ID: 392

In Vitro Comparative Activities of Four Essential Oils on Mycelial Radial Growth of *Magnaporthe Oryzae* B.C. Couch, a Rice Blast Pathogen in Burkina Faso

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Abstract

Caused by *Magnaporthe oryzae* B.C. Couch, rice blast is considered as the main fungal disease in rice fields in Burkina Faso. Pure essential oils of *Cymbopogon schoenanthus, Ocimum americanum, Ocimum gratissimum* and *Lippia multiflora* were tested in vitro to assess the radial growth and inhibition rate of the fungus using the contact and fumigation methods. For each essential oil, five doses were developed: D2 (0.1 µl/ml), D4 (0.6 µl/ml), D6 (1.2 µl/ml), D8 (1.8 µl/ml) and D10 (2.4 µl/ml). In both tests, two synthetic fungicides namely mancozeb and azoxystrobin at recommended doses of 6.67 µl/ml and 3.33 µl/ml respectively, and an absolute control were used. The results showed that in the contact method, pure essential oils of *O. americanum, L. multiflora, C. schoenanthus* and *O. gratissimum* inhibited 100% of the mycelial radial growth of the fungus at doses D10 (2.4 µl/ml), D4 (0.6 µl/ml), D6 (1.2 µl/ml) and D4 (0.6 µl/ml) respectively. As for the fumigation method, oils of *O. americanum, L. multiflora, C. schoenanthus* and *O. gratissimum* inhibited mycelial radial growth of the fungus by 100% at doses D10 (2.4 µl/ml), D2 (0.3 µl/ml), D6 (1.2 µl/ml) and D8 (1.8 µl/ml) respectively. Mancozeb and azoxystrobin inhibited radial mycelial growth by 100% and 74.1% respectively. These essential oils can be used to control rice blast in the field. This will also reduce environmental pollution.

Key words : *Essential oils, Efficacy, Oriza sativa, Magnaporthe oryzae, Burkina Faso*

Introduction

Rice blast, caused by the fungus *Magnaporthe oryzae*, remains the most damaging fungal constraint to rice production, with damage that can cause yield losses of up to 100% if no protective measures are taken (Zhan *et al.,* 2018 ; Li *et al.,* 2019 ; Savary *et al.,* 2019 ; Kassankogno *et al.,* 2021). The use of chemicals such as tricyclazole to control M. oryzae has been successful in increasing yields (Moinina *et al.,* 2018). However, their application has numerous consequences for human and animal health and the environment. It is therefore necessary to use alternative control methods such as biological control through the use of biopesticides (Yarou *et al.,* 2017 ; Gamsore *et al.,* 2018 ; Toundou *et al.,* 2020). Studies have shown that essential oils possess insecticidal, bactericidal or fungicidal properties (Amarti *et al.,* 2010 ; Hamdani *et al.,* 2015). Also, other authors have shown that Lippia multiflora essential oil has strong antifungal activity, totally inhibiting the mycelial growth of Fusarum moniliforme and *Bipolaris oryzae* at 100 ppm and 400 ppm respectively (Tiendrebeogo *et al.,* 2017). However, the use of essential oils from aromatic plants for the control of M. oryzae remains largely unexplored. With this in mind, the present study was initiated to evaluate the *in vitro* efficacy of *Cymbopogon schoenanthus*, *Ocimum americanum*, *Lippia multiflora* and *Ocimum gratissimum* essential oils for the control of *M. oryzae*. Specifically, the aim is to evaluate the *in vitro* comparative activities of four essential oils on mycelial radial growth of *Magnaporthe oryzae* B.C. Couch, a rice blast pathogen in burkina faso.

Materials and methods

Fungal material

The fungal material used was *Magnaporthe oryzae* strain BF075 from rice leaves collected at the Farakô-Bâ site and isolated at INERA Farakô-Ba's phytopathology laboratory. The choice of this strain is justified by its virulence level (Kassankogno, 2016).

