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Effects of three essential oils on the phytohormones production against *Magnaporthe oryzae* B.C. Couch, A rice blast pathogen

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ABSTRACT

This study aimed to evaluate the effect of three essential oil formulations on the defence mechanism of rice plants against *Magnaporthe oryzae*, a rice blast pathogen in Burkina Faso. The experimental set-up was a randomised block of five treatments, including T0, T1, T2, T3 and T4. Rice plants were inoculated with *M. oryzae* on the 14th day after transplanting and essential oils were applied one week after inoculation. The biomass of the rice plants was collected at the full tillering (FT) and paniclar initiation (PI) stages and then sent to the laboratory. The parameters assessed were the levels of signalling and defence molecules. The results showed that for signalling molecules, high ethylene levels were recorded by treatments T2 in FT (6.61 mg EA/g) and T4 in PI (5.59 mg EA/g). The highest salicylic acid contents were obtained by treatment T1 with 106.42 mg/100 g in FT and 96.98mg/100 g in PI. In terms of defensive phytocompound content, treatment T4 recorded the highest levels of polyphenols (256.33 mg EA/100 g) and flavonoids (74.58 mg EA/100 g) in FT. The highest alkaloid and chlorophyll contents were recorded in treatment T2. These were 33.9 mg EA/g in FT and 26.35 mg EA/g in PI for alkaloids, 1.54 g/100 g in FT and 1.78 g/100 g in PI for chlorophyll a and 1.73 g/100 g in FT and 1.77 g/100 g in PI for chlorophyll b. These results are importants because they will enable to control *M. oryzae* in rice fields.

Key words: *Oryza sativa*, Defence mechanism, *Magnapothe oryzae*, Essential oils, Burkina Faso

INTRODUCTION

Rice cultivation in Burkina Faso faces many constraints, including fungal diseases such as blast caused by Magnaporthe oryzae. This pathogen is responsible for major production losses (Bouet *et al.,* 2012; Kassankogn, 2016). The work of Adjou and Aoumanou (2013) has shown that several control methods are used, including the use of essential oil formulations for biocontrol of fungal diseases as alternatives to synthetic fungicides. Essential oils are a very promising alternative without being a source of danger to human health or environmental pollution (Gamsore *et al.,* 2018). According to Isman *et al.* (2011), the use of essential oils in plant protection is still in its infancy, but these products have the fungicidal potential to replace synthetic fungicides. Previous studies on certain oils with antifungal potential have shown that their ability to counter the pathogen is explained partly by their chemical composition and partly by their ability to stimulate cells producing defence compounds under stress (Ambindei *et al.,* 2014). The essential oils of *Cymopogon schoenanthus* and *Lippia multiflora* and their combination applied to rice plants infested by *M. oryzae* considerably reduced the incidence and severity of the disease (Ouattara *et al.*, 2023). Unfortunately, their synergistic effects and their mechanisms of action in stimulating the biosynthesis of defence compounds by the rice plant have not yet been clearly established. However, knowledge of the mechanisms of action of these oils may enable a good pathogen management strategy to be put in place. The fundamental question underlying this study is to determine the effect of essential oils of *C. schoenanthus* and *L. multiflora* on the biosynthesis of rice signalling and defence molecules. The aim was to assess the effect of applying essential oils on the levels of signalling and defence molecules in rice plants.

MATERIALS AND METHODS

Biological material

The plant material used was the FKR64 variety, which is known to be susceptible to blast. The *M. oryzea* strain BF201 used was isolated from rice leaves collected at the Farakô-Bâ site and was chosen because of its high level of virulence.

Essential oils

The essential oils of *C. schoenanthus* (Lm) and *L. multiflora* (Cs) used for the tests were extracted from the leafy branches. These oils were used pure (100%) and then in combination (50% Lm + 50% Cs).

