

Alternative control of helminthosporium leaf spot on wheat using essential oils of *Origanum compactum* and *Thymus satureioides*

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The production of cereals in Morocco has experienced fluctuations due to various factors, including cryptogamic diseases. The control of these diseases still relies heavily on chemical products that harm the environment. The objective of this study, was to test an alternative method that improves yield and is environmentally friendly in controlling helminthosporium leaf spot disease caused by *Bipolaris sorokiniana*, using essential oils extracted from medicinal plants. The effect of two essential oils, *Origanum compactum* and *Thymus satureioides*, was tested against helminthosporium leaf spot on four varieties of durum wheat (*Triticum durum*) and common wheat (*Triticum aestivum*). These two essential oils were applied to the aerial part at a concentration of 0.31 µl/ml for *Origanum compactum* essential oil and 1.25 µl/ml for *Thymus satureioides* essential oil. The different parameters evaluated included the types of infection, the greenness rate on the top two leaves at the flowering stage, grain weight, the number of grains per spike, and the weight of a thousand grains. The results showed that both essential oils significantly reduced disease severity by 48% and increased grain yield by an average of 25% across all varieties used. *Thymus satureioides* was found to be more effective than *Origanum compactum*, reducing the infection rate by 52% and improving productivity by 50%. These results indicate that the control of this disease can be achieved without the use of synthetic chemical products.

Keywords: Helminthosporium leaf spot, Wheat, *Bipolaris sorokiniana*, Alternative control, Essential oils, *Origanum compactum*, *Thymus satureioides*

INTRODUCTION

Cereals play a predominant role in Moroccan agriculture. For this reason, Morocco has adopted projects and programs through the Green Morocco Plan to improve its production. Cereals and their derivatives have an undeniable nutritional, social, and economic significance, serving as the staple food of the population, providing approximately two-thirds of the energy needs, with 67% of the intake being carbohydrates and 75% being protein intake in an average diet. Cereals, primarily including soft wheat, barley, durum wheat, and maize, account for 25% of food expenses. Sorghum and rice are also cultivated but to a lesser extent (Ait El Mekki, 2006).

Despite the importance and predominance of cereal cultivation, Morocco has yet to achieve self-

sufficiency in this regard, as national production and yields remain significantly below the country's actual potential. Consequently, imports annually cover nearly 40% of the country's total cereal needs. The low production and yields of cereals are primarily attributed to environmental factors, techniques used, and biotic factors such as cryptogamic diseases, pests, and weeds (El Yousfi, 1984; Zidane et al., 2010; El Fatihi et al., 2011).

As a result of these various constraints, the annual production of cereals, in general, and wheat specifically, has seen a remarkable decline, contributing to the economic and social deficit of the country. Therefore, it is essential to reconsider the production strategy by employing a rational approach to the methods used, such as methods of diseases and pests control.

Control methods include the management of agricultural lands, such as the selection of genetically resistant varieties and healthy seeds, as well as crop rotation, among other practices.

Chemical control is also an option using chemicals like insecticides, fungicides, herbicides, and nematicides. However, their intensive and unregulated use poses significant problems for human health and the environment. Therefore, biological control offers a solution to the challenges associated with chemical control.

Biological control is defined by the use of antagonistic organisms to control pathogenic microorganisms. This approach also involves the use of natural substances extracted from medicinal plants, such as essential oils.

Hmiri et al. (2011) and Zahraoui et al. (2017, 2023) demonstrated in their studies that essential oils extracted from aromatic plants, *Mentha pulegium*, *Eucalyptus camaldulensis*, *Origanum compactum* and *Thymus satureioides* have antifungal activity against fungi responsible cryptogamic diseases.

The aim of this study is to investigate the control of the cryptogamic disease "helminthosporium leaf spot," a foliar disease caused by the pathogenic fungus *Bipolaris sorokiniana*, on different varieties of durum and soft wheat, using a biological control method involving essential oils extracted from *Origanum compactum* and *Thymus satureioides*.

MATERIAL AND METHODS

Plant Material

The plant material used consisted of four varieties, including two varieties of durum wheat: Carioca and Ourgh, and two varieties of soft wheat: Marchouch and Wafia. All four varieties are generally resistant to septoria leaf blotch and brown rust. The thousand grain weight for Ourgh is estimated to be between 38 to 41 grams, for Carioca it is between 45 to 50 grams, it is average for Marchouch, and ranges from 41 to 44 grams for Wafia.

