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Original article

Effect of the Essential Oil of *Mentha Piperita* and *Thymus Numidicus* on the Growth of *Fusarium Sp* [1]

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Abstract

The polluting substances are of industrial or agricultural origin, at the level of the different ecosystems. These are the effects of synthetic products on the fauna and flora we are interested in. So our use of biopesticides based on plant extracts, namely essential oils as a natural insecticide is our concern. These bioactive substances or molecules are of great ecological interest because they are very harmful to the ecosystem.

The objective of this study is the use of the essential oil of *Mentha peperita* harvested from the area of Larabaa (Blida) and *Thymus numidicus* recovered from the area of Bouira as biofungicides with respect to a fungal strain isolated and identified from the leaves of the zucchini crop in Boudouaou-El Bahri (Boumerdes). The isolated species belongs to the genus Fusarium.

The extraction method is the hydraudistilation which allowed us to have yields of essential oil of 2.02% for *M. peperita* and 1.2% for *T. numidicus*. The study of the antifungal activity of these two oils tested was carried out by the direct contact method. The evaluation of the antifungal effect of these two extracts shows that the essential oil of *T. numidicus* has an inhibitory capacity on mycelial growth. *T. numidicus* oil is moderately active for concentrations; 400 µl 700 µl is inhibition rates; 43.33% and 50%. This natural substance is active with the 1000 µl dose, with an inhibition rate of 53.33%. It appears from this study that *M. piperita* oil is not very active on *Fusarium sp* with the following concentrations; 400 µl 700 µl and 1000 µl are inhibition rates respectively of 23%, 21.66%, and 23%. The essential oil of Peppermint has a slightly inhibitory activity on *Fusarium sp* with an inhibition zone diameter of 4.6 cm, 4.7 cm and 4.6 cm respectively for doses 400 µl 700 µl and 1000 µl. So, it is found that the increase in *M. piperita* oil concentration has no effect on the growth of *Fusarium sp*.

Fusarium sp also shows an allergy to *T. numidicus* oil, while this plant pathogen is resistant to *M. piperita* oil.

Keywords: *Thymus numidicus, Mentha piperita, Fusarium sp,* Extracts, Essential oil.

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INTRODUCTION

Fusarium wilt is one of the most common plant diseases with the most severe impact on crops (Nelson et al., 1983).

In the search for alternative methods of fighting, the plant kingdom offers a lot of possibilities. Currently, many studies show the existence of plant-based substances with fungicidal activity (Lichtenstein, 1996).

Thus, it is the aromatic and medicinal plants that contain these substances and molecules with very interesting biological properties and having many applications in various fields, namely medicine, pharmacy, and cosmetology.

Many species are used in traditional medicine because they contain several molecules endowed with therapeutic activities. Among the best known species are *T. numidicus* and *M. piperita*. These plants have been the subject of some studies. It is important to note the work of (Nelson et al., 1983). These latter carried out the study of the chemical composition and the antimicrobial activity of the essential oils of *M. piperita* in France. Thus, Rasooli et al. (2006) worked on the essential oils and aromatherapy of *T. nimudicus* in Europe. Also, we can note the work of Mendes-Giannini et al. (1989) on the effect of aromatic and medicinal plants in England.

In Algeria, we can be referred to the work of et al. (2006) who dealt with the chemical composition, the antimicrobial and antioxidant activity of the essential oil of Thyme. Thus, Benchabane et al. (2012) studied the analysis and antioxidant activity of the essential oil of Thyme. Behidj et al. (2017) discussed the antimicrobial effect of aqueous and organic extracts of *T. numidicus*.

The present study, which is included in this context, aims at evaluating the antifungal activity of differents extracts of *T. numidicus* and *M. piperita* on *Fusarium sp*.

Material and Methods

Biological material

During this study, in a market gardening area in Boudouaou El Bahri (Boumerdes), green house courgette plants at harvest stage with symptoms of wilting were recovered.

Also, the fresh aerial parts, namely the stems and leaves of *T. numidicus*, were collected in Chréa (Blida) during the month of April 2016. Thus, *M. piperita*'s flowering tops were harvested at the beginning of flowering between the end of March and the beginning of April (2016) in the region of Larbaa (Blida).

Study methods

Extraction of the essential oil

The essential oil of *T. numidicus* and *M. piperita* was extracted by hydrodistillation.

Identification of mushrooms

In situ observation of the morphology of the spore chains and of the mycelium is referred to the work of (Gubler et al., 1999). It is done either by a mounting between the blade and lamella and the addition of a drop of methylene blue or a drop of sterile water, or by sampling of mushroom with the aid of the tape and put them on a blade containing a drop of methylene blue. Thus, to identify this phytopathogen, one proceeds to its isolation and its purification. Then, the two steps, namely the comparison and the evaluation of the antifungal activity of the oils tested, are carried out.