Experimental design and plant inoculation

The trial was conducted in a semi-controlled environment using a completely randomised block design with three replicates each. The treatments consisted of formulations of essential oils of *L. multiflora*, *C. schoenanthus* and their combination. A control inoculated (T1) with *M. oryzae* and a neutral control (T0) were used. After pre-sprouting the seeds, they were transplanted into pots previously filled with sterilised potting soil at a rate of one plant per pot. Inoculum was then prepared to obtain a final concentration of 10^4 spores/ml. Inoculation was carried out on the 14^{th} day after transplanting by spraying the conidial suspension onto the leaves of the rice plants.

Dosage and application technique for essential oils

The essential oils of *C. schoenanthus* and *L. multiflora* and their combination, recognised as highly effective at minimum inhibition doses of 0.6 µl/ml, 1.5 µl/ml and 0.3 µl/ml respectively in the *in vitro* antifungal evaluation of mycelial growth using the direct contact method, were used at the same doses for the in planta tests. The essential oils were applied one week after inoculation of the rice plants to allow them to be infected by *M. oryzae* (Kassankogno, 2016).

Rice plant biomass collection

Fresh biomass from rice plants was collected at the full tillering (FT) stage and at the panicular initiation (PI) stage. It was then sent directly to the Biochemistry, Food Technology and Nutrition Laboratory at Joseph KI-ZERBO University, where it was ground in a fresh state for the determination of phytocompounds according to the treatments carried out.

Phytocompound extraction and assay technique

Signalling molecules

Salicylic acid and ethylene were extracted and assayed using the method described by Yang *et al.* (2018).

Defence molecules

Chlorophyll pigments were extracted and assayed using the method described by Lazzarotto and Maherou (2010). Flavonoids and polyphenols were extracted and assayed using the method described by Sombié *et al.* (2018). Alkaloids were extracted and assayed using the spectrophotometric method described by John *et al.* (2014). Ferric Reducing Antioxidant Power (FRAP) was determined using the method described by Sombié *et al.* (2019).

Statistical analysis

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

RESULTS

Effect of essential oils on levels of signalling molecules

Figs 1 and 2 show, respectively, the ethylene and salicylic acid contents of fresh rice leaves at the FT and PI stages as a function of the treatments applied.

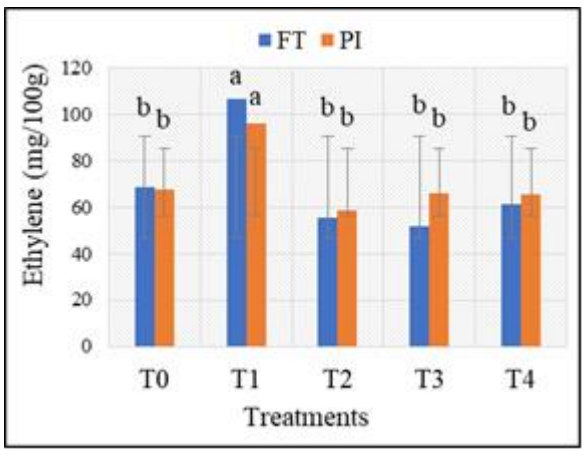

Fig. 1. Ethylene content

Analysis of variance shows a significant difference between treatments. For ethylene, the results show that the highest levels were recorded by treatments T2 at the FT stage and T4 at the PI stage, with values of 6.61 mg EA/g and 5.59 mg EA/g respectively. Treatments T4 and T0 recorded the lowest levels with respective values of 2.15 mg EA/g in FT and 2.92 mg EA/g in PI. For salicylic acid, the results showed that the highest levels were recorded by treatment T1 at the FT stage (106.42 mg/100g) and at the PI stage (96.98mg/100g). The lowest levels were recorded by treatments T2 and T3 with values of 58.65 mg/100g at the PI stage and 51.73 mg/100g at the FT stage respectively.