Experimental Setup

The experiment comprised 96 pots, all of the size 17x18.5x13 cm, filled with 3 kg of natural soil collected from the Sidi El Aidi experimental station located 13 km from Settat. Planting was carried out on November, with 10 seeds of each variety per pot. The experiment was set up using a "split-plot" experimental design with three main blocks. Each block represented a treatment and was further divided into four replicates for the four varieties. The experiment was repeated twice.

Irrigation and fertilizers were applied based on the crop's requirements. Typically, ammonium nitrate, potassium sulfate, and triple superphosphate were the fertilizers used in the experiment. These inputs were provided as needed to support the growth and development of the crops in the

study.

Inoculum preparation

After 15 days in the incubator, the culture media containing *Bipolaris sorokiniana* are scraped with a brush after adding a quantity of water mixed with a detergent "Twin 20" (one drop per 100 ml). Then, the solution is filtered to remove the mycelium, leaving only the spores. The concentration of the inoculum is determined by counting the spores under a binocular microscope. On a slide, 10 drops of 5 μ l each are placed using a micropipette, and the spores in each drop are counted. The counting and dilution process is repeated several times until a concentration of 30×10^3 spores/ml is achieved.

The treatment with essential oils (EO) of *Origanum compactum* and *Thymus satureioides* was performed during the flowering stage. Two dilutions were prepared, 0.31 μ l/ml for *Origanum compactum* EO and 1.25 μ l/ml for *Thymus satureioides* EO. These values were determined based on previous in vitro studies Zahraoui et al. (2017). Using a manual sprayer, only the two blocks were sprayed with these essential oils at a volume of 10 ml per pot. The third block was left untreated.

Inoculation

After 48 hours of treatment, the plants in the experiment were inoculated with a volume of 10 ml per pot of the *Bipolaris sorokiniana* inoculum using a manual sprayer. The pots were then covered with plastic bags for 5 days to maintain a high relative humidity, promoting spore germination and infection.

Evaluations

Fifteen days after inoculation, various assessments were conducted on the aboveground parts of the plants, including measuring the stem, spike, and awns using a ruler. The types of infection were determined on a scale of 1 to 9, and the level of greenness in the first and second leaves, as well as the percentage of disease attack on each spike and for the entire pot, were recorded. These evaluations were performed on 5 randomly selected plants from each pot.

When the trial plants reached maturity, the spikes from each pot were harvested, and their awns were removed. They were placed in labeled plastic bags, noting the trial, treatment type, repetition, and the number of spikes.

After the wheat plants reached maturity, an electric thresher was used to perform threshing to determine the number of grains and estimate the grain yield.

RESULTS

The analysis of variance for the data on foliar infection types caused by *Bipolaris sorokiniana* demonstrated a highly significant effect of the variety, the essential oil used, and their interaction. Regarding this leaf, a similar result to that of the flag leaf was obtained after analyzing the data on infection types induced by *Bipolaris sorokiniana* (Table 1 and 2).

Table 3 presents the average infection types revealed by each of the four varieties in the case of each of the two treatments with essential oils of *Origanum compactum* and *Thymus satureioides*, in addition to the control. It was observed that the infection types vary, for the same essential oil treatment, depending on the wheat varieties used on one hand, and depending on the essential oil for the same wheat variety on the other hand. The infection types were also lower when treated with both essential oils compared to the control. It is noticeable that treatment with *Thymus satureioides* resulted in the most significant reduction in infection types compared to *Origanum*

compactum. The Ourgh variety is the most sensitive to infection, as indicated by the high infection types it exhibited in the control, unlike Wafia.

The analysis of variance for the percentages of greenness at the flag leaf level indicated a highly significant effect of the variety, essential oils, and their interaction (Table 4). The percentage of greenness on the flag leaf differs significantly based on the variety and the essential oil used.

The average percentage of greenness at the flag leaf level for each of the four wheat varieties in the case of the control and the two tested essential oils on table 3, highlighting the significant green surface in the case of preventive treatment with *Thymus satureioides* across all varieties compared to *Origanum compactum*. The green area in the case of treatment with *Thymus satureioides* also remains larger compared to the control.

The same trend of results was obtained for the percentages of greenness at the flag leaf -1 level, as presented in table 5, except that the interaction between the variety and treatment was not significant ($p = 0.145$). This indicates that the green surface of this leaf is generally similar among the wheat varieties studied for each of the treatments with essential oils.

