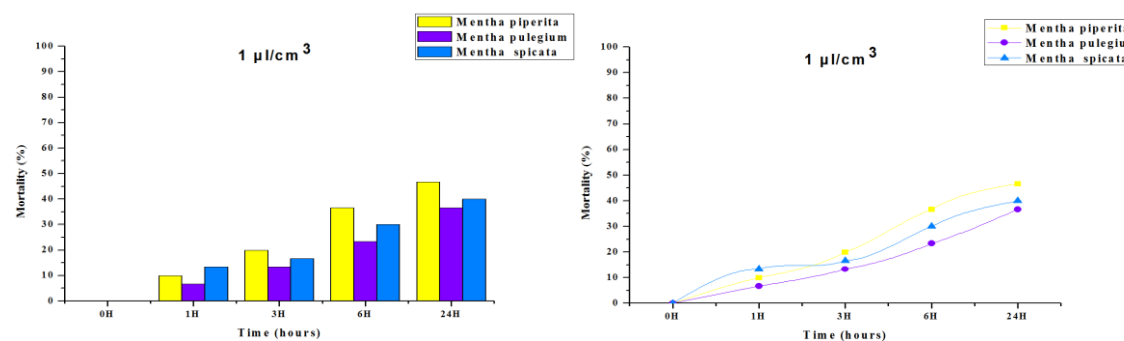
Specie	Dose of oils	1 µl/cm <sup>3</sup>																									
	Time (hours)	0h			1h			3h			6h			24h													
		Mortality (%)																									
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	10	10	10	Repetitions	20	20	20	Repetitions	30	40	40	Repetitions	50	40	50							
	Averages	0			Averages			10			Averages			36.66			Averages			46.66							
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	0	10	10	Repetitions	10	10	20	Repetitions	30	20	20	Repetitions	30	40	40							
	Averages	0			Averages			6.66			Averages			13.33			Averages			23.33			Averages			36.66	
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	10	20	10	Repetitions	20	10	20	Repetitions	30	30	30	Repetitions	50	30	40							
	Averages	0			Averages			13.33			Averages			16.66			Averages			30			Averages			40	

Table 3. The results activity of three essential oils on *Sitophilus granarius* for concentration 1.5µl/cm<sup>3</sup>.

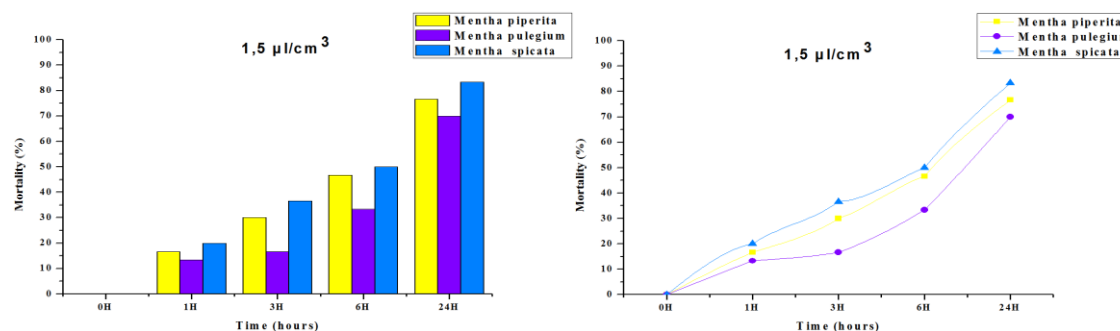
Specie	Dose of oils	1.5 µl/cm <sup>3</sup>																									
	Time (hours)	0h			1h			3h			6h			24h													
		Mortality (%)																									
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	20	20	10	Repetitions	30	30	30	Repetitions	50	50	40	Repetitions	80	70	80							
	Averages	0			Averages			16.66			Averages			30			Averages			46.66			Averages			76.66	
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	20	10	10	Repetitions	20	10	20	Repetitions	30	40	30	Repetitions	70	80	60							
	Averages	0			Averages			13.33			Averages			16.66			Averages			33.33			Averages			70	
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	20	20	20	Repetitions	40	30	40	Repetitions	50	50	50	Repetitions	80	80	90							
	Averages	0			Averages			20			Averages			36.66			Averages			50			Averages			83.33	

**Table 4.** The results activity of three essential oils on *Sitophilus granarius* for concentration 2µl/cm<sup>3</sup>.