Evaluation of the antifungal activity of Peppermint and Thyme of Numidia oils

The antifungal effect of the extracts tested against *Fusarium sp* is determined by measuring the growth rate after incubation at 25° C for 7 days using the Ebbot formula.

$$T = (DK-D0) / DK \times 100$$

DK: Diameter of the control fungal colony in (cm).

D0: Diameter of the fungal colony in the presence of the extract in (cm).

T: Mycelial growth inhibition rate in percent.

The extract is called very active when it has an inhibition of between 75% and 100%, the fungal strain is said to be sensitive. It is active when it has an inhibition of between 50% and 75%, the fungal strain is said to be sensitive. It is considered moderately active when it has an inhibition of between 25% and 50%, the fungal strain is said to be the limit. Finally, it is little or not active when it has an inhibition of between 0% and 25%, the fungal strain is said to be weakly sensitive or resistant (Motiéjunaité and Peiculyté, 2004).

Growth rate

To calculate the growth rate of Oidium, the following law was used:

$$V: D / T$$

V: growth rate in cm/ Day. T: growth time in day. D: growth diameter in cm

Results

Evaluation of the antifungal activity of Peppermint and Thyme of Numidia oils

In this study, the antifungal activity is evaluated in vitro for essential oils of *M. piperita* and *T. numidicus* against the *Fusarium sp*.

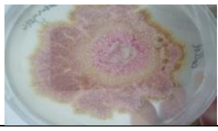
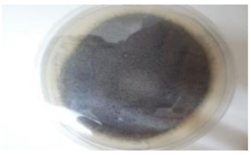
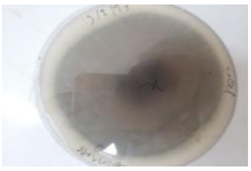
Isolation and identification of the phytopatogenic fungus Fusarium sp

Characterization of isolates

Evaluation of the macroscopic characteristics of the isolates

Purified strains are selected for morphological characterization of colonies. The results revealed a variation between the macroscopic characters of the isolates. The following table summarizes the macroscopic appearance of the purified strains.

Table 1. Macroscopic identification of purified strains

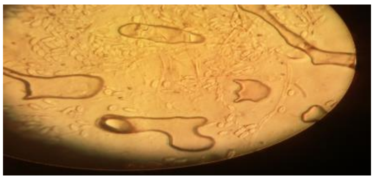
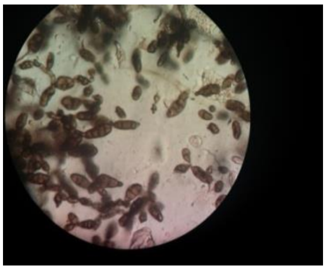
Isolates	Macroscopic appearance	
1- Collard of zucchini	 	- The colonies are flaky. - They are at the beginning of pink color which will become after red to purple
2- Root of zucchini	 	- Fluffy colonies of gray to dark black color.

Through macroscopic identification, we find two different fungal strains by the various aspects, namely the texture and color of the colonies from the roots and Collard of the zucchini.

Evaluation of the microscopic characteristics of the isolates

The microscopic study (Table 2) concerns the observation of the characteristic structures of the different strains studied.

Table 2. Microscopic appearance of the different strains studied

Isolates	Microscopic appearance	
1- Collard of zucchini		Under optical microscope (Gr X100). - Fusiform, septate, curved and pointed macroconidia
2- Root of zucchini		Under optical microscope (Gr X100). - The mycelium is of the non-septate type associated with the presence of multicellular conidia in irregular brown chains.

From the macroscopic and microscopic results and their studied characters, we have the genus of the two isolates; *Fusarium sp* and *Alternaria sp*.

Evaluation of the antifungal activity of the two extracts on Fusarium sp

Evaluation of the antifungal activity of M. piperita essential oil

The effect of Peppermint oil is evaluated by the direct contact method. After 9 days of incubation at 25°C, mycelial growth is measured in (cm).

The results obtained are recorded in Figures 1 and 2.

