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ANTIFUNGAL EFFICIENCY OF PLANTS AQUEOUS EXTRACTS WITH POTENTIAL FOR CONTROL OF *Cercospora oryzae* MIYAKE IN RICE

H.B. SOURA[1, 2], L.S.A. GNANCADJA[2], E. GOUDJO[2], A. ATTADEOU[2], L. BABA-MOUSSA[3] and A. SAVADOGO[4]

[1] University of Fada N'Gourma, Institut Superieur for Substainable Development, Fada N'Gourma, Burkina Faso
[2] University of Abomey-Calavi, Department of Plant Biology, Laboratory of Plant Physiology and Study of Environmental Stresses, Faculty of Science and Technology, Cotonou, Benin
[3] University of Abomey-Calavi, Department of Biochemistry and Cell Biology, Laboratory of Biology and Molecular Typing in Microbiology, Faculty of Science and Technology, Cotonou, Benin
[4] University of Joseph Ki-Zerbo, Department of Biochemistry Microbiology, Laboratory of Biochemistry and Applied Immunologies, Training and Research Unit in Life and Earth Sciences, Ouagadougou, Burkina Faso
Corresponding author: sbanemane@yahoo.fr

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ABSTRACT

Rice (*Oryza* spp.) is the third most cultivated cereal crop in Benin, and major staple in the population's daily diets. Its production, though is heavily impaired by fungal diseases, of which cercosporios accounts for about 60% yield loss. The objective of this study was to examine the efficacy of plant aqueous extracts for control of *Cercospora oryzae* diseases for improved rice production in Benin. We assessed the *in vitro* effectiveness of plant extracts on the sporulation and mycelial growth of *C. oryzae*, by evaluating the effectiveness of extracts on the foliar and panicular incidence, as well as the epidemic of *C. oryzae* in the field. A randomised complete block design arranged in split plots, was laid out with three spraying replications. The study combined phytiatric tools. Extracts from *Lippia multiflora, Azadirachta indica* and *Eucalyptus camaldulensis* at concentrations of 10 and 15% showed antifungal efficacy on *Cercospora oryzae in vitro* and in the field. Extracts of *Cymbopogon citratus* and *Citrus limon* did not exhibit antifungal efficacy against *Cercospora oryzae*.

Key Words: *Azadirachta indica*, *Cercospora oryzae*, phytiatric tools

RÉSUMÉ

Le riz (*Oryza* spp.) est la troisième céréale la plus cultivée au Bénin et l'aliment de base de l'alimentation quotidienne de la population. Sa production, cependant, est fortement altérée par les maladies fongiques, dont les cercosporios représentent environ 60% de perte de rendement. L'objectif de cette étude était d'examiner l'efficacité des extraits aqueux de plantes pour le contrôle des maladies de *Cercospora oryzae* pour l'amélioration de la production de riz au Bénin. Nous avons évalué l'efficacité *in vitro* d'extraits végétaux sur la sporulation et la croissance mycélienne de *C. oryzae*, en évaluant l'efficacité d'extraits sur l'incidence foliaire et paniculaire, ainsi que sur l'épidémie de *C. oryzae* au champ. Une conception en blocs complets randomisés disposés en parcelles divisées a été disposée avec trois répétitions de pulvérisation. L'étude a combiné des outils phytiatriques. Des extraits de *Lippia multiflora*, *Azadirachta indica* et *Eucalyptus camaldulensis* à des concentrations de 10 et 15% ont montré une efficacité antifongique sur *Cercospora oryzae in vitro* et au champ. Les extraits de *Cymbopogon citratus* et *Citrus limon* n'ont pas montré d'efficacité antifongique contre *Cercospora oryzae*.