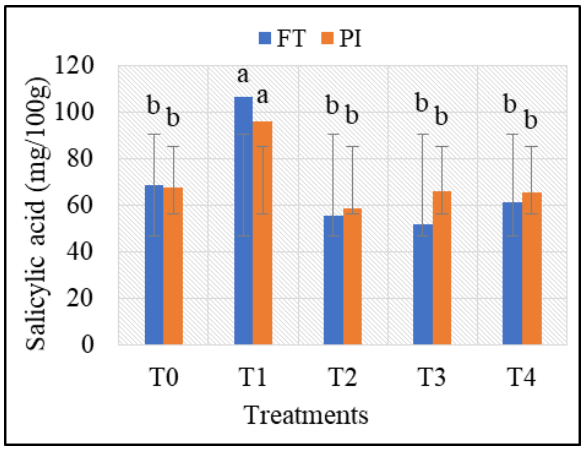

Fig. 2. Salicylic acid content

Effect of essential oils on the content of defence molecules

Figs 3, 4, 5 and 6 show the contents of total polyphenols, flavonoids, alkaloids and proteins in the fresh aerial organs of rice plants at the FT IP stages as a function of the essential oil formulations applied.

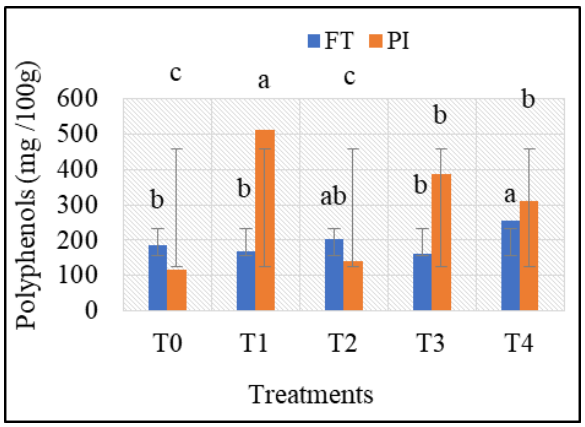

Fig. 3. Polyphenol content

Analysis of variance showed a significant difference between the treatments, except for proteins, which showed no significant difference. The determination of total polyphenols revealed that the highest levels were recorded by treatments T4 at the FT stage and T1 at

the PI stage, with values of 256.33 mg EA/100 g FM and 512.34 mg EA/100 g FM respectively. The lowest levels were recorded by the To control with values of 186.47 mg EA/100 g FM at the FT stage and 116.86 mg EA/100 g FM at the PI stage. For flavonoid content, the results show that treatments T4 and T2 recorded the highest values, respectively 74.58 mg EA/100 g FM in FT and 60.56 mg EA/100 g FM in PI. Treatments T2 and To recorded the lowest levels of FT (60.57 mg EA/100 g MF) and PI (49.61 mg EA/100 g FM) respectively.