Essential oils

The essential oils of the *C. schoenanthus, O. americanum, L. multiflora* and *Ocimum gratissimum* aromatic plants used for the tests were extracted from the leafy twigs and used in pure without any combination.

Effects of essential oils on mycelial growth of M. oryzae on PDA medium

The essential oils were tested using two methods: the contact or “poisoned food” method (Adam *et al.,* 1998), which involved mixing the essential oils directly into the Potato Dextrose Agar (PDA) culture medium, and the fumigation or micro-atmosphere method, where the essential oils were poured and then spread on the underside of the Petri dish lid (Tonzibo *et al*., 2013). For each type of essential oil, five (05) doses (D) were prepared in the following proportions : D2 (0.1 µl/ml), D4 (0.6 µl/ml), D6 (1.2 µl/ml), D8 (1.8 µl/ml) and D10 (2.4 µl/ml). In both tests, two synthetic fungicides, mancozeb and azoxystrobin, at recommended doses of 6.67 µl/ml and 3.33 µl/ml respectively, and an absolute control were used. Petri dishes were then placed in an incubation chamber. For each method, the thallus diameters (in mm) of colonies in each Petri dish were measured for 14 days after incubation (JAI) at two-day intervals (Sirima *et al.,* 2020 ; Kassankogno, 2016).

- Assessment of mycelial growth : from seven (07) days of pure culture of *M. oryzae*, one (01) explant measuring six (06) mm was removed and placed in the center of the Petri dish containing the culture medium. Mycelial radial growth was assessed by averaging the two (02) perpendicular diameters passing through the middle of the explant (Sirima *et al*., 2020). Three replicates were performed for each concentration.

- Minimum inhibition doses : the inhibition rate (IR) was calculated according to the following formula: $\boldsymbol{TI}_{(\%)} = \left(\frac{T_0 - T}{T_0}\right) \times 100$.. With T0 = mean value recorded in control medium, T = mean value recorded in culture medium containing essential oil or fungicide (Sirima et al., 2020) From these inhibition rates, minimum inhibition doses were determined.

Results

Effects of essential oils on M. oryzae growth on PDA medium

A variation in the radial mycelial growth of *M. oryzae* as a function of the oils and the method used is shown in Figures 1 and 2. Analysis of variance showed a highly significant difference between the radial mycelial diameters of the different essential oils compared with the controls. In the contact method, pure essential oils of *O. americanum, L. multiflora, C. schoenanthus* and *O. gratissimum* inhibited mycelial radial growth of the fungus by 100% at doses D10 (2.4 µl/ml), D4 (0.6 µl/ml), D6 (1.2 µl/ml) and D4 (0.6 µl/ml) respectively. As for the fumigation method, oils of *O. americanum, L. multiflora, C. schoenanthus and O. gratissimum* inhibited 100% of the mycelial radial growth of the fungus respectively from doses greater than or equal to D10 (2.4 µl/ml), D2 (0.3 µl/ml), D6 (1.2 µl/ml) and D8 (1.8 µl/ml). Fungicides based on mancozeb and azoxystrobin inhibited radial mycelial growth by 100% and 25.9% respectively.

