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EFFECTS OF POWDERY FORMULATIONS OF ESSENTIAL OILS AGAINST *MAGNAPORTHE ORYZAE* B.C COUCH, RICE BLAST PATHOGEN AND ON THE GERMINATION CAPACITY OF RICE SEED IN BURKINA FASO

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ABSTRACT

Introdution: Fungi transmitted by *Oryza sativa* L rice seed cause considerable production losses. The aim of our work was to evaluate the effect of essential oil formulations against *Magnaporthe oryzae* and on the germination capacity of rice seeds. Contaminated rice seeds for antifungal tests and uncontaminated rice seeds for viability tests were coated in powdery formulations of essential oils of *Lippia multiflora, Cymbopogon schoenanthus* and *Ocimum americanum* in pure form and four of their combinations at a dose of 10g/kg of seed. A fungicidal control and a neutral control were used. For the antifungal test, the results showed that among the formulations, the essential oil of *L. multiflora* recorded the highest percentage of germinated grains (96%) and the lowest percentage of damaged grains (4%). After 45 days of storage, the results showed that the combination of *L. multiflora* and *C. schoenanthus* oils recorded the highest germination and viability percentages of 91.87% and 97.85% respectively. These essential oil formulations can be further improved and used in the conservation of seeds in stock.

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INTRODUCTION

Rice production in Burkina Faso has increased significantly in recent years, both in terms of areas sown and quantities produced. Because of its economic and food value, rice is one of the most widely grown cereals in all the country's agro-ecological zones. However, its production faces a number of constraints, including fungal diseases, which account for 70% of crop diseases. The most important of these in the rice-growing sectoris blast disease (Bouet *et al*., 2012; Bhaskar *et al.*, 2018). It can cause seedling damping-off, leaf spot and even panicle blotch (Mahomed *et al*., 2022). Infection can reach the seeds before harvest via the panicle, or after harvest, during drying and storage. These diseases are among the main causes of deterioration in production quality and reduced yields (Kassankogno, 2016; Dong *et al*., 2020).

The disease has many economic, food and health-related consequences. The use of chemical products remains the main means of combatingthisdisease, but their abusive use leads to health problems such as food poisoning, infertility problems, cancers, immune deficiency, disturbances in neurological and behavioural development, metabolic disturbances and diabetes (Aicha *et al.*, 2017). An alternative control method that respects human health and the environment therefore needs to be considered (Toundou *et al.*, 2020). Ouedraogo *et al.* (2016) have highlighted the fungicidal potential of essential oils. *C. schoenanthus and L. multiflora* oils also have fungicidal properties (Koïta *et al*., 2012 ; Bonzi *et al*., 2013 ; Sirima *et al*.,2020). However, the use of essential oils from aromatic plants for the control of *M. oryzae* in stock conservation remains largely unexplored. With this in mind, the present study aims to evaluate the *in vitro* effect of essential oils of *C. schoenanthus, O. americanum, L. multiflora* and their combinations against *M. Oryzae* and on the germinative capacity of grains.

MATERIAL AND METHODS

Biological material: Seeds of the FKR64 (TS2) variety were used for the experiment. For the essential oil efficacy tests, a strain of *M. oryzae*wasused.

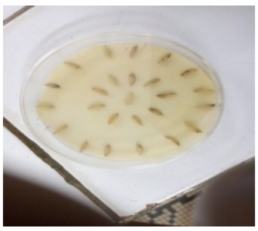
Essential oil formulations: The essential oils of *C. schoenanthus, O. americanum* and *L. multiflora* in pure form and four of their combinations were used at the minimum inhibitory doses (MID) determined. These minimum inhibitory doses were incorporated into rice flour in hermetically sealed 50 ml bottles and then vortexed to obtain a homogeneous mixture. The flour was sterilised in an oven.