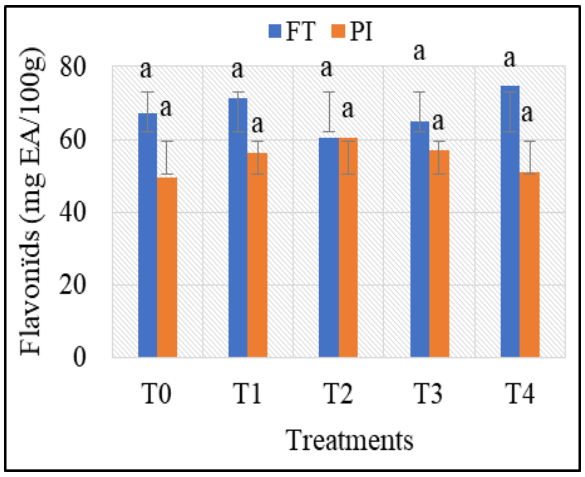

Fig. 4. Flavonoid content

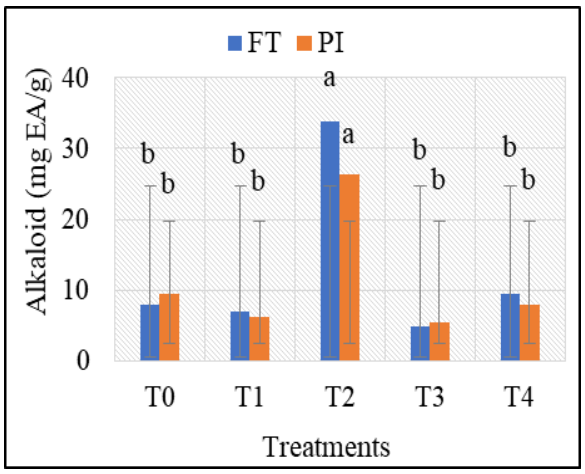

Fig. 5. Alkaloid content

With regard to alkaloid levels, the results show that the highest levels were recorded in treatment T2, with values of 33.9 mg EA/g FM at the FT stage and 26.35 mg EA/g FM at the PI stage. The lowest levels were observed in treatment T3 with values of 4.94 mg EA/g at the FT stage and 5.53 mg EA/g at the PI stage. As for protein, the results show that the highest levels were recorded by treatment To with 6.54 mg EA/g of FM at the FT stage and 6.26 mg EA/g of FM at the PI stage. The lowest levels were recorded by treatments T3 at the FT stage and T2 at the PI stage, with values of 6.07 mg EA/g FM and 6.23 mg EA/g FM respectively.

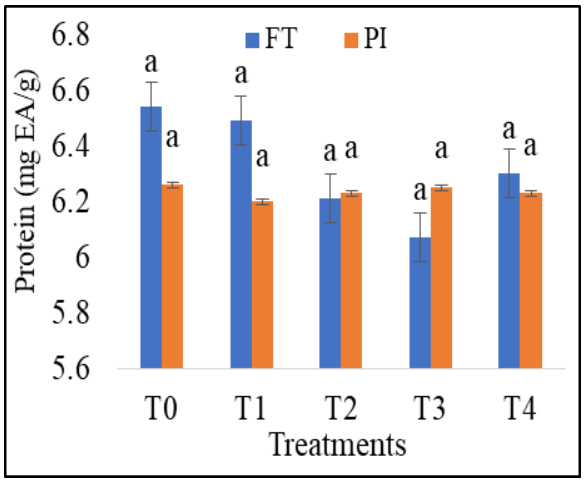

Fig. 6. Protein content

The chlorophyll a and b contents and antioxidant activity (FRAP) of fresh rice leaves in FT and PI according to the treatments applied are presented in Table 1. The results show that treatment T2 recorded the highest chlorophyll a and b levels. These contents were 1.54 g/100 g FM in FT and 1.78 g/100 g FM in PI for chlorophyll a and 1.73 g/100 g MF in FT and 1.77 g/100 g FM in PI for chlorophyll b. Treatment To recorded the lowest levels of chlorophyll a and chlorophyll b in FT, with values of 0.39 g/100 g FM and 0.38 g/100 g FM respectively. In PI, the lowest chlorophyll a and b contents were recorded by treatment T1 with values of 0.55 g/100 g FM and 0.61 g/100 g FM respectively. The results show that treatments T3 and T4 recorded the highest FRAP values of 115.23 mg EAA/L in FT and 107.28 mg EAA/L in PI respectively. Treatment To recorded the lowest values for FT (83.23 mg EAA/L) and PI (66.67 mg EAA/L).