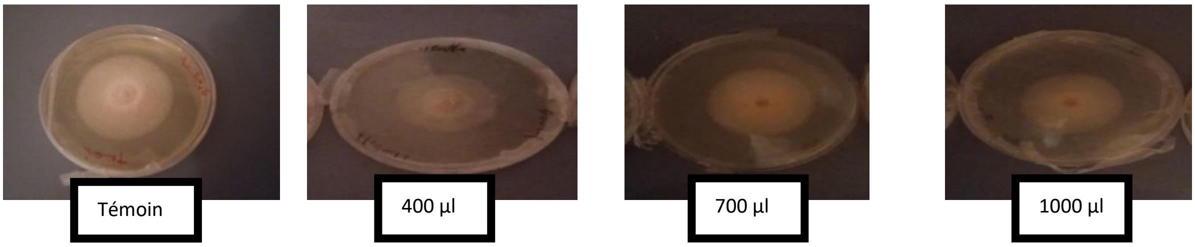


Figure 1. Inhibitory effect of M. piperita essential oil against Fusarium sp

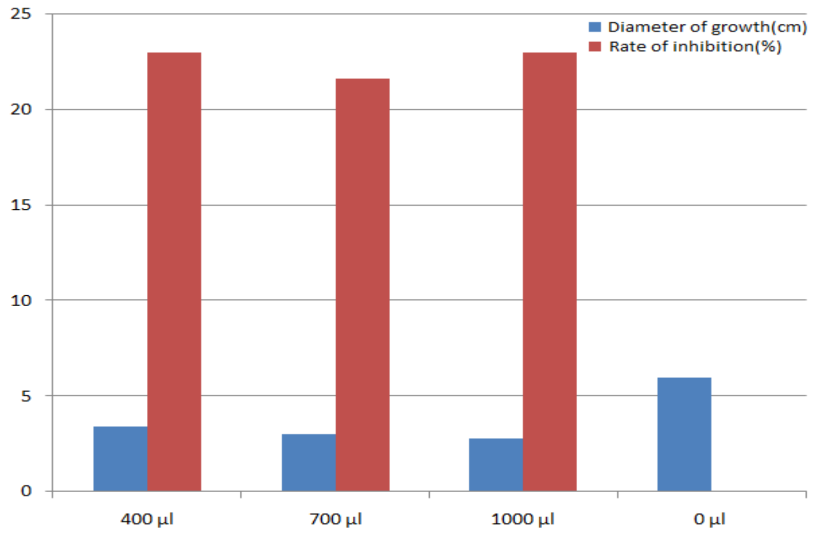


Figure 2. Growth diameters and inhibition rates of *Fusarium sp* against *M. piperita* essential oil

Evaluation of antifungal activity for T. numidicus oil

The effect of *T. numidicus* oil is evaluated by the direct contact method. After 9 days of incubation at 25°C, mycelial growth is measured in (cm).

The results obtained are recorded in Figures 3 and 4.

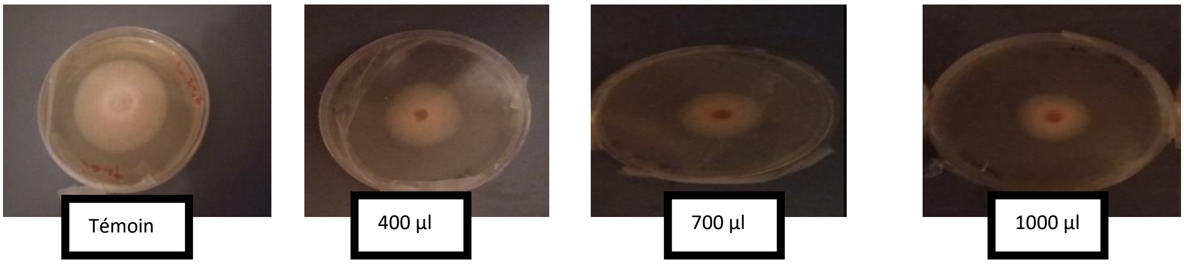


Figure 3. Inhibitory effect of *T. numidicus* essential oil against *Fusarium sp*

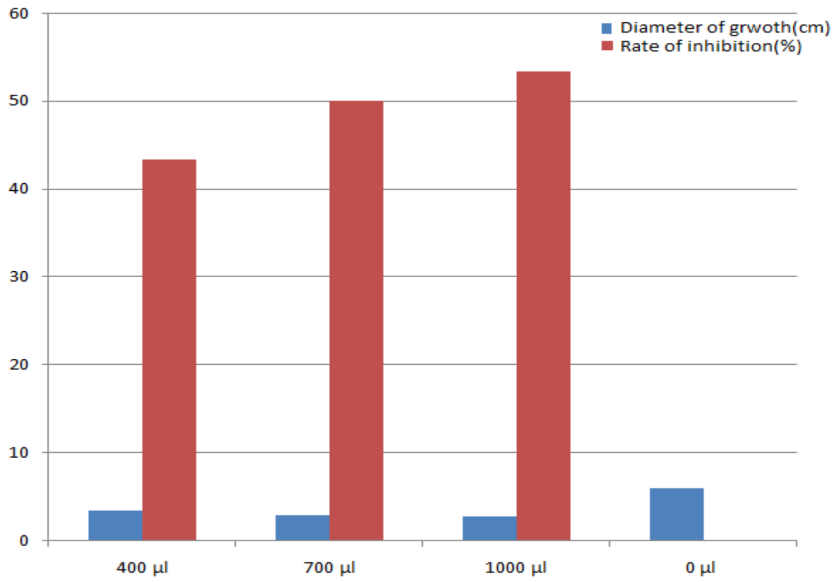


Figure 4. Growth diameters and inhibition rates of *Fusarium sp* against *T. numidicus* essential oil

Discussion

It appears from this study that *M. piperita* oil is not very active on *Fusarium sp* with the following concentrations; 400 µl 700 µl and 1000 µl are inhibition rates respectively of 23%, 21.66%, and 23%.

