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Effect of essential oils from Thymus satureioides and Origanum compactum on wheat root rot induced by Fusarium culmorum and Bipolaris sorokiniana

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Durum wheat, which is a major crop in Moroccan agriculture, faces significant constraints in its production. Among these constraints, root rot is disease is one of the most important and of which control is more or less ineffective, leading to the search for alternative control methods. The aims of the present study is to investigate the effect of essential oils extracted from *Thymus satureioides* and *Origanum compactum* on wheat root rot caused by *Fusarium culmorum* and *Bipolaris sorokiniana*. The plant material used was the durum wheat variety *Ourgh*. Foliar treatments were carried out using the essential oils of *Origanum compactum* and *Thymus satureioides*. Disease assessment focused on severity in the root system and at the base of the stems, specifically at the first nodes. The application of *Origanum compactum* at a concentration of 0.31 µl/ml and *Thymus satureioides* at 1.25 µl/ml reduced disease severity and increased yield. However, *Thymus satureioides* essential oil showed a significantly higher activity in increasing yield and both essential oils resulted in a similar reduction in disease severity. Based on these results, the evaluated essential oils represent a promising alternative for controlling wheat root rot disease.

Keywords: Durum wheat, *Ourgh*, essential oils, *Thymus satureioides*, *Origanum* compactum, *Fusarium culmorum*, *Bipolaris sorokiniana*

INTRODUCTION

Durum wheat, known for its hard and vitreous grain, is an important crop in cereal production. In 2008, on average, 37% of farmland was dedicated to durum wheat cultivation, ranking first with a self-sufficiency rate of 45.1% compared to other cereal species. Considering the increasing demand for durum wheat products in both urban and rural areas, concerns about quality arise. Indeed, in recent years, the issue of quality has gained increasing importance in research (Nassif et al., 2012).

Regarding plant health, fungal diseases receive great attention due to their detrimental effects on wheat production. Examples of these diseases include rust (yellow or brown), seedling blight, powdery mildew, fusarium head blight, helminthosporium and root rot. Root rot, in particular, is a common disease of durum wheat that often affects the roots and crown, resulting in a direct decrease in the number of spikes and a subsequent yield reduction (Boulif, 2013; Duezek, 1984).

In Morocco, this disease is primarily caused by two soilborne pathogens: Bipolaris sorokiniana and Fusarium culmorum (Sturz and Bernier, 1987). Fusarium culmorum causes cortical root rot in durum wheat crown (Liddell et al., 1985), seedling blight, crown rot, and fusarium head blight (Dyer et al., 2009). This species is more dominant in arid and semi-arid regions of Morocco and reproduces asexually through conidia. Root rot can also be induced by Bipolaris sorokiniana, which infects the leaves and roots, causing seedling blight (Wagacha and Muthomé, 2006; Matusinsky et al., 2010). The latter is abundant in drier brown soil areas (Fernandez and Jefferson, 2004).

Several control methods can be adopted to manage diseases caused by these two pathogens, particularly root rot. These methods include crop rotation (Conner et al., 1996), biological control (Cook, 1992), conservation practices (Conner et al., 1987) and chemical control (Singh et al., 2002). Chemical control is the most commonly used method, but it has negative effects on the environment, and its intensive use can lead to increased resistance of pathogens due to poor control (Chang et al., 2008).

One alternative to chemical control is the use of natural plant products, which are simple to apply and non-toxic to humans, animals and treated plants. They also exhibit fungitoxic activity (Naeini et al., 2010) and have a long tradition of use in protecting stored products (Koul et al., 2008). One such natural product is essential oils (EOs), which are complex volatile compounds synthesized by various parts of the plant and have great potential in combating root rot-causing agents (Akhtar et al., 2013).

In this context, the present study aims to investigate the effect of essential oils, specifically Thymus satureioides and Origanum compactum, on root rot caused by F. culmorum and B. sorokiniana.

MATERIAL AND METHODS

Plant material

The plant material used is the durum wheat variety “Ourgh”, which was registered in the official catalog of Morocco in 1995. This variety is sensitive to pathogen attacks but responds well to fungicide treatment.

Inoculum preparation

For the preparation of the inoculum of F. culmorum and B. sorokiniana, under sterile conditions and using a brush, the developed colonies of the fungus were crushed with sterile distilled water. After filtration, they were transferred into a petri dish and incubated for 20 days with a photoperiod of 12 hours. The mycelium clusters were added to barley grains that were previously sterilized in Erlenmeyer flasks.

After incubating the barley seeds for two months under the previously described conditions, they were air-dried in the greenhouse at room temperature for 15 days. Subsequently, the grains were ground to obtain a fine powder of inoculum, referred to as inoculated organic matter (IOM), for future use. The inoculum was stored at a temperature of 5°C.