Specie	Dose of oils	2 µl/cm <sup>3</sup>																		
	Time (hours)	0h			1h			3h			6h			24h						
		Mortality (%)																		
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	20	20	30	Repetitions	50	50	70	Repetitions	70	70	70	Repetitions	100	100	90
	Averages	0			23.33			56.66			70			96.66						
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	20	20	10	Repetitions	50	50	50	Repetitions	50	60	80	Repetitions	90	80	90
	Averages	0			16.66			50			63.33			86.66						
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	20	20	20	Repetitions	60	60	50	Repetitions	70	80	80	Repetitions	100	100	100
	Averages	0			20			56.66			76.66			100						



**Figure 1.** Activities of three essential oils after 24 hours of treatment on *Sitophilus granarius* for concentration 1µl/cm<sup>3</sup>.



**Figure 2.** Activities of three essential oils after 24 hours of treatment on *Sitophilus granarius* for concentration 1.5µl/cm<sup>3</sup>.

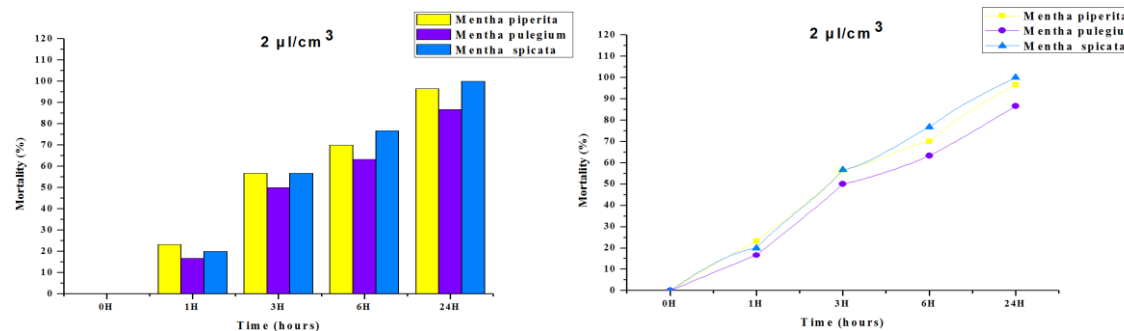


Figure 3. Activities of three essential oils after 24 hours of treatment on *Sitophilus granarius* for concentration  $2\mu\text{l}/\text{cm}^3$ .

Table 5. The results witness for *Sitophilus oryzae*.

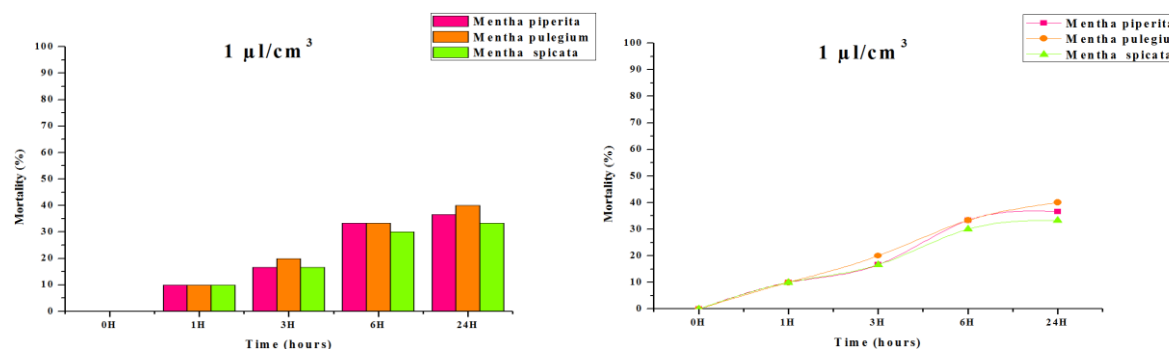
Specie	Dose of oils	0 µl/cm <sup>3</sup>														
		0h			1h			3h			6h			24h		
		Mortality (%)														
<i>Mentha piperita</i>	Repetitions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Averages	0			0			0			0			0		
<i>Mentha pulegium</i>	Repetitions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Averages	0			0			0			0			0		
<i>Mentha spicata</i>	Repetitions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Averages	0			0			0			0			0		

Table 6. The results activity of three essential oils on *Sitophilus oryzae* for concentration  $1\mu\text{l}/\text{cm}^3$ .

