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In vitro assessment of the efficacy of three essential oils of aromatic plants against *Magnaporthe oryzae* B.C. Couch, a rice blast pathogen in Burkina Faso

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ABSTRACT

Blast, caused by *Magnaporthe oryzae* B.C.Couch, is the main fungal disease in the rice fields in Burkina Faso. Three pure essential oils at different concentrations of *Cymbopogon schoenanthus*, *Ocimum americanum* and *Lippia multiflora*, and four of their combinations were tested *in vitro* to evaluate their inhibition properties on mycelial growth, sporulation, and spore germination of *M. oryzae*. The contact method and the fumigation method were used for the different tests at doses of 0.05 µl/ml, 0.1 µl/ml, 0.3 µl/ml, 0.6 µl/ml, 0.9 µl/ml, 1.2 µl/ml, 1.5 µl/ml, 1.8 µl/ml, 2.1 µl/ml and 2.4 µl/ml. Two chemical fungicides, mancozeb and azoxystrobin and an absolute control were used in the trials. The results showed that the pure essential oil of *L. multiflora* and its combination with that of *C. schoenanthus* presented the most effective minimum doses of inhibition in the contact and fumigation method. These doses ranged from 0.1 µl/ml to 0.3 µl/ml for 100% inhibition of radial growth, sporulation, and spore germination of *Magnaporthe oryzae*. Mancozeb inhibited 100% of all parameters evaluated, while azoxystrobin was ineffective. The essential oil of *L. multiflora* and its combination with that of *C. schoenanthus* can be tested in a real environment to control rice blast.

Keywords: Burkina Faso, Essential oils, *Oryza sativa* L, *Magnaporthe oryzae*.

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INTRODUCTION

Rice, *Oryza sativa* L, is a widely grown cereal in Burkina Faso. Its production faces many abiotic and biotic constraints (Bouet et al., 2012; Adamou et al., 2020). Among the biotic constraints, blast caused by the fungus *Magnaporthe oryzae*, remains the most explosive and potentially damaging fungal constraintfor rice production (Kassankogno, 2016; Zhang et al., 2016; Kassankogno et al., 2016). Damage from this pathogen can cause yield losses of 80% to 100% without any protective measures (Singh et al., 2018; Savary et al., 2019). *M. oryzae* mainly attacks aerial organs (leaves and panicles) and its severity depends on the growth stage of the rice plant, the variety and especially the ecology (Bouet, 2008; Bahous et al., 2010). Globally, economic losses related to blast are estimated at 66 billion dollars each year (Nalley et al., 2016). It poses a threat to the food security of about 03 billion people who depend on rice as their main food (Cruz and Valent, 2017). The use of chemicals such as tricyclazole for the control of *M. oryzae* has been successful in increasing yields (Moinina et al., 2018).

However, their application has many consequences on human and animal health and on the environment (Deguine and Ferron, 2006). It is therefore necessary to use alternative control methods such as biological control using biopesticides. (Yarou et al., 2017; Gamsoré et al., 2018; Toundou et al., 2020). Several studies indicated that essential oils have insecticidal, bactericidal or fungicidal properties (Amarti et al., 2010; Zohra et al., 2015; Asma, 2021). Sirima et al. (2020) reported the effectiveness of essential oils of *L. multiflora, C. schoenanthus, O. americanum* and *O. basilicum* in the inhibition of the radial growth of *Alternaria sp.* which is responsible for tomato alternariosis in Burkina Faso.

This study aimed to evaluate *in vitro* the inhibitory effect of essential oils at different concentrations of *C. schoenanthus, O. americanum, L. multiflora* and their combinations on mycelial growth, sporulation and spore germination of *M. oryzae*.

MATERIALS AND METHODS

Fungal material

The *M. oryzae* strain BF201 was used as fungal material to evaluate the essential oils efficacy. It was isolated from rice leaves, collected on the experimental site of Farakô-Bâ. The choice of this strain is justified by its high level of virulence.