Soil preparation

The soil used is natural soil placed in plastic pots. Each pot is filled with 1.3 kg of soil. Initially, the soil is filled up to halfway in the pot. Then, 10 grains were placed in each pot and covered with the same soil until it reached three-fourths of the pot’s height. Next, the surface of each pot’s soil was filled with 0.4 g of the inoculated organic matter (IOM) containing F. culmorum or B. sorokiniana, depending on the experiment. Finally, the pot was topped with an additional 1 cm of the same soil. (Figure 1).

The first irrigation was carried out using a 100 ml of water to initiate germination. Subsequently, irrigation and fertilization were performed as per the plant's requirements.

Experimental protocol

The experimental design used is a four-replicate trial, consisting of three blocks, with one treated by Origanum, the second by Thymus satureioides, and the third as a control treated only with water. Each block comprises three rows, one treated with F. culmorum, the second with B. sorokiniana, and the third without inoculation (control). This trial was repeated twice. The experiment was conducted in a glass greenhouse. Figure 2 describes the experimental protocol used.

Essential oil concentrations

According to the in vitro previous study conducted by Zahraoui et al., (2017), it has been found that Origanum compactum essential oil inhibits the pathogens F. culmorum and B. sorokiniana at a concentration of 0.31 µl/ml and Thymus satureioides inhibits the same pathogens but at a concentration of 1.25 µl/ml. In order to achieve an effect of the essential oils on the pathogens, it is necessary to treat the plants with a sufficient quantity that covers the entire leaf surface. This quantity is estimated to be 5 ml/pot of the treatment containing the concentration of each essential oil.

We prepared 60 ml of the treatment to treat each block, but the 3rd block (control) is treated only with water. The foliar treatments were carried out twice by spraying plants using a manual sprayer. The first treatment is done at the heading-flowering stage, and the second treatment is done 15 days after the first treatment.

Severity assessment

Since the roots are located in the underground part, it is obvious that evaluating the severity of the disease requires rinsing the root system in water and then washing it so that the observation of symptoms indicating the attack of seminal, collar, and sub-collar roots can be clearly seen. The evaluation started around the flowering stage of the plants.

After washing the root system of both trials, specifically the first two repetitions, symptoms caused by the two pathogens can be distinguished. We start with those that are attached to the roots, starting from the sub-collar and reaching the collar. Then, we assess the severity level based on Table 1.

After observing and evaluating the severity on each plant in each pot, they are classified according to the severity class. The severity index is obtained using the following formula:

Xi: Number of class i, Ni: Number of plants in class i, i: Ranges from 0 to 5.

Severity on the nodes

The evaluation of severity on the nodes is based on the disease development on the number of infected nodes in each plant, specifically in the first two repetitions. Starting from ground level, the disease is assessed on the first three nodes of each plant. If the disease has reached the first node, it is assigned a value of 1. If it reaches the second node, the value is 2. And if it surpasses the third node, the value is 3. Intermediate values can be assigned to severity for intermediate levels of node infection.

Yield

The samples that were washed and evaluated are air-dried in a greenhouse for one week, and then they are weighed to determine their dry weights. The ears from all the pots in the trial were harvested, and using an ear thresher, the ears from each pot were threshed to remove the grains. The obtained grains were subsequently weighed to obtain the grain yield (g). To calculate the number of seeds per ear, the number of seeds per pot was counted and then divided by the number of ears in the same pot.

Statistical analysis

The results were compared using an analysis of variance (ANOVA), followed by a comparison of means using the Duncan test at a 5% probability level, using SPSS 22 software.

RESULTS

The analysis of variance showed that the treatments have a highly significant difference ($p<0.0001$) on the severity index caused by F. culmorum and B. sorokiniana, while these pathogens themselves have no significant effect ($p=0.955$). It was also noted that there was no significant interaction between the treatments and these pathogens ($p=0.230$) (Table 2). The treatments with Thymus satureioides and Origanum compactum have reduced the mean severity index by 41% (Table 3). This reduction is evident in Figure 3, which shows that both essential oils act in a similar manner on the mean severity index compared to the untreated.

Regarding the severity on the nodes, there is a highly significant effect of the treatments ($p<0.0001$). Additionally, there is a significant difference between the trials ($p=0.006$). Similarly, there is no difference between the pathogens ($p=0.496$) and no interaction ($p=0.468$) between these pathogens and the treatments (Table 4). Furthermore, Figure 4 shows that the mean severity undergoes a reduction during the treatments with Thymus satureioides and Origanum compactum, which act in a similar manner on node severity compared to the untreated. This reduction is estimated to be 54% compared to the control (Table 3).