Table 1. Chlorophyll content and antioxidant activity (FRAP)

Treatments	Chlorophyll a (mg/100 mg)		Chlorophyll b (mg/100 mg)		FRAP (µg EAA/g MS)	
	FT	PI	FT	PI	FT	PI
T1	0.90[a]	0.55[b]	0.93[a]	0.61[a]	101.60[a]	87.22[a]
T2	1.54[a]	1.78[a]	1.73[a]	1.77[a]	109.26[a]	107.28[a]
T3	1.00[a]	1.64[a]	0.94[a]	1.51[a]	98.87[a]	99.63[a]
T4	1.64[a]	1.58[a]	1.26[a]	1.63[a]	115.23[a]	69.19[a]
To	0.39[b]	1.62[a]	0.38[a]	2.03[a]	83.23[a]	79.52[a]
Pr > F	0.003	0.003	0.074	0.074	0.563	0.563
Signification	HS	HS	NS	NS	NS	NS

DISCUSSION

Treatments with *L. multiflora* and *C. schoenanthus* essential oils and their combination increased the production of ethylene, alkaloids and chlorophyll a and b and phenolic compared with the neutral and inoculated controls. This ability of essential oils to stimulate the production of ethylene, alkaloids, chlorophyll a and b and phenolic compounds can be explained by their content of chemical elements that act on the plant's production cells under conditions of biotic stress (Ambindei *et al.*, 2014). For Derra *et al.* (2022), the mode of action of phenolic compounds is linked to their antimicrobial properties, their role in strengthening plant cell walls and their ability to modulate and induce host defence reactions. The increase in the levels of these phytocompounds could also be explained by their involvement in the process of plant defence against *M. oryzae*. According to Sombié *et al.* (2019) and Vagiri *et al.* (2017), phenolic compounds are involved in constitutive defence mechanisms and levels may increase during infection. The work of Xu *et al.* (2011) has shown that chlorophyll pigments can play an essential role in the defence of plants against pathogens. Faced with a pathogen, plants deploy a defensive arsenal by stimulating cells that produce defence phythormones. A drop in alkaloid and ethylene content from the full tillering stage to the panicle initiation stage was recorded in all treatments. The more the plant develops, the better integrated it is into its environment, with the development of other defence systems. The content of these phytocompounds therefore decreases with the age of the plant. This decrease can be explained by the fact that the plant is able to adapt to its environment by modifying its cell walls, creating a barrier or preventing the penetration of pathogens. According to Rouxel (1989), plants have the capacity to react to bio-aggressors through numerous physiological and biochemical modifications, including disruption of cell permeability and stimulation of the production of phytohormones such as ethylene, a low molecular weight antimicrobial compound. For flavonoid content, no significant difference was recorded between treatments. This indicates that the essential oils have no effect on the production of flavonoids within the plant. For Ferric Reducing Antioxidant Power, no significant difference was recorded between the treatments of essential oils and the inoculated control. This indicates that the essential oils have no effect on the Ferric Reducing Antioxidant Power within the plant. Furthermore, the results showed that Ferric Reducing Antioxidant Power and flavonoid content were more abundant during the Full tillering stage when the plants were young. These results confirm those of Nanema *et al.* (2017) who showed through a study carried out on aerial organs of *Faidherbia albida,* that phytohormone contents were more abundant in the leaves of the youngest plants compared with the oldest plants. In 2015, these authors also reported the existence of correlations between different phytohormones, showing that the production of some had a negative impact on that of others (Nanema *et al.,* 2015).

CONCLUSION

This study enabled us to understand that the essential oils of *L. multiflora* and *C. schoenanthus* and their combination have a mode of action on the physiological functioning of rice plants under conditions of biotic stress by stimulating the production of defence and signalling phytohormones.

The application of essential oils leads to an increase in the levels of defence phytocompounds such as alkaloids, ethylene and chlorophyll a and chlorophyll b. This effect is particularly strong in young plants, which are more fragile and more susceptible to fungal attack. The levels of these phytocompounds decrease as the plant develops and ages. These results are of vital importance for fundamental research, as they will provide a better understanding of the mechanism and enable the development of defence and protection strategies at the physiological level of plants against fungal agents in particular and bioaggressors in general.

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