M. oryzae was identified on the basis of symptoms observed in the field. Infested leaves showing symptoms were washed with clean water. These leaves were cut into small fragments measuring 2.5 cm. The leaf fragments were disinfected for a few seconds in 2% sodium hypochlorite and rinsed with distilled water for 30 seconds to eliminate exogenous microflora. These fragments were placed on sterile filter paper in Petri dishes, which were incubated for 3 to 4 days at a temperature between 25 and 30°C, alternating 12 hours in the light and 12 hours in the dark, in order to induce sporulation and the formation of conidia. After incubation, the leaf fragments were examined under a binocular magnifying glass to identify the conidia. Under a binocular magnifying glass, conidia were picked individually using a sterile plastic needle and cultured on PDA (Potato, Dextrose, Agar) medium in 90 mm diameter Petri dishes to obtain a pure culture of the isolate.

Essential oils

The essential oils of *C. schoenanthus, O. americanum* and *L. multiflora* used for the tests were extracted from leafy branches. Their extraction was made by distillation at the Laboratory of the Natural Substances Department of the Institute for Research in Applied Sciences and Technologies of Ouagadougou located in Kossodo. The essential oils were extracted by humid steam distillation through a still. Four combinations of the essential oils were set at the following proportion: 50% *O. americanum* + 50% *L. multiflora*, 50% *O. americanum* + 50% *C. schoenanthus*, 50% *L. multiflora* + 50% *C. schoenanthus* and 1/3 (*O. americanum* + *L. multiflora* + *C. schoenanthus)*.

Antifungal tests methods

Two methods were used to test the essential oils and their combinations efficacies: the contact method or « poisoned food » (Thompson, 1989) and the fumigation method or micro-atmosphere method. In the first method, the essential oils and their combinations were mixed directly into the culture medium Potato Dextrose Agar (PDA), while in the second method, the essential oils and their combinations were poured and spread on the inner side of Petri dishes' covers (Amvam et al., 1998).

The different proportions of each essential oil and combination of essential oils prepared for the medium culture dosage were: 0.05 µl/ml, 0.1 µl/ml, 0.3 µl/ml, 0.6 µl/ml, 0.9 µl/ml, 1.2 µl/ml, 1.5 µl/ml, 1.8 µl/ml, 2.1 µl/ml and 2.4 µl/ml. The chemical fungicides used in both methods were mancozeb and azoxystrobin. An absolute control without essential oils in the PDA medium was used as well. The Petri dishes were incubated in the laboratory at room temperature (around 25 to 28°C). For each Petri dish, in both methods, the thallus diameters (mm) of the colonies were measured at the 12th day after incubation (DAI) as well as sporulation assessment. The germination rates of spores on PDA medium were assessed 12 hours after sowing.

Mycelial growth assessment

The mycelial growth was evaluated by taking the average of the two (02) perpendicular diameters passing through the middle of the disc. Three repetitions were performed for each concentration.

Sporulation assessment

From each Petri dish used for the mycelial growth evaluation, three explants of 06 mm size of diameter were collected and put in 03 ml of distilled water contained in test tubes. Tubes were shaked for 15 seconds to separate spores from conidiospores. The mycelial fragments were eliminated from the suspensions by filtration with mousseline. The count of the number of spores was carried out using the Malassez cell. The number of spores per unit area (mm²) was counted followed by the number of particles in 1 ml.

Spores' germination assessment

From a seven (07) days young mycelial culture, 03 explants were collected and placed in tubes containing 03 ml of distilled water. The spores released after shaking, were counted by Malassez Hematimeter and then adjusted to 10^5 spores/ml after dilution. According to the method used, suspensions of 100 µl were spread on PDA medium in Petri dishes at 03 repetitions. Control Petri dishes without essential oils and fungicides in the PDA medium, were assessed as well. After 12 hours, the number of germinated spores was evaluated. For each parameter, the inhibition rate (IR) has been determined by the following formula:$IR_{(\%)} = \left(\frac{T_0 - T}{T_0}\right) \times 100$.where, T0 = the mean value from control medium, T = the mean value from essential oil or fungicide medium.