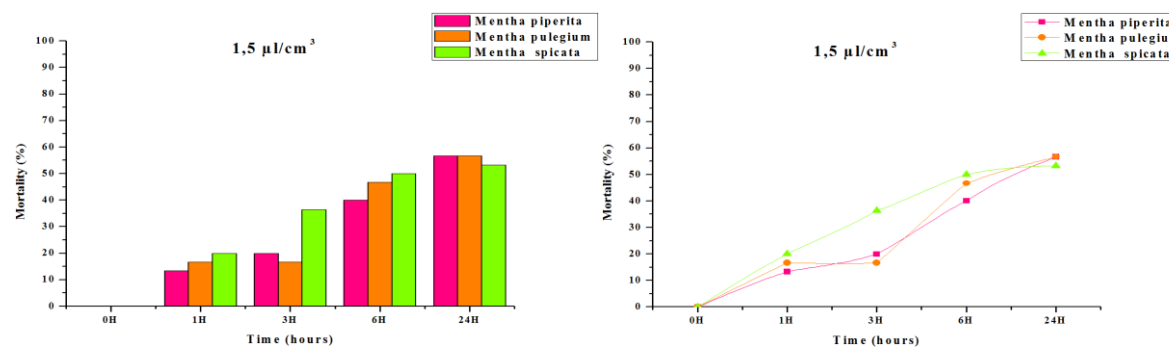
Specie	Dose of oils	1 µl/cm <sup>3</sup>														
		0h			1h			3h			6h			24h		
		Mortality (%)														
<i>Mentha piperita</i>	Repetitions	0	0	0	10	10	10	10	20	20	30	40	30	40	40	30
	Averages	0			10			16.66			33.33			36.66		
<i>Mentha pulegium</i>	Repetitions	0	0	0	10	10	10	20	20	20	30	30	40	40	40	40
	Averages	0			10			20			33.33			40		
<i>Mentha spicata</i>	Repetitions	0	0	0	10	10	10	20	10	20	30	30	30	30	30	40
	Averages	0			10			16.66			30			33.33		

**Table 7.** The results activity of three essential oils on *Sitophilus oryzae* for concentration 1.5µl/cm<sup>3</sup>.

Specie	Dose of oils	1.5 µl/cm <sup>3</sup>																		
	Time (hours)	0h			1h			3h			6h			24h						
		Mortality (%)																		
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	10	20	10	Repetitions	20	20	20	Repetitions	40	40	40	Repetitions	60	50	60
	Averages	0			13.33			20			40			56.66						
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	20	20	10	Repetitions	20	10	20	Repetitions	40	40	30	Repetitions	50	60	60
	Averages	0			16.66			16.66			46.66			56.66						
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	20	20	20	Repetitions	30	30	40	Repetitions	50	50	50	Repetitions	50	50	60
	Averages	0			20			36.33			50			53.33						



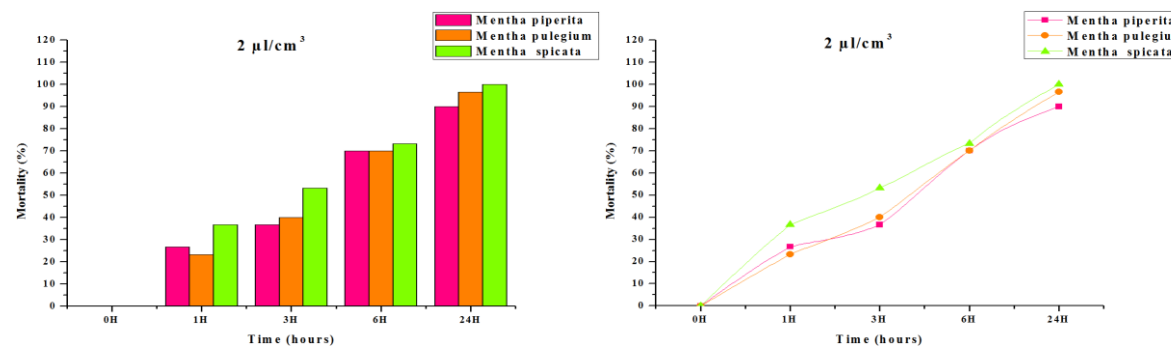
**Figure 4.** Activities of three essential oils after 24 hours of treatment on *Sitophilus oryzae* for concentration 1µl/cm<sup>3</sup>.



**Figure 5.** Activities of three essential oils after 24 hours of treatment on *Sitophilus oryzae* for concentration 1.5µl/cm<sup>3</sup>.