Preparation of inoculum and treatment of rice grains: For tests against *M. oryzae*, the inoculum solution was prepared at 10^4 conidia/ml for inoculation of rice grains by soaking. 250g of rice was disinfected with 15% sodium hypochlorite for 10 minutes, followed by a sanitarye valuation (Photo A). A first batch of these seeds was soaked in the inoculum for 24 hours (Photo B) and then dried under a fume hood in the laboratory for 48 hours. These inoculated seeds were coated in powdered formulations of essential oils at a dose of 10g/kg of seed (Mahomed *et al*., 2022). The second batch of uninoculated seeds was also coated in the powder formulations (Photo C). The inoculated seeds were used to evaluate the efficacy of the essential oil formulations against *M. oryzae*. An inoculated control was used. Twenty-five (25) grains from each seed lot and for each essential oil formulation were placed on blotting paper in Petri dishes and incubated at 25°C in the dark (Logeshwari *et al*., 2022). The uninoculated seeds were stored to assess the effect of the essential oil formulations on their germinative capacity over time. A neutral control were used

Table 1. Dosage of essential oil formulations

Essential oils	Minimal inhibition dose	Dose (µl/100g of flour)
L. multifmora (Lm)	0,6 µl/ml	30 µl/100g
C. schoenanthus (Cs)	1,5 µl/ml	75 µl / 100g
O. americanum (Oa)	2,4 µl/ml	120 µl /100g
Combinaison *Lm.Cs*	0,3 µl /100g	15 µl /100g
Combinaison *Lm.Oa*	0,6 µl/100g	30 µl/100g
Combinaison *Oa.Cs*	1,2 µl/100g	60 µl/100g
Combinaison *Lm.Cs.Oa*	0,9 µl/100g	45 µl/100g

Experimental set-up: The experimental set-up is a completely randomised block comprising eight treatments (T) with three replicates, including an absolute control. T0: absolute control; T1: treatment with *L. multiflora* essential oil ; T2 : treatment with *C. schoenanthus* essential oil; T3: treatment with *O. americanum* essential oil; T4 : treatment with combination of *L. multiflora* and *C. schoenanthus* essential oils; T5 : treatment with combination of *L. multiflora* and *O. americanum* essential oils; T6 : treatment with combination of *C. Schoenanthus* and *O. americanum* essential oils T7 : treatment with combination of essential oils of *L. multiflora*, *C. schoenanthus* and *O. americanum*.



A : Sanitary test

B : Soakedrice grains

C: Rice flour

Parameters assessed: To assess the efficacy of essential oils against *M. oryzae*, the percentages of germinated seeds and rotted grains were evaluated at 7^{th}day after inoculaton. For the germination test, the percentage of germinated grains, the percentage of sound ungerminated grains and the viability percentage of rice grains were assessed at 15^{th}, 30^{th} and 45^{th} day after storage (DAS). The viability percentage is obtained by adding the percentages of germinated grains (GG%) and sound ungerminated grains (GSNG%).

Statistical analysis: The data collected were entered into Microsoft Excel 2013 and then analysed using XLSTAT.2016 softwares. Analysis of variance and comparison of means were performed using the Fisher test at the 5% probability threshold.

RESULTS

Efficacy of essential oils against *M. oryzae*.

Percentage of germination: Figure 1 shows the effect of the different essential oil formulations on the percentages of germinated seeds. The analysis of variance shows a very highly significant difference between treatments. All the essential oil formulations showed a higher germination percentage than the control. The *L. multiflora* formulation recorded the highest germination percentage (96%) comparedwith the other formulations. The lowest germination percentage was recorded by the inoculated absolute control with a germination rate of 92%.

Rice seed rot: Figure 2 shows the effect of different essential oil formulations on the percentage of rotten seeds. Analysis of variance shows a highly significant difference between treatments. The *L. multiflora* formulation recorded the lowest percentage of rotted seeds (4%) compared with the other treatments. The absolute control recorded a value of 7.33% rotten seeds.

Germination capacity

Percentage of sprouted grains. Table 2 shows the percentage of germinated grains at the 15^{th}, 30^{th} and 45^{th}day after storage at the different doses of the powder formulations. The analysis of variance showed a highly significant difference between the different oil formulations at the 5% probability using Fisher's test. On the 15^{th} day after storage, the results showed that among the essential oil formulations, the highest percentage of germinated seeds was recorded by O. americanum essential oil (96.63%). At the 30^{th} and 45^{th} DAS, the formulation resulting from the combination of *L. multiflora* and *C. schoenanthus* essential oils recorded the highest percentage of germinated seeds, with values of 93.65% and 91.87% respectively. The formulation of pure essential oil of *L. multiflora* recorded the lowest percentage of germinated kernels through out the storage period, with 94.16% the 15^{th} DAS, 91.45% the 30^{th} DAS and 88.45% the 45^{th}DAS.