Data analysis

The data were entered and organised using Excel 2013. The results were analyzed by the variance method (ANOVA) using XLSTAT 2016 software, followed by a comparison of means using the test Fisher at 5% probability limit.

RESULTS

Antifungal effects of essential oils on mycelial radial growth of *M. oryzae*

Table 1 presents the antifungal effects of essential oils on the mycelial radial growth of *M. oryzae* according to the doses and the method used. Statistical analysis indicated a significant difference between the treatments. The results obtained at the contact method showed that the pure essential oils of *O. americanum*, *L. multiflora*, and *C. schoenanthus* inhibited 100% the mycelial radial growth of the fungus, respectively from doses D10 (2.4 µl/ml), D4 (0.6 µl/ml), and D7 (1. 5 µl/ml). For the combinations of essential oils, the results showed that the doses of the

combinations of essential oils of *O. americanum* and *L. multiflora*, *O. americanum* and *C. schoenanthus*, *L. multiflora* and *C. schoenanthus* as well as that of the three essential oils inhibited 100% the mycelial radial growth of the fungus respectively from doses greater than or equal to D4 (0.6 µl/ml), D6 (1.2 µl/ml), D3 (0.3 µl/ml) and D5 (0.9 µl/ml).

As for the fumigation method, the results revealed that the oils of *O. americanum*, *L. multiflora*, and *C. schoenanthus*, inhibited 100%the mycelial radial growth of the fungus, respectively from doses greater than or equal to D10 (2.4 µl/ml), D3 (0.3 µl/ ml), and D6 (1.2 µl/ml). Regarding the combinations of essential oils, the results showed that the combination of essential oilsof *O. americanum* and *L. multiflora*, *L. multiflora* and *C. schoenanthus*, O. *americanum* and *C. schoenanthus*, then that of the three essential oils inhibited mycelial radial growth respectively from doses D10 (2.4 µl/ ml), D6 (1.2 µl/ml), D10 (2.4 µl/ml), and D7 (1.5 µl/ml). Mancozeb and azoxystrobin fungicides inhibited mycelial radial growth respectively 100% and 25.9%, regardless of dose.

Antifungal effects of different essential oils on *M. oryzae* sporulation

Table 2 presents the antifungal effects of essential oils and their combinations on the sporulation of *M. oryzae* using the contact and fumigation methods. The analysis of variance shows that in the contact method, among the essential oils and their combinations, the oil of *O. americanum* recorded the lowest inhibition rates from dose D1 (0.05µl/ml) to dose D8 (1.8 µl/ml) with respective variations of 1.2% to 98.9%. On the other hand, *L. multiflora* essential oil recorded the highest inhibition rates from dose D1 (0.05 µl/ml) to dose D3 (0.3 µl/ml) with respective variations of 74.1% to 100%. The combination of *L. multiflora* and *C. schoenanthus* essential oils and that of *O. americanum*, *L. multiflora* and *C. schoenanthus* inhibited sporulation from dose D5 (0.9%). *C. schoenanthus* essential oil, the combination of essential oils of *O. americanum* and *L. multiflora* and that of *O. americanum* and *C. schoenanthus* inhibited 100% the sporulation of *M. oryzae* respectively from doses D6 (1.2 µl/ml), D4 (0.6 µl/ml) and D6 (1.2 µl/ml).

In the fumigation method, among pure essential oils and their combinations, the essential oil of *O. americanum* recorded the lowest inhibition percentages ranging from 0.9% to 97.9%, respectively, from dose D1 (0.05 µl/ml) to D8 (1.8 µl/ml). *L. multiflora* essential oil and the combination of *L. multiflora* and *C. schoenanthus* essential oils inhibited 100% the sporulation of *M. oryzae* from dose D3 (0.3µl/ml). The essential oil of *C. schoenanthus* and its combination with *O. americanum* oil inhibited sporulation 100% from dose D5 (0.9 µl/ml). The combination of essential oils of *O. americanum* and *L. multiflora* and then that of the three essential oils inhibited sporulation 100% from dose D4 (0.6 µl/ml). Mancozeb and azoxystrobin fungicides recorded 100% and 67.33% sporulation inhibition rates, respectively.