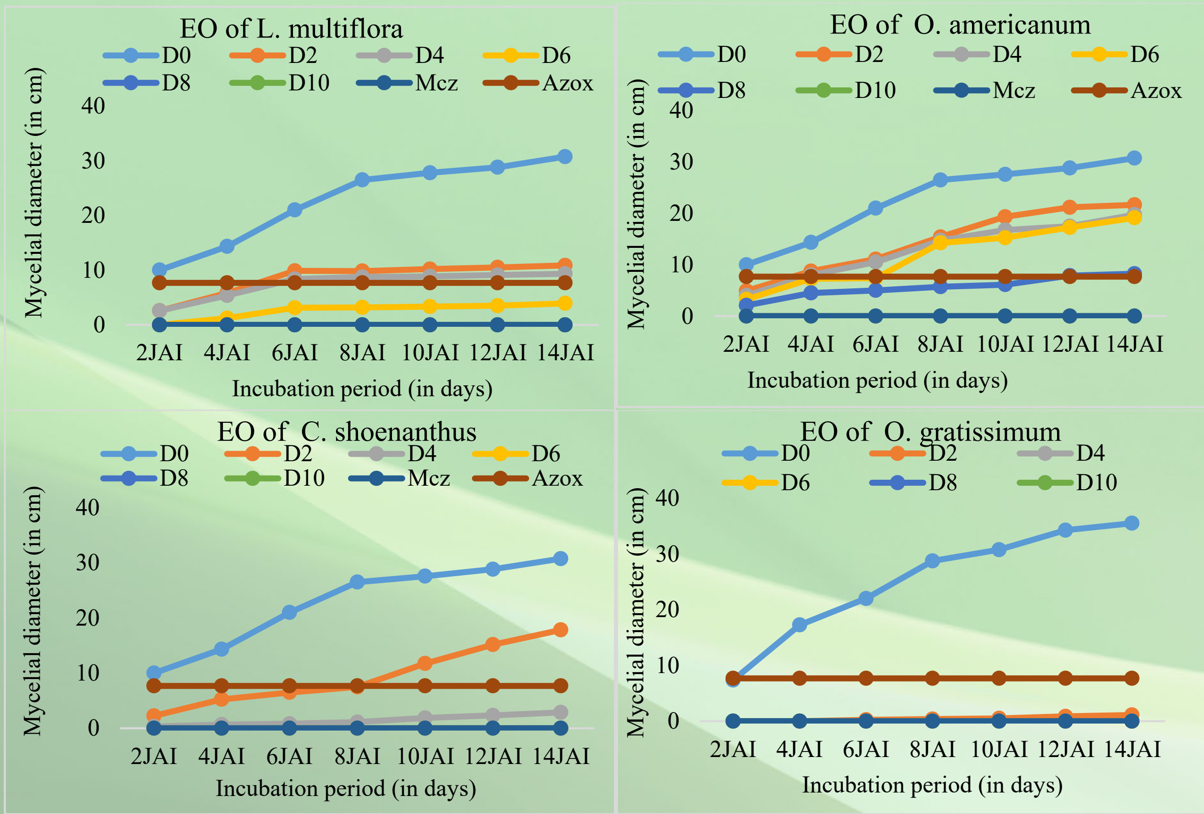

Figure 1 : Evolution of *M. oryzae* mycelial diameter according to incubation time using the contact method.
Legend: JAI = Day after incubation, EO = Essential oil, *Oa = Ocimum americanum, Lm = Lippia multiflora, Cs = Cymbopogon shoenanthus, Og = Ocimum gratissimum.*