The presentation of the variation in the average percentage of greenness of the flag leaf-1 for each variety and each treatment (Table 6) reveals that, for 3 out of 4 varieties, this percentage is close to the same value for each treatment. It is approximately 40% in the case of *Thymus satureioides* for Ourgh, Carioca, and Marchouch, and around 20% for Ourgh, Marchouch, and Wafia in the case of treatment with *Origanum compactum*.

It turns out that the greenness at the level of this leaf, as indicated by the wheat varieties, is more significant when treated with *Thymus satureioides*, while with *Origanum compactum*, the greenness was more reduced. In any case, these percentages of greenness remain higher compared to the control for all the varieties studied.

The significant probability values regarding the analysis of variance for spike infection data indicate a significant effect of the variety, essential oils, and their interaction on this component (Table 7). The effect of treatments with essential oils on the ear scald rate varies among the varieties. Table 6 shows that *Thymus satureioides* and *Origanum compactum* led to a significant reduction in ear scald compared to the control. It is noteworthy that this reduction was similar for both treatments at 10%.

The analysis of variance for the percentage of attack on the entire plants per pot by *Bipolaris sorokiniana* indicated that the effect of treatment with essential oils, as well as the interaction between treatment and variety, were highly significant (Table 8). However, the varietal effect was not found to be significant ($p = 0.637$).

The averages of attack rates per pot based on varieties and the type of treatment showed that the varieties recorded similar attack percentages, estimated at 36%. On average, both *Origanum compactum* and *Thymus satureioides* treatments reduced the severity of the disease by 47% compared to the control. Specifically, *Thymus satureioides* reduced the attack by 52%, while *Origanum compactum* reduced it by 44%. Table 6 clearly illustrates this reduction in severity after the use of these essential oils.

According to table 3, the Marchouch variety was the most sensitive to infection by *Bipolaris sorokiniana*, both at the level of the two leaves L1 and L2 and on the ear. The green surface of the upper leaves of this variety was one of the most significant (50% and 20% respectively for L1 and L2). The infection values were 3 for both L1 and L2 leaves, and 24% for the ears. The variety showing the lowest infection was Wafia, with the lowest green surface as well.

Regardless of the wheat variety, Table 6 highlights that infection by *Bipolaris sorokiniana* in the

case of treatment with *Thymus satureioides* essential oil is the lowest compared to treatment with *Origanum compactum* essential oil. Indeed, *Thymus satureioides* reduced the infection rate on both upper leaves L1 and L2 by 28% and 35%, respectively. The green surface of these leaves is also the largest in the case of this treatment, being 29% larger for L1 and 72% larger for L2. The effect of treatment with both essential oils on the percentage of ear attack was similar; both treatments reduced this percentage by 59% compared to the control.

On the other hand, treatment with *Thymus satureioides* essential oil reduced the infection type by 49% for L1 and L2. Meanwhile, the greenness increased by 64% for L1 and 78% for L2 compared to the control.

The result of the analysis of variance for grain yield presented in table 9 indicates that there was no significant difference between the results of the two experimental trials ($p = 0.602$). The effect of treatments with essential oils on yield was also similar in both trials ($p = 0.216$). However, there is a highly significant varietal effect on grain yield ($p = 0.0001$). The effect of treatments with both essential oils also had a significant impact ($p = 0.037$).

The same trend in the results of the analysis of variance was obtained for the number of grains per ear (Table 10) and the thousand grain weight (Table 11).

According to table 12, grain yield increased in plants treated with *Thymus satureioides* compared to the control. The marginal means of grain weight for each variety (Table 13) show the highest yield in the Marchouch variety, compared to the other varieties

The number of grains per ear also increased with treatment using *Thymus satureioides* essential oil (15 grains) compared to approximately 12 grains for *Origanum compactum* and the control. The number of grains also varies according to the varieties. The Marchouch variety has the highest number of grains, at around 19 grains.

The mean values for grain yield, number of grains per ear, and thousand grain weight presented in table 12 show that they differ depending on the essential oil used. Treatment with *Thymus satureioides* resulted in the highest averages compared to the control, increasing grain weight by 50%, the number of grains per ear by 30%, and the thousand grain weight by 16%. The averages in the case of *Origanum compactum* were similar to those of the control.

Regarding the thousand grain weight, *Origanum compactum* led to the lowest weight, while treatment with *Thymus satureioides* produced the highest thousand grain weight.

The analysis of mean values for yield and its components by variety (Table 13) shows that they differ depending on the plant material. They were similar for the three varieties Wafia, Ourgh, and Carioca, while Marchouch had the highest mean values for yield components.