Antifungal effects of different essential oils on spore germination of *M. oryzae*

Table 3 presents the antifungal effects of essential oils and their combinations on the germination of *M. oryzae* spores using the contact and fumigation methods. In the contact method, the essential oil of *O. americanum* recorded the lowest sprouting inhibition rate at doses D1 (0.05 µl/ml), D2 (0.1 µl/ml), D3 (0.3 µl/ml) and D4 (0.6 µl/ml) with respective rates of 6.42%, 35.3%, 78.6% and 96.9% compared to other treatments. The essential oil of *L. multiflora* recorded the highest inhibition rates at dose D1 (0.05 µl/ml) and D2 (0.1 µl/ml) with respective inhibition rates of 70, 8% and 98.9%. This oil was the most effective because it recorded a very marked inhibitory effect on the spores' germination rate. From dose, D3 (0.3 µl/ml), *L. multiflora* essential oils and the combination of *L. multiflora* and *C.*

schoenanthus essential oils inhibit 100% the germination of *M oryzae*. The essential oil of *C. schoenanthus*, the combination of essential oils of *O. americanum* and *L. multiflora* and then that of the three essential oils recorded 100% inhibition rates from the dose D4 (0.6 µl/ml).

In the fumigation method, the results showed that the essential oil of *O. americanum* had less inhibitory effect on *M. oryzae* spore germination by recording the lowest inhibition rates at doses D1 (0.05 µl/ml), D2 (0.1 µl/ml), D3 (0.3 µl/ml) and D4 (0.6 µl/ml) with respective rates of 21.9%, 41.4%, 68.3% and 93.9%. The essential oil of *L. multiflora* recorded the highest germination inhibition rates at all doses, except dose D1 (0.05µl/ml) with 23.40%. The percentage of germination inhibition reached 100% from dose D3 (0.3 µl/ml) for *C. schoenanthus* essential oils and the combination of *L. multiflora* and *C. schoenanthus* essential oils. The combination of essential oils of *O. americanum* and *L. multiflora* and that of the three essential oils recorded 100% germination inhibition rate from dose D4 (0.6 µl/ml). The essential oil of *O americanum* and its combination with the essential oil of *C. schoenanthus* recorded 100% inhibition of germination from dose D5 (0.9 µl/ml). Fungicides consisting of mancozeb and azoxystrobin recorded 100% and 85.5% sprout inhibition rates, respectively.

Figure 1 presents the measurement of mycelial radial growth, the sporulation, conidia and spore germination of *M. oryzae*.