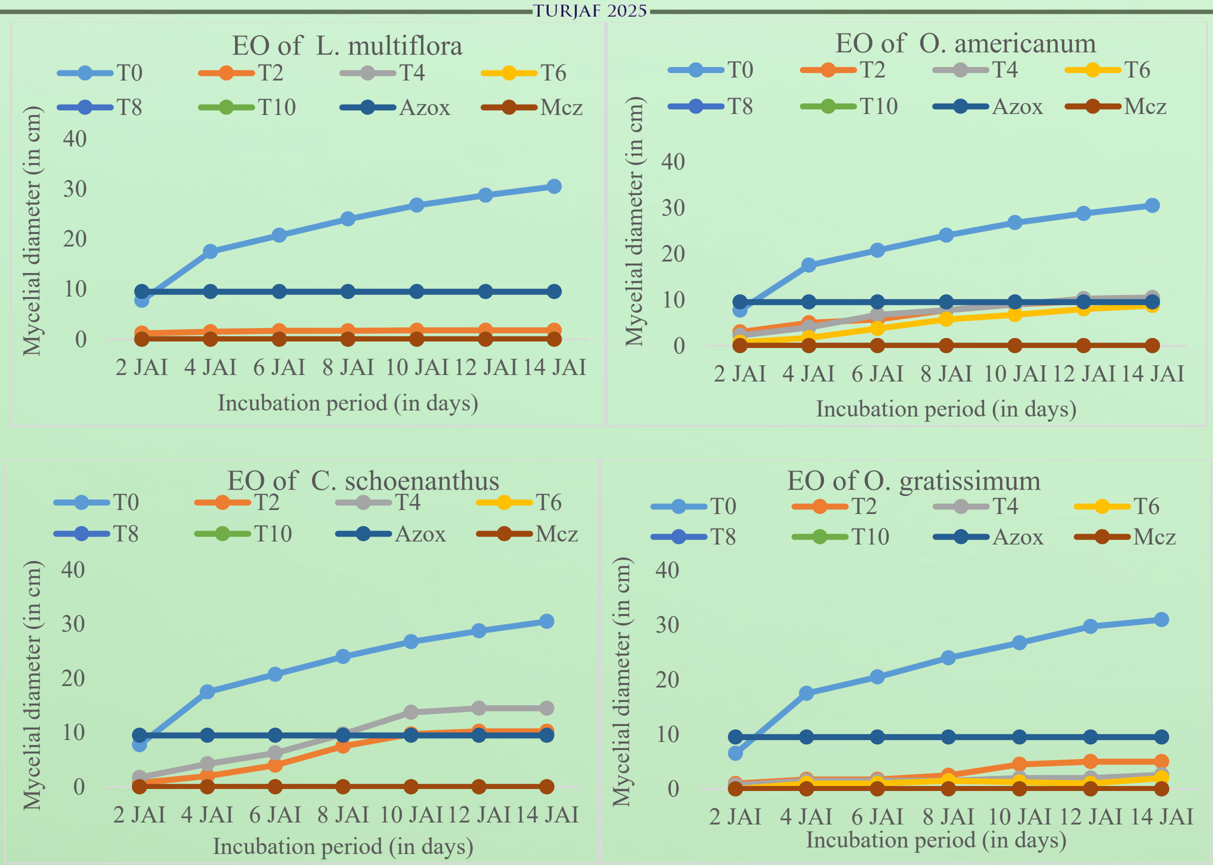

Figure 2 : Evolution of *M. oryzae* mycelial diameter according to incubation time using the fumigation method.
Legend: JAI = Day after incubation, EO = Essential oil, *Oa = Ocimum americanum, Lm = Lippia multiflora, Cs = Cymbopogon shoenanthus, Og = Ocimum gratissimum.*

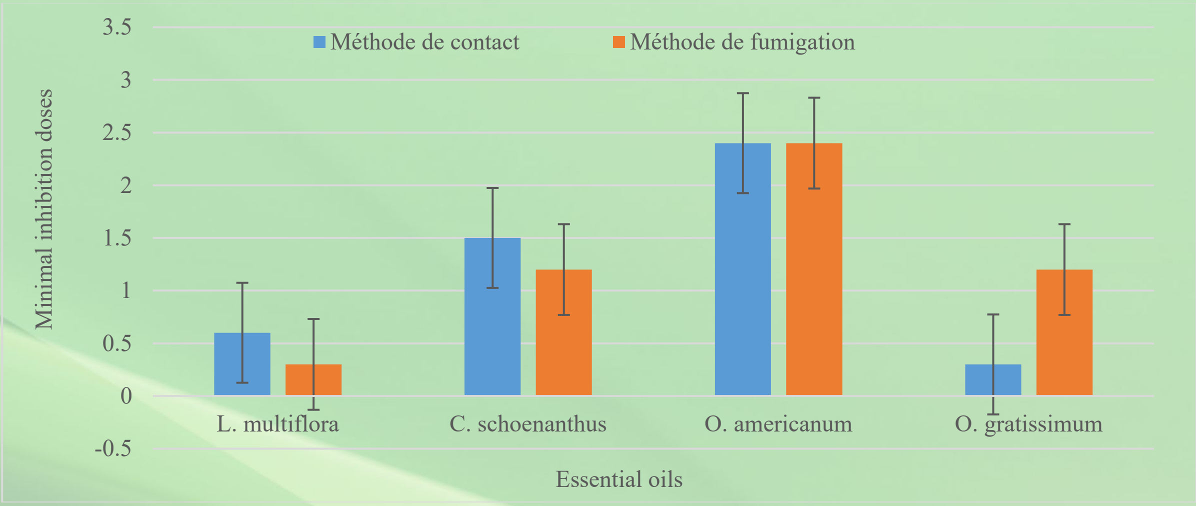

Figure 3 : Minimal inhibition doses of essential oils on radial mycelial growth of *M. oryzae*.