The essential oil of Peppermint has a slightly inhibitory activity on *Fusarium sp* with an inhibition zone diameter of 4.6 cm, 4.7 cm and 4.6 cm respectively for doses 400 µl 700 µl and 1000 µl.

It is found that the increase in *M. piperita* oil concentration has no effect on the growth of Fusarium sp.

Abdeli et al., (2016), when investigating the chemical composition, the antimicrobial, insecticidal and antioxidant activities of the essential oil recovered from the leaves of *M. pulegium* show that this essential oil has a significant effect on the resistance of mushrooms.

The 3 µL dose has no effect on all fungi. In contrast, the 5.5 µL dose inhibits the growth of Rhizopus and Alternaria. Thus, the 10 µL dose has a significant effect on both genres (Rhizopus, Alternaria). However, for the other species no antifungal effect has been reported.

Rhizopus and Alternaria showed sensitivity to *M. piperita* essential oil. While, the other mushrooms do not show any sensitivity towards this natural substance.

T. numidicus oil is moderately active for concentrations; 400 µl 700 µl is inhibition rates; 43.33% and 50%. This natural substance is active with the 1000 µl dose, i.e. an inhibition rate of 53.33%.

Fusarium sp showed sensitivity to oil of *T. numidicus*. Thus, a growth diameter of 3.4 cm is obtained for the 400 µl dose. For concentration 700 µl, a growth diameter of 3 cm is noted. Finally, for the 1000 µl dose, there is a growth diameter of 2.8 cm.

Henceforth, it is found that more the concentration of the oil of *T. numidicus* is higher, more the inhibitory effect is marked.

The essential oil of *T. numidicus* is more active than Peppermint oil. Indeed, it has caused an inhibition of the growth of the strain from 400µl.

For this purpose, it is very important to note that the antifungal activity of *T. numidicus* oil also depends on the dose used.

Through these results, it is deduced that the oil of *T. numidicus* exerts an inhibitory activity on *Fusarium sp.* While *M. piperita* oil does not exert a remarkable effect on *Fusarium sp.*

It is also noted that the inhibition of mycelial growth in the presence of the essential oil of *T. numidicus* is greater comparing to the oil of Peppermint.

The antibacterial and antifungal activity of essential oils of Thyme has been demonstrated in several studies. We can note those of Kulevanova and Panovska (2002). Thus, these authors mention that the essential oil of *T. numidicus* has a significant antimicrobial effect. Therefore according to Rasooli and Abyaneh (2004), all species of Thyme have a very good antifungal activity.

Behidj-Benyounes (2016), who tested the oil of *T. inodorus* on a range of microorganisms showed that this oil has a significant activity on these living creatures. As a result, the results obtained during this study are close to those of the works cited above.

The difference in the antifungal power of the essential oils tested can be attributed to their chemical compositions. Indeed, the molecules that have a very wide spectrum of antimicrobial activity are the natural compounds present in the essences of most species, especially Thyme (Ahmadi et al., 2015).

Thus, the activity of the essential oil is the result of its major compounds and also of the synergistic effect of the minority compounds (Ouraïni et al., 2007).

According to these observations, it can be speculated that the strong antifungal activity observed in the essential oils of Thyme comes from the effectiveness of its components. It can be the result of synergy between the different constituents of oils (El Ajjouri et al., 2008).

Conclusion

The present work investigated the antifungal effect of the essential oil of Peppermint and Numidian Thyme on *Fusarium sp* isolated from infested leaves, roots and snares of courgettes to develop a strategy to fight Fusarium wilt.

The oils studied were recovered from the flowering tops of Peppermint and stems and fresh leaves of *T. numidicus* by the hydrodistilation method. The results of macroscopic and microscopic examination of pathogenic fungi showed the presence of two genres; *Fusarium sp* and *Alternaria sp*. It was chosen to test the activity of the *Fusarium sp* strain by the direct contact method. This revealed the antifungal power of the essential oil of *T. numidicus* and Peppermint with respect to *Fusarium sp.*

The results obtained show that the essential oil of *T. numidicus* has an inhibitory capacity on mycelial growth. Thus, the inhibition rate varies between 40 and 50%. While the oil of *M. piperita* is not very active on *Fusarium sp* with an inhibition rate ranging between 20 and 25%.

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