**Table 8.** The results activity of three essential oils on *Sitophilus oryzae* for concentration 2µl/cm<sup>3</sup>.

Specie	Dose of oils	2 µl/cm <sup>3</sup>																		
	Time (hours)	0h			1h			3h			6h			24h						
	Mortality (%)																			
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	20	30	30	Repetitions	30	40	40	Repetitions	70	70	70	Repetitions	90	90	90
	Averages	0			26.66			36.66			70			90						
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	30	20	20	Repetitions	40	40	40	Repetitions	70	70	70	Repetitions	90	100	100
	Averages	0			23.33			40			70			96.66						
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	30	40	40	Repetitions	60	50	50	Repetitions	70	70	80	Repetitions	100	100	100
	Averages	0			36.66			53.33			73.33			100						



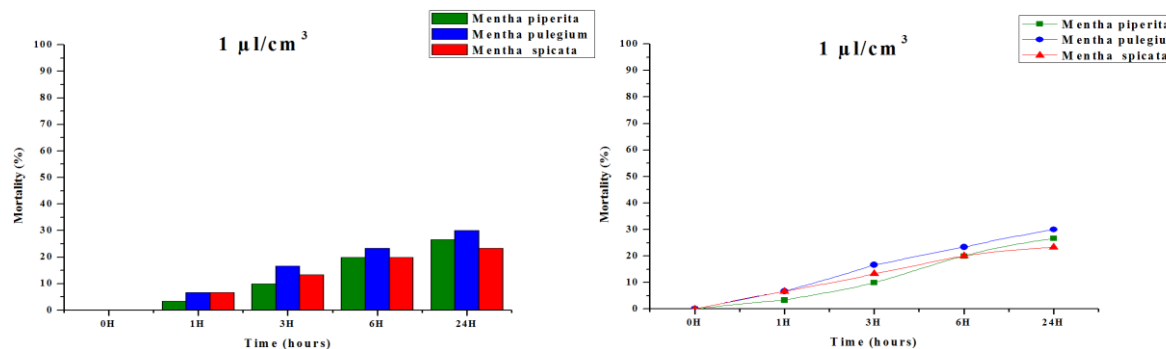
**Figure 6:** Activities of three essential oils after 24 hours of treatment on *Sitophilus oryzae* for concentration 2 µl/cm<sup>3</sup>.

**Table 9.** The results witness for *Sitophilus zeamais*.

Specie	Dose of oils	0 µl/cm <sup>3</sup>																		
	Time (hours)	0h			1h			3h			6h			24h						
	Mortality (%)																			
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0
	Averages	0			0			0			0			0						
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0
	Averages	0			0			0			0			0						
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0	Repetitions	0	0	0
	Averages	0			0			0			0			0						

**Table 10.** The results activity of three essential oils on *Sitophilus zeamais* for concentration 1µl/cm<sup>3</sup>.

Specie	Dose of oils	1 µl/cm <sup>3</sup>																		
	Time (hours)	0h			1h			3h			6h			24h						
Mortality (%)																				
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	0	0	10	Repetitions	10	10	10	Repetitions	20	20	20	Repetitions	30	30	20
	Averages	0			3.33			10			20			26.66						
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	0	10	10	Repetitions	10	20	20	Repetitions	30	20	20	Repetitions	30	30	30
	Averages	0			6.66			16.66			23.33			30						
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	10	0	10	Repetitions	10	10	20	Repetitions	20	20	20	Repetitions	20	20	30
	Averages	0			6.66			13.33			20			23.33						



**Figure 7:** Activities of three essential oils after 24 hours of treatment on *Sitophilus zeamais* for concentration 1µl/cm<sup>3</sup>.

**Table 11.** The results activity of three essential oils on *Sitophilus zeamais* for concentration 1.5µl/cm<sup>3</sup>.

Specie	Dose of oils	1.5 µl/cm <sup>3</sup>																		
	Time (hours)	0h			1h			3h			6h			24h						
Mortality (%)																				
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	10	0	10	Repetitions	20	10	20	Repetitions	40	20	40	Repetitions	40	50	60
	Averages	0			6.66			16.66			33.33			50						
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	10	10	10	Repetitions	20	20	20	Repetitions	30	40	30	Repetitions	30	60	60
	Averages	0			10			20			33.33			50						
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	10	10	10	Repetitions	20	20	20	Repetitions	20	30	30	Repetitions	40	50	50
	Averages	0			10			20			26.66			46.66						