Determination of minimum inhibition doses

The minimum inhibition doses of the various essential oils on radial mycelial growth using the contact and fumigation methods are shown in figure 3.

Analysis of variance showed a highly significant difference between the minimum inhibition doses of the different essential oils. In terms of the parameters evaluated, *O. americanum* essential oil recorded the highest minimum inhibition dose (2.4 µl/ml) in both contact and fumigation methods. In the contact method, *Ocimum gratissimum* oil recorded the lowest minimum inhibition dose (0.3 µl/ml) for all parameters assessed. In the fumigation method, the essential oil of *L. multiflora* applied in pure form recorded the lowest minimum inhibition dose of 0.3 µl/ml on mycelial growth.

Discussion

The effect of essential oils on the growth of *M. oryzae* in vitro varied according to the type of oil and the method used. *O. gratissimum* essential oil was the most effective in inhibiting radial mycelial growth of the fungus. This effectiveness in reducing radial mycelial growth can be explained by its content of major elements, its antifungal properties and its chemical composition, which enable it to stop or slow down the fungus' development (Mohamed *et al.,* 2012). The inhibitory effect of these oils can be attributed to their major compounds, such as 1,8-cineole for *O. americanum* essential oil, or to the synergistic or additive effect of these compounds (Ambindei *et al.,* 2014). According to Sirima *et al.* (2020), the essential oil of *C. schoenanthus* and *L. multiflora* exhibited strong antifungal activity, stopping the mycelial growth of *Alternaria sp* isolates at doses ranging from 5% to 100%.
For Tiendrebeogo *et al*. (2017), the essential oil of *L. multiflora* exhibits strong antifungal activity, totally inhibiting the mycelial growth of *P. oryzae* at 600 ppm. They also showed that this essential oil totally reduced the mycelial growth of F. moniliforme and *B. oryzae* at 100 ppm and 400 ppm respectively. Their work also showed that essential oils were more effective in inhibiting fungal mycelial growth than aqueous plant extracts.
These results can be explained by a probable deactivation or disruption of the fungus's operating system by essential oils. The work of Hamdani *et al* (2015) showed strong 100% inhibition of Fusarium spore germination by *C. aurantium sp* and *F. oxysporum* by *C. sinensis*, while essential oils of *C. limon* and *C. reticulata* stimulated spore germination by 48% and 53% respectively in Alternaria sp. *Magnaporthe oryzae* is a fungus that can infect rice seeds in storage.

Conclusion

The various tests carried out in this study on essential oils and their combinations enabled us to verify their efficacy against *M. oryzae*. All essential oil formulations were effective in inhibiting *M. oryzae*. However, *L. multiflora* oil and *C. schoenanthus* oil used in pure form showed better minimum inhibitory doses on mycelial radial growth of *M. oryzae* spores, with values of 0.6 µl/ml and 1.5 µl/ml respectively. The essential oil of *O. gratissimum* was most effective with a minimum inhibition dose of 0.3 µl/ml. These results attest to the potential of essential oils as a natural means of controlling the pathogen responsible for rice blast. These essential oil formulations can now be tested in real-life conditions to assess their effectiveness in controlling rice blast.

Acknowledgments
We thank the managers of the Institute for Research in Applied Sciences and Technology and the Biosciences Laboratory of Joseph KI-ZERBO University for facilitating the work.

Author Contributions
OS planned the experiments, interpreted the results, analysed the data statistically, SA and KK supervised the project.

Conflict of interest
The authors declare no conflicts of interest.

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