DISCUSSION

The alternative control of foliar disease caused by *Bipolaris sorokiniana* using essential oils of *Origanum compactum* and *Thymus satureioides* has shown promising results, which are supported by the findings of this study. Treatment with essential oils of *Origanum compactum* and *Thymus satureioides* led to a reduction in disease severity. According to the results, they reduced the average disease severity by a rate of 48%. Specifically, *Thymus satureioides* reduced the infection by 52%, while *Origanum compactum* reduced it by 44%. The reduction in disease severity varied depending on the tested wheat varieties, with Carioca, Ourgh, and Marchouch being found to be sensitive. Marchouch, in particular, was the most vulnerable, but it responded strongly to both essential oil treatments. Furthermore, the two treatments improved yield to varying degrees. *Thymus satureioides* increased grain weight by 50%, the number of grains per spike by 30%, and

the weight of a thousand grains by 16%.

These observations are consistent with other studies that have used essential oils extracted from various plants, such as *Origanum compactum* and *Thymus satureioides*, and demonstrated their significant antifungal activity against a wide range of fungal pathogens (Kivanc, 1991). According to Ultee and Smid (2001), essential oils from *Origanum compactum* and *Thymus satureioides* are among the best inhibitors of pathogenic fungi due to the presence of phenolic compounds like carvacrol and *Thymus satureioides*, which are the major constituents that can disrupt the fungal cell membrane.

The effect of these two essential oils is attributed to their chemical profiles, which are rich in different bioactive compounds. The essential oil used in this study is derived from *Thymus satureioides*, primarily dominated by Borneol (29.4%), while Carvacrol (36.5%), followed by *Thymus satureioides* (29.7%) and p-cymene (24.3%), are the major compounds in *Origanum compactum* essential oil (El Babili et al., 2011). The results obtained by El Ajjouri et al. (2008), along with other research, have demonstrated that *Thymus satureioides* and carvacrol are among the most active compounds against fungi. Bornyl acetate, found in Borneol, has shown antifungal activity against *Candida albicans* and *Aspergillus Niger* in vitro (Tae-Ju Park et al., 2003).

Furthermore, Karmen et al. in 2003 tested 22 pure compounds from essential oils against *Coriolus versicolor* and *Coniophora puteana* and found that *Thymus satureioides* and carvacrol were the most active against these two wood-decaying fungi. Many studies have emphasized the antifungal efficacy of terpene phenols, especially *Thymus satureioides* and/or carvacrol (Crespo et al., 1990; Cosentino, 1999). The mechanism of phenols' toxicity to fungi is based on the inactivation of fungal enzymes containing the Hydrogen Sulfide group in their active site (Farak et al., 1989; Celimene et al., 1999; Cowan, 1999). Carvacrol can destabilize the cytoplasmic membrane and act as a proton exchanger, reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually leads to cell death (Ultee et al., 2002). Moreover, it has been reported that essential oils rich in phenolic compounds exhibit high levels of antimicrobial activity.

The major chemical compounds in these oils have demonstrated action against fungi. It is also possible that the action of the main components is modulated by other minor molecules (Hoet et al., 2006). This synergy between major and minor compounds has also been shown in studies conducted by Bouhdid et al. (2008), indicating that the compounds present in smaller proportions are not necessarily responsible for the overall activity; the involvement of less abundant compounds should also be considered (Cimanga et al., 2002). According to these studies, a synergistic effect was observed between carvacrol and p-cymene at low concentrations. P-cymene likely acts in synergy with carvacrol, making it more easily transported into the cell (Ultee et al., 2002).

CONCLUSION

According to the results of this study, the effect of the two essential oils, *Origanum compactum* and *Thymus satureioides*, on foliar disease caused by *Bipolaris sorokiniana* is expressed through the reduction in disease severity and an increase in yield, generally across the four varieties used. Similarly, treatment with *Thymus satureioides* exhibited strong antifungal activity compared to *Origanum compactum*.

Since these two essential oils have the potential to reduce infection rates caused by this pathogen, they deserve further studies to explore their antifungal properties and evaluate the long-term effectiveness of the treatment.

Furthermore, field studies under natural conditions would be necessary to thoroughly examine the effect of these essential oils and understand the reaction of this pathogenic fungus to the

successive use of these oils. Testing these essential oils on plant material consisting of other wheat varieties, as well as against other pathogens, would be a significant asset.

It is also recommended to study the action of the blend of the two essential oils to assess the possible existence of synergism or antagonism between the chemical compounds of these oils and, consequently, their effect on the disease.

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