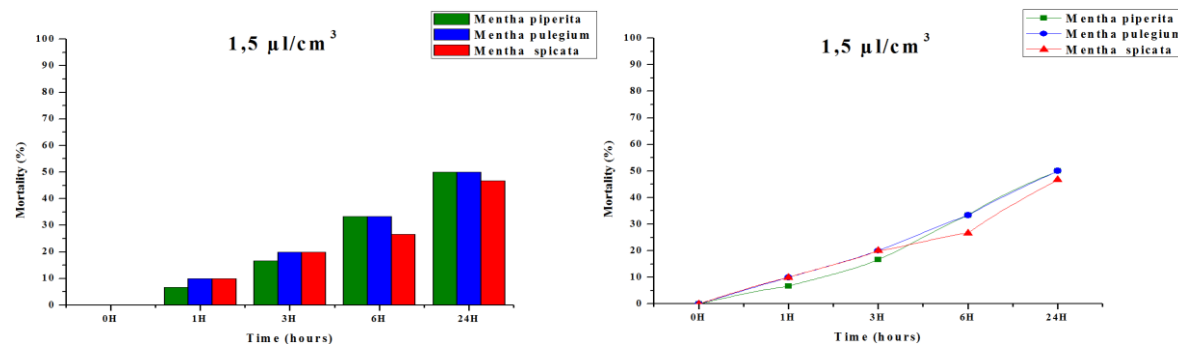


Figure 8. Activities of three essential oils after 24 hours of treatment on *Sitophilus zeamais* for concentration 1.5µl/cm³.

Specie	Dose of oils Time (hours)	2 µl/cm3																		
		0h			1h			3h			6h			24h						
Mortality (%)																				
<i>Mentha piperita</i>	Repetitions	0	0	0	Repetitions	40	30	30	Repetitions	70	80	70	Repetitions	90	90	100	Repetitions	100	100	100
	Averages	0			Averages	33.33			Averages	73.33			Averages	93.33			Averages	100		
<i>Mentha pulegium</i>	Repetitions	0	0	0	Repetitions	40	40	40	Repetitions	70	60	70	Repetitions	80	80	70	Repetitions	100	100	100
	Averages	0			Averages	40			Averages	66.66			Averages	76.66			Averages	86.66		
<i>Mentha spicata</i>	Repetitions	0	0	0	Repetitions	40	40	50	Repetitions	60	70	70	Repetitions	70	90	90	Repetitions	100	100	100
	Averages	0			Averages	43.33			Averages	66.66			Averages	83.33			Averages	100		

Table 12. The results activity of three essential oils on *Sitophilus zeamais* for concentration 2µl/cm³.

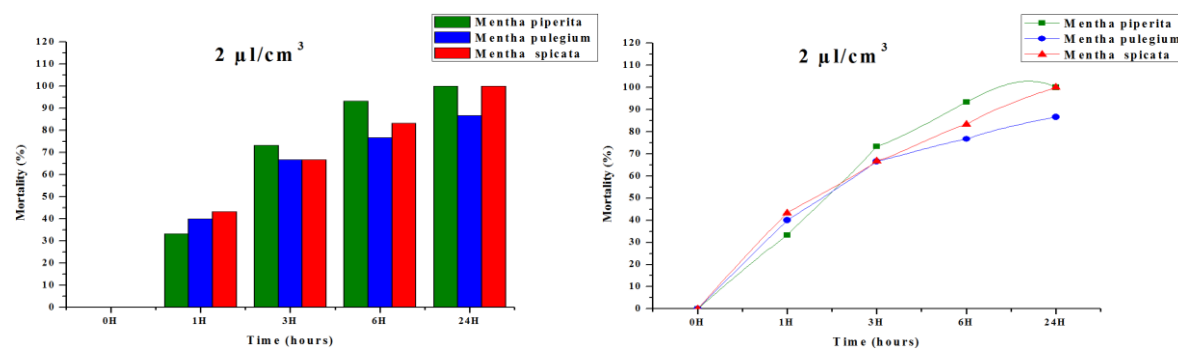


Figure 9. Activities of three essential oils after 24 hours of treatment on *Sitophilus zeamais* for concentration 2µl/cm³.

#### 4. Conclusion:

From the analysis of the chemical composition of the essential oils, it appears that the volatile extract contains a high rate of deadly compounds against pests. The essential oil of *Mentha piperita* is rich in Menthone (42.97%) and Menthol (27.64%), the most abundant chemical compounds in the essential oil of *Mentha pulegium* are piperien (31.27%) and piperienne (22.98). %) and for *Mentha spicata* we have the presence of carvones (33.14%) and trans-carveol (20.06%). Since the insecticidal activity of essential oils has shown that they can be used as phyto-chemical biopesticides to preserve the environment as well as human and animal health, alternative solutions are always sought, particularly in the African flora, teeming with many plants with insecticidal effect or insect repellent biodegradable.

This work could therefore be continued with a view to producing practical use of these essential oils in the protection of stocks.

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