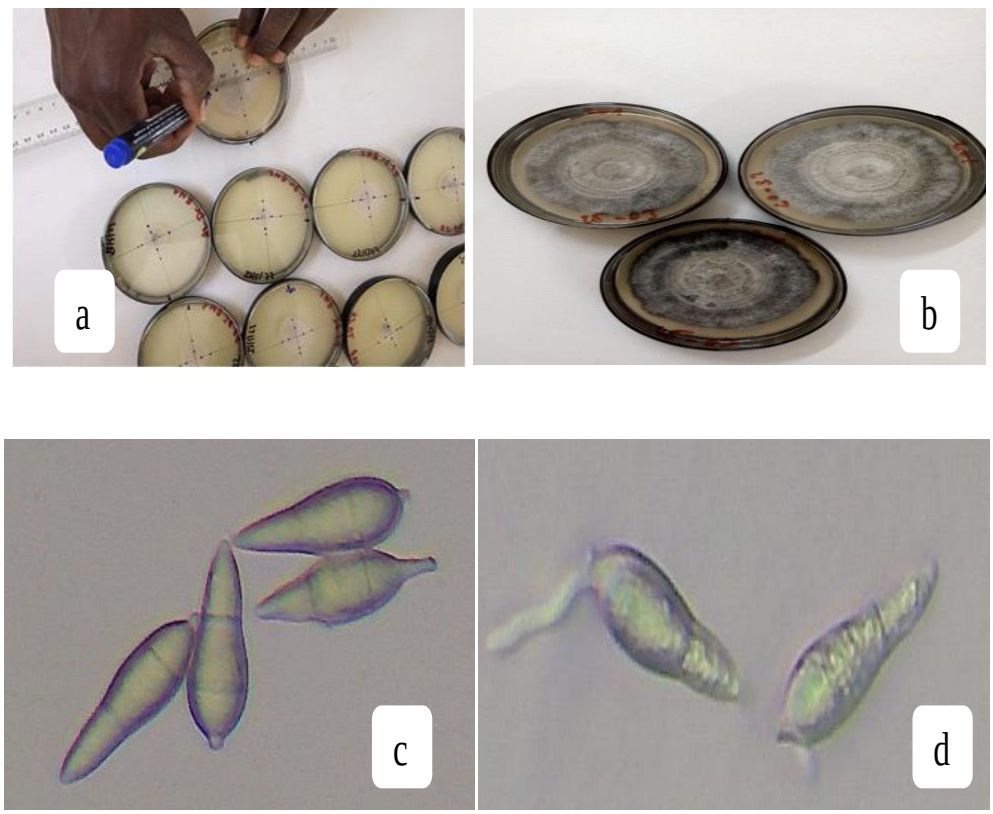

Figure 1: **a** : Measurement of mycelial radial growth; **b** : Sporulation of *M. oryzae*, **c** : Conidia of *M. oryzae* viewed under a light microscope at GX40 magnification, **d** : *M. oryzae* spores germinated on culture medium.

Table 1 : Essential oils and their combinations effects on *Magnaporthe oryzae* mycelial radial growing.

Doses (µl/ml)	*Oa*		*Lm*		*Cs*		*Oa/Lm*		*Oa/Cs*		*Lm/Cs*		*Oa/Lm/Cs*		Fongicides	
	CM	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**Mz**	**Az**
D1 (0.05)	37.5^{g}	17.9^{i}	33.5^{d}	81.3^{c}	15.4^{f}	70.7^{e}	3.1^{d}	13.1^{i}	15.1^{f}	23.7^{i}	59.4^{c}	84.17^{f}	35.3^{e}	85.6^{e}	100^{a}	25.9^{a}
D2 (0.1)	37.8^{g}	23.5^{h}	48.8^{c}	92.3^{b}	71.8^{e}	$73.8d^{e}$	57.4^{c}	68.4^{h}	86.8^{e}	35.3^{h}	88.5^{b}	86.42^{e}	85.3^{d}	89.2^{d}	100^{a}	25.9^{a}
D3 (0.3)	42.2^{f}	31.8^{g}	89.1^{b}	100^{a}	72.7^{de}	76.9d	84.2^{b}	70.6^{g}	87.1^{de}	74.8^{g}	100^{a}	91.71^{d}	88.5^{c}	89.4^{d}	100^{a}	25.9^{a}
D4 (0.6)	43.3^{f}	55.4^{f}	100^{a}	100^{a}	74.4^{cd}	87.1^{c}	100^{a}	82.2^{f}	96.4^{c}	76.9^{f}	100^{a}	94.96^{c}	94.3^{b}	93.3^{c}	100^{a}	25.9^{a}
D5 (0.9)	47.9^{ef}	70.65^{e}	100^{a}	100^{a}	75.8^{c}	92.8^{b}	100^{a}	94.8^{e}	97.8^{bc}	77.7^{f}	100^{a}	95.68^{bc}	100^{a}	93.9^{c}	100^{a}	25.9^{a}
D6 (1.2)	50.2^{de}	72.4^{de}	100^{a}	100^{a}	88.8^{b}	100^{a}	100^{a}	95.1^{d}	100^{a}	82.7^{e}	100^{a}	100^{a}	100^{a}	94.9^{b}	100^{a}	25.9^{a}
D7 (1.5)	53.7^{d}	75.6^{d}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	95.7^{cd}	100^{a}	94.9^{d}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	25.9^{a}
D8 (1.8)	76.3^{c}	79.3^{c}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	96.5^{bc}	100^{a}	95.7^{cd}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	25.9^{a}
D9 (2.1)	81.6^{b}	86.5^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	97.8^{b}	100^{a}	97.7^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	25.9^{a}
D10 (2.4)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	25.9^{a}
Pr > F	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**> 0.05**	**> 0.05**
Signification	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**NS**	**NS**