Percentage of ungerminated sound seeds: Table 3 shows the percentages of ungerminated sound seeds at the 15^{th}, 30^{th} and 45^{th} DAS. The results showed that the highest percentages of ungerminated sound grains at 15^{th} and 30^{th} DAS were recorded for the essential oil of *L. multiflora*, with values of 4.58% and 6.52% respectively. At the 45^{th} DAS, the results showed that the highest percentage of ungerminated sound grains was recorded by the combination of *O. americanum* and *L. multiflora*oils essential oil (6.89%). On the 15^{th}, 30^{th} and 45^{th} DAS, the lowest percentages of ungerminated sound grains were recorded by the essential oils of *C. schoenanthus* (2.45%), *O. americanum* (4.13%) and *C. schoenanthus* (5.07%) respectively.

Percentage of grain viability: Table 4 shows the viability percentages of the grains at the $15^{th,}$ 30^{th} and 45^{th} DAS. At 15^{th} DAS, the highest viability percentage was recorded by the formulation of *O. americanum* essential oil (99.33%). The combination of *L. multiflora* and *C. schoenanthus* essential oils recorded the highest viability percentages at 30th and 45th DAS, with values of 98.89% and 97.85% respectively. The combination of *O. americanum* and *C. schoenanthus* oils, the combination of *O. americanum* and *L. multiflora* oils and the pure essential oil of *C. schoenanthus* recorded the lowest viability percentages at 15th DAS (97.78%), 30th DAS (96.97%) and 45th DAS (94.75%) respectively.

Graphs assigned the sameletter are not statistically different at the 5% probability level.

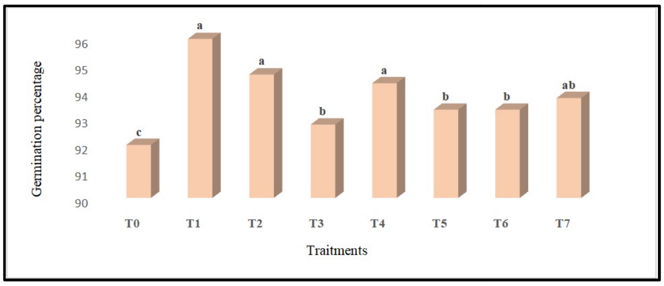


Figure 1. Germination percentage

Graphs assigned the sameletter are not statistically different at the 5% probability level.

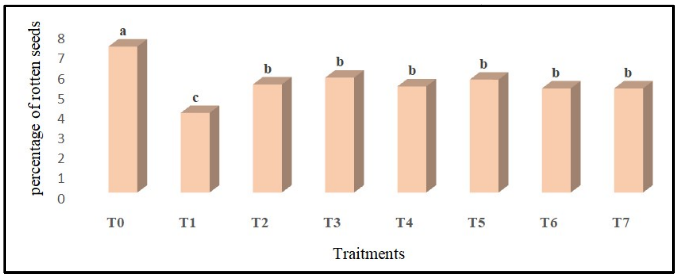


Figure 2. Percentage of rotted grains

Table 2. Percentage of germination of grains

Formulation	15 DAS	30 DAS	45 DAS
T0	100^{a}	100^{a}	99,33^{a}
Oa	96,63^{b}	93,10^{b}	90,33^{d}
Lm	94,16^{g}	91,45^{f}	88,45^{h}
Cs	96,12^{b}	92,79^{c}	89,68^{fg}
Oa.Lm	94,55^{ef}	91,54^{e}	88,78^{gh}
Oa.Cs	94,76^{de}	92,69^{cd}	89,97^{ef}
Lm.Cs	95,33^{c}	93,65^{b}	91,87^{b}
Oa.Lm.Cs	94,43^{f}	92,01^{d}	90,73^{c}
Prob	*0,001*	*0,001*	*0,001*
Sign	*THS*	*THS*	*THS*

Legend : Values in the same column marked with the same letter are not statistically different at the 5% probability level according to the Fisher test.

Table 3. Percentage of ungerminated sound grains

Formulation	15 DAS	30 DAS	45 DAS
T0	0^{g}	0^{f}	0^{f}
Oa	2,67^{e}	4,13^{e}	5,64^{d}
Lm	4,58^{a}	6,52^{a}	6,78^{b}
Cs	2,45^{f}	4,19^{d}	5,07^{de}
Oa.Lm	4,12^{b}	5,43^{b}	6,89^{a}
Oa.Cs	3,02^{d}	5,14^{c}	5,78^{cd}
Lm.Cs	3,87^{c}	4,24^{d}	5,98^{c}
Oa.Lm.Cs	4,33^{ab}	5,12^{c}	6,78^{b}
Prob	*0,001*	*0,001*	*0,001*
Sign	*THS*	*THS*	*THS*

Legend: Values in the same column marked with the same letter are not statistically different at the 5% probability level according to the Fisher test.