For grain yield, there is a significant effect of the treatments ($p=0.016$) and the absence of any interactions (Table 5). Interestingly, the treatment with Origanum compactum significantly decreased the mean grain yield compared to the control in both non-inoculated samples and those inoculated with B. sorokiniana. However, it increased the grain yield in plants inoculated with F. culmorum. On the other hand, the treatment with Thymus satureioides resulted in an increased grain yield in all inoculation cases (Figure 5).

The essential oils of Thymus satureioides and Origanum compactum increased the mean grain yield by 30% compared to the untreated control (Table 3).

Subsequently, there was a non-significant effect of the pathogens ($p=0.463$) and treatments ($p=0.073$) on the number of seeds per ear. However, there is an interaction between the treatments and pathogens ($p=0.021$) (Table 6). The essential oils had an effect on the number of seeds per ear similar to their effect on the mean grain yield (Figure 6). Specifically, Origanum compactum has decreased the mean number of seeds per ear by 22% compared to the untreated control (Table 3).

On the contrary, pathogens ($p=0.641$) and treatments ($p=0.191$) have no significant effect on the thousand grain weight (Table 7).

DISCUSSION

The study on the fungicidal effect of Origanum compactum and Thymus satureioides essential oils on F. culmorum and B. sorokiniana demonstrates that they have reduced the severity index, but with different intensities. Origanum compactum caused a 43% reduction compared to the control,

while Thymus satureioides only reduced it by 39%. Similarly, both essential oils reduced the severity on the nodes respectively by 59% and 49%, compared to the control. As there is an interaction between treatments and pathogens for the number of grains per ear, the treatment's action differs depending on the targeted pathogen. No difference was found between the essential oil treatments in the case of inoculation by F. culmorum and B. sorokiniana. However, for the non-inoculated control, Origanum compactum reduced the number of grains per ear by 48%, compared to 58% by Thymus satureioides. The effect of Origanum compactum and the untreated control was the same on grain yield, where Origanum compactum decreased it by 34% compared to Thymus satureioides, while it decreased by 27% compared to the untreated control.

Other studies on essential oils have shown antimicrobial and antioxidant activities (Ipek et al., 2005) as well as activity against plant pathogens (Koul et al., 2008). Various species of Thymus satureioides have been found to possess antifungal properties (Pina-Vaz et al., 2004).

Our present study demonstrated that both Origanum compactum and Thymus satureioides essential oils have an identical fungicidal effect, considering that Thymus satureioides and Origanum compactum essential oils have similar antimicrobial activity (Emiroglu et al., 2010). Similarly, both essential oils reduced the severity of the disease in a similar manner.

The major components of these essential oils could act directly on the mycelial growth of these two pathogens. It should be noted that T. sateroiedes species produces an essential oil rich in borneol, which has antimicrobial activity (Lattaoui et al., 1993).

Thymus satureioides essential oil increased the yield more than Origanum compactum, indicating that the concentration of Thymus satureioides is higher than that of Origanum compactum, which makes a difference. It is also likely that Thymus satureioides does not reach the roots completely and remains on the leaves, thus stopping the invasion of pathogens on the reproductive part of the plant and protecting the ears. Consequently, the pathogens are limited at the collar and roots level. However, Origanum compactum may have migrated from the leaves to the roots and halted the growth of these pathogens.

Thymus satureioides essential oil has been found to be the most effective in reducing root rot caused by Rhizoctonia solani and increasing plant survival rates (Mahmoud et al., 2013). T. satureoiedes essential oil has shown strong fungitoxic efficacy (Ouraini et al., 2007). Similarly, according to Bouhdid et al. (2008), O. compactum produces an essential oil with a better antifungal effect, which supports our results. However, the action of Origanum compactum essential oil showed a fungicidal activity that depends on the targeted pathogens, such as F. culmorum and B. sorokiniana.

CONCLUSION

The application of Origanum compactum essential oil at a concentration of 0.31 µl/ml and Thymus satureioides essential oil at a concentration of 1.25 µl/ml on the durum wheat variety Ourgh inhibits the development and vegetative growth of F. culmorum and B. sorokiniana.

Regarding disease severity, the evaluation of Thymus satureioides essential oil's effect showed similar efficacy to Origanum compactum essential oil, but Thymus satureioides significantly increased yield compared to Origanum compactum.

Since the durum wheat variety Ourgh is susceptible to pathogen attack, it allows us to reject the hypothesis of plant resistance. However, this plant may be capable of absorbing the essential oils and transferring them to the roots to inhibit the pathogenic fungi, or it may acquire tolerance when treated.

Based on this study, it is recommended to use these essential oils as fungicides instead of chemical products, which have harmful effects on health and the environment. This recommendation is specifically for large-scale application. In the near future, it is suggested that this experiment be reproduced in order to further confirm our conclusions.

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