CM = Contact Method, **FM** = Fumigation Method, ***Oa*** = *Ocimum americanum*, ***Lm*** = *Lippia multiflora*, ***Cs*** = *Cymbopogon shoenanthus*, ***Oa/Lm***= Combination of *Oa*and *Lm*, ***Oa/Cs*** = Combination of *Oa*and *Cs*, ***Lm/Cs*** = Combination of *Lm* and *Cs*, ***Oa/Lm/Cs*** = Combination of *Aa*, *Lm* and *Cs*, **Mz** = Mancozeb, **Az** = Azoxystrobin. *The same columns values with the same letter are not different statistically at 5% limit of probability.*

Table 2 : Essential oils and their combinations effects on *Magnaporthe oryzae* sporulation.

Doses (µl/ml)	*Oa*		*Lm*		*Cs*		*Oa/Lm*		*Oa/Cs*		*Lm/Cs*		*Oa/Lm/Cs*		Fungicides	
	CM	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**Mz**	**Az**
D1 (0.05)	1.2^{i}	0.9^{i}	74.1^{c}	8.3^{c}	11.1^{f}	16.8^{e}	11.9^{e}	62.1^{c}	9.5^{f}	11.3^{e}	16.7^{d}	9.7^{d}	35.97^{e}	7.5^{d}	100^{a}	67.33^{a}
D2 (0.1)	7.3^{h}	8.6^{h}	97.5^{b}	26.3^{b}	21.2^{e}	33.9^{d}	42.1^{d}	93.9^{b}	25.7^{e}	29.8^{d}	68.3^{c}	45.3^{c}	85.27^{d}	39.1^{c}	100^{a}	67.33^{a}
D3 (0.3)	17.9^{g}	12.8^{g}	100^{a}	100^{a}	49.3^{d}	67.3^{c}	72.7^{c}	100^{a}	41.4^{d}	57.2^{c}	89.1^{b}	83.9^{b}	88.49^{c}	89.7^{b}	100^{a}	67.33^{a}
D4 (0.6)	37.3f	49.7^{f}	100^{a}	100^{a}	74.6^{c}	89.7^{b}	93.3^{b}	100^{a}	77.3^{c}	89.4^{b}	100^{a}	100^{a}	94.24^{b}	100^{a}	100^{a}	67.33^{a}
D5 (0.9)	59.7^{e}	71.5^{e}	100^{a}	100^{a}	92.9^{b}	100^{a}	100^{a}	100^{a}	91.2^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	67.33^{a}
D6 (1.2)	79.8^{d}	81.9^{d}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	67.33^{a}
D7 (1.5)	89.1^{c}	92.8^{c}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	67.33^{a}
D8 (1.8)	98.9^{b}	97.9^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	67.33^{a}
D9 (2.1)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	67.33^{a}
D10 (2.4)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	67.33^{a}
Pr > F	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**> 0.05**	**> 0.05**
Signification	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**NS**	**NS**

CM = Contact Method, **FM** = Fumigation Method, ***Oa*** = *Ocimum americanum*, ***Lm*** = *Lippia multiflora*, ***Cs*** = *Cymbopogon shoenanthus*, ***Oa/Lm***= Combination of *Oa*and *Lm*, ***Oa/Cs*** = Combination of *Oa*and *Cs*, ***Lm/Cs*** = Combination of *Lm* and *Cs*, ***Oa/Lm/Cs*** = Combination of *Aa*, *Lm* and *Cs*, **Mz** = Mancozeb, **Az** = Azoxystrobin. *The same columns values with the same letter are not different statistically at a 5% limit of probability.*

Table 3: Essential oils and their combinations effects on the germination inhibition of *Magnaporthe oryzae* spores.