Tableau 4. Percentage of grain viability

Formulation	15 DAS	30 DAS	45 DAS
T0	100^{a}	100^{a}	99,33^{a}
Oa	99,33^{b}	97,23^{d}	95,97^{cd}
Lm	98,74^{c}	97,97^{c}	95,23^{f}
Cs	98,57^{d}	96,98^{d}	94,75^{g}
Oa.Lm	98,67^{cd}	96,97^{d}	95,67^{e}
Oa.Cs	97,78^{e}	97,83^{cd}	95,75^{de}
Lm.Cs	99,2^{b}	98,89^{b}	97,85^{b}
Oa.Lm.Cs	98,76^{c}	98,13^{bc}	97,51^{bc}
Prob	*0,001*	*0,001*	*0,001*
Sign	*THS*	*THS*	*THS*

Legend: Values in the same column marked with the sameletter are not statistically different at the 5% probability level according to the Fisher test.

DISCUSSION

M. oryzae is a fungus that can infest stored rice seeds. The application of powdery essential oil formulations of *L. multiflora*, *C. schoenanthus* and their combination to seeds artificially contaminated by this fungus demonstrated the antifungal effect of these essential oil formulations resulting in a reduction in seed infection compared to untreated contaminated seeds. The inhibitory effect of these essential oil formulations could be attributed to the majority compounds in the chemical molecules, such as piperitone (69.01%) in *L. multifmora* essential oil (Koffi *et al*., 2006), or to the synergistic or additive effect of the chemical compounds (Ambindei*et al.*, 2014). The efficacy of essential oils in seed protection was reported by Mahomed *et al.* (2022), who showed the fungicidal effect of three formulations based on essential oils of *L. multiflora*, *C. schoenanthus* and *O. americanum* in protecting cowpea seeds. Also, the work of Adjou & Aoumanou (2013) on fungi isolated from groundnuts in Benin, showed antifungal activity of essential oils and aqueous extracts of *O. americanum* on several toxigenic strains of the genus *Aspergillus* and *Fusarium oxysporum*. Another specie of the same genus, *O. basilicum*, inhibited at different concentrations, fungi influencing the germination of maize and cowpea (Koffi *et al.,* 2006). Zidda *et al*. (2008) also showed the efficacy of *C. schoenanthus* essential oils against sorghum and millet seed-borne fungi. Similar effects of essential oils from other plants belonging to the *Cymbopogon* genus have also been observed. Bonzi *et al.* (2013) also showed the antifungal efficacy of essential oils on various seeds. It should be considered that treatments with essential oil formulations could constitute an alternative to chemical products in the fight against rice fungi in storage. The work of Gbogbo *et al.* (2006) carried out on maize and cowpea seeds in the presence of phytopathogenic and toxinogenic fungi in Togo showedthat the essential oils of *O. basilicum* and *C. schoenanthus* showed better antifungal activity in inhibiting the reduction in the germination rate of the seeds of these two crops. According to Hannin *et al*. (2003), infection of grains by major pathogensoften affects their germination rate and leads to a reduction in seedling vigour and even to dieback. According to these authors, seed deterioration increases when humidity and temperature conditions are favourable to the development of microorganisms. The percentage of viability of sound seeds under the effect of essential oils decreases as the seeds age. Seed viability declines slowly at first, then more rapidly as the seeds age (Kameswara*et al.,* 2006).

CONCLUSION

The tests carried out in this study on essential oil formulations against *M. oryzae* in seed treatment showed that all the essential oils had an impact in inhibiting the development of *M. oryzae*. Formulations of pure oils of *L. multiflora* and *C. schoenanthus* and their combination showed the best antifungal results. These formulations had an effect on rice seed germination by inhibiting the action of *M. oryzae*. However, they had a dormant effect on rice seeds, reducing their germinative capacity over time. These results attest to the essential oils potential as a natural means of controlling *M. oryzae* in seed treatments. These essential oil formulations can be further improved and used to preserve seeds in storage.

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