Doses (µl/ml)	*Oa*		*Lm*		*Cs*		*Oa/Lm*		*Oa/Cs*		*Lm/Cs*		*Oa/Lm/Cs*		Fongicides	
	CM	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**CM**	**FM**	**Mz**	**Az**
D1 (0.05)	6.42^{e}	21.9^{e}	70.8^{b}	76.3^{b}	21.2^{d}	56.3^{c}	12.2^{d}	38.7^{d}	10.5^{e}	48.68^{d}	18.8^{c}	88.5^{b}	18.7^{d}	51.9^{d}	100^{a}	86.4^{a}
D2 (0.1)	35.3^{d}	41.4^{d}	98.9^{ab}	100^{a}	80.4^{c}	88^{b}	63.7^{c}	83.5^{c}	61.7^{d}	83.7^{c}	95.4^{ab}	93.6^{ab}	63.2^{c}	78^{c}	100^{a}	86.4^{a}
D3 (0.3)	78.6^{c}	68.3^{c}	100^{a}	100^{a}	96.8^{bc}	100^{a}	90.5^{b}	92.3^{bc}	81.15^{c}	89^{bc}	100^{a}	100^{a}	94.3^{b}	98.9^{b}	100^{a}	86.4^{a}
D4 (0.6)	96.9^{b}	93.9^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	98.6^{bc}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	86.4^{a}
D5 (0.9)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	86.4^{a}
D6 (1.2)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	86.4^{a}
D7 (1.5)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	86.4^{a}
D8 (1.8)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100a	100^{a}	86.4^{a}
D9 (2.1)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	86.4^{a}
D10 (2.4)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	86.4^{a}
Pr > F	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**> 0.05**	**> 0.05**
Signification	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**THS**	**NS**	**NS**

CM = Contact Method, **FM** = Fumigation Method, ***Oa*** = *Ocimum americanum*, ***Lm*** = *Lippia multiflora*, ***Cs*** = *Cymbopogon shoenanthus*, ***Oa/Lm***= Combination of *Oa*and *Lm*, ***Oa/Cs*** = Combination of *Oa*and *Cs*, ***Lm/Cs*** = Combination of *Lm* and *Cs*, ***Oa/Lm/Cs*** = Combination of *Aa*, *Lm* and *Cs*, **Mz** = Mancozeb, **Az** = Azoxystrobin. *The same columns values with the same letter are not different statistically at a 5% limit of probability.*

DISCUSSION

The effectiveness of essential oils and their combinations on the growth of *M. oryzae in vitro* varies according to the doses and the method tested. The results showed that the essential oil of *L. multiflora* and its combination with that of *C. schoenanthus* recorded the best percentages of inhibition of the mycelial radial growth of the fungus. This effectiveness recorded in reducing radial growth is explained by their content of major elements, their antifungal properties, and their chemical compositions, which allow them to stop or slow down the development of the fungus (Doumbouya et al., 2012; Adjou and Soumanou, 2013). The inhibitory effect of these essential oils can be attributed to the majority compounds of the chemical molecules, such as 1,8-cineole for the essential oil of *O. americanum* or even to the synergistic or additive effect of the chemical compounds (Ambindei et al., 2014). Moreover, Sirima et al. (2020) studies had also showed that *C. schoenanthus* and *L. multiflora* essential oils stop *Alternaria alternata* mycelial growing with doses ranging from 5% to 100%; revealing the high antifungal activity of these essential oils. These results confirm those of Tiendrebeogo et al. (2017) who concludes that *L. multiflora* essential oils have a high antifungal activity by inhibiting the mycelial growth of *P. oryzae* at 600 ppm. They also showed that this essential oil 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 fungi's mycelial growth than aqueous plant extracts. The combination of *L. multiflora* and *C. schoenanthus* essential oils showed a synergistic effect in inhibiting radial growth by contact and by fumigation. Studies conducted by Nazzaro et al. (2007) showed possible synergistic effects between different essential oils and/or their components and synthetic molecules. According to Serghat et al. (2004), certain synthetic chemical fungicides tested *in vitro* such as carboxinethirams as well as their combinations were proved to be effective against the development of *P. grisea* by strongly reducing their mycelial growth.

Essential oils and their combinations also inhibited the sporulation of *M. oryzaein vitro* using the contact and fumigation method. The results showed that in contact method, all the essential oils and their mixtures presented an inhibiting effect on sporulation with an intense activity at the level of the essential oil of *L. multiflora* as well as its combination with that of *C. schoenanthus* with rates of inhibition reaching 100% respectively from D3 (0.3 µl/ml) and D4 (0.6 µl/ml). In the fumigation method, the essential oil of *L. multiflora* and the combination of the essential oils of *L. multiflora* and *C. schoenanthus* completely inhibited (100%) the sporulation of the fungus from dose D3 (0.3 µl/ ml). Similar work carried out by Kassankogno (2016) showed that the aqueous extracts of *Agave sisalana* and *C. citratus* had significantly influenced the sporulation of *M. oryzae* by inhibiting it respectively at 3% and 20%. According to Chutia et al. (2009), the essential oils of *Citrus sp* recorded sporulation inhibition percentages of 74% and 83% respectively for *Alternaria alternata* and *Fusarium oxysporum*, contrary to the work of Ouraïni et al. (2005) who revealed that the essential oils of *T. saturejoides*, *M. pulegium* and *R. officinalis* have an effect promoting the production of spores of *M. gypseum*, *M. nanum* and *M. canis.*

For spore germination, in the contact and fumigation method, the highest percentages of germination inhibition were recorded by the essential oil of *L. multiflora* and its combination with that of *C. schoenanthus*. These two formulations inhibited spore germination 100% at the same level as the fungicide mancozeb and exerted a higher inhibition rate than the fungicide azoxystrobin from dose D3 (0.3 µl/ml). In the contact method, the inhibition rate reached 100% from dose D3 (0.3 µl/ml) for the essential oil of *L. multiflora* and its combination with the essential oil of *C. schoenanthus*. In the fumigation method, the essential oil of *L. multiflora* alone recorded a germination inhibition rate of 100% from D2 (0.1 µl/ml). These results are explained by the essential oils' possible deactivation or disruption of the fungus' functioning system. According to Freiesleben and Jäger (2014), antifungal products can deactivate fungal

function by disrupting the structure and function of fungal cell membranes or organelles and/or by inhibiting nuclear material or protein synthesis. Work carried out by Bourahli and Traka (2021) showed a high inhibitory activity of the essential oils of *T. pallescens* and *C. citratus* on the sporal germination of *Trichoderma sp* at concentrations of 15 and 10 µL/ml. In addition, Koïta et al. (2012) reported that aqueous extracts of *L. multiflora* at 40 g/l had recorded a very high rate of inhibition on the spore germinations of *P. arachidis* Speg., the pathogen of peanut rust. A high inhibitory rate of 100% had been also scored by *C. aurantium sp* and *C. sinensis* essential oils on *Fusarium oxysporum* spores' germination in Zohra et al. (2015) studies. This stimulation can be explained by a mechanism developed by some fungal pathogens which consist to use secondary metabolites as signals to induce germination.

Conclusion

The various tests carried out in this study on essential oils and their combinations made it possible to verify their effectiveness against *Magnaporthe oryzae*. All the essential oils and their mixtures had an impact in inhibiting the parameters studied. However, *Lippia multiflora* essential oil and its combination with *Cymbopgon schoenanthus* essential oil showed the best antifungal results on mycelial radial growth, sporulation, and spore germination of *Magnaporthe oryzae*. These results attest the potential of essential oils as a natural means of combating this rice blast pathogen. These essential oils can be tested in a real environment to assess their effectiveness in the fight against rice blast in the farm conditions.

COMPETING INTERESTS

The authors declared that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SO conceived the project and carried out the research and laboratory work. IS and AS participated in finalising the project and correcting the manuscript. AIK took part in finalising the laboratory work and drafting the manuscript. ASI participated in the correction of the manuscript. SZ participated in the analysis of the data and the correction of the manuscript. KK was involved in supervising the work and correcting the manuscript, and MS, Laboratory Manager, supervised the project.

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