Mots Clés : *Azadirachta indica, Cercospora oryzae*, outils phytiatriques

INTRODUCTION

Rice (*Oryza* spp.) cultivated mainly for its grains, is a fundamental food security crop the world over (Zeigler and Barclay, 2008; CCR-B, 2014). The main cause of rice crop yields decline either in quantity or in quality in sub-Saharan Africa is due to pathogenic microorganisms, which develop and severely disrupt plant development (Bouet *et al.*, 2012). Fungi constitute the main causative agents of the majority of pathotypes associated with poor rice yields (Elbouchtaoui *et al*., 2015). Fungal diseases can damage crops and lead to damping of the inflorescences largely caused by the genus *Curvularia* and *Nigrospora* and also leaf diseases such as downy mildew.

The contol of fungal diseases in rice production is mostly by use of synthetic fungicides (Harris and Mohammed, 2003). Although effective and accessible, synthetic fungicides remain expensive for for low resourced rice producers mainly in sub-Saharan Africa. Additionally, their use induces significant ecological risks by decimating otherwise vital on flora and fauna, water and air pollution leading to human health problems, such as poisoning, skin and eye irritations, and cancer (Babar *et al*., 2011). These risks are exacerbated by the limited knowledge among rural populations on the safe use of these synthetic fungicides.

Promotion of biological pesticides sourced from local plants constitutes an interesting alternative method for controlling crop diseases without disrupting the environmental balance (Amadioha, 2000; Iftikhar *et al*., 2010). Several studies focusing on natural pesticides, sourced from plant compounds have shown potent activities such as for *A. indica* and *E. camaldulensis* used against parasitic fungi of cereals (Somda *et al*., 2007; Uddin *et al*., 2010; Katooli *et al*., 2011). In addition, the antifungal properties of some plants such as *Cassia occidentalisis* (Bonzi, 2005), *A. indica*, *S. longepedonculata* and *P. oleracea* (Kabore *et al*., 2007), and *Lippia multiflora* (Koita *et al*., 2012) have been approved. Extracts of many plants available locally in sub-Saharan Africa seem to have prominent fungicidal properties that can be leveraged for containment of pathological problems in rice. Because they are natural, local plant pesticides do not threaten their biotope and could be less costly because of their local availability. Several plants endowed with such potential fungicidal activity are yet to be explored for rice production in sub-Saharan Africa. The objective of this study was to explore antifungal efficacy of local plant aqueous extracts against cercosporiosis caused by *Cercospora oryzae* in rice production in Benin.

MATERIALS AND METHODS

Study site. The study was conducted in the Docomey village, located in Zinvié district, in Abomey-Calavi municipality in South Benin.

The site is found at an altitude of 12 m above see level and is inhabited by a population of 307,745 inhabitants (INSAE, 2016).

Rice varieties used. The intraspecific variety TS2, cercosporiosis sensitive, was in this study. This choice was made due to its ability to express disease symptoms and thus ease of efficacy assessment.

Plant extracts used. Five aqueous plant extracts from leaves of *Cymbopogon citratus*, *Lippia multiflora*, *Eucalyptus camaldulensis*, *Citrus limon* and *Azadirachta indica* (Table 1), were used in the study (Photos 1, 2, 3, 4 and 5). These plant species have shown antifungal and antibacterial efficacy on other pathogens and on a variety of crops.

The leaves of the plant species were dried in the shade, and then pounded into power using porcelain mortar (Koita *et al*., 2012). Sieving was carried out for the powders, which still contained coarse particles. Aqueous extracts were obtained from maceration of each plant sample powder in distilled water, for 72 hours, at room temperature. At the end of the maceration, the mixture was filtered using a fine cloth.

The control fungicide. Benomyl was used during the study as a reference control. It is a systemic fungicide recommended for field use on aerial parts of rice (Hassikou *et al*., 2002). It was applied at the recommended rate of 3 kg ha^{-1}, equivalent to 40 g per 20 litre sprayer.

Preparation of isolates and *in vitro* treatment methods. *Cercospora oryzae* isolates were obtained from leaf lesions and cultivated on rice flour culture medium. After solidification of the prepared media, a fungus explantate was taken from a 14-day-old colony, and placed in the centre of each Petri dish, where 1 ml of every vegetable extract solution to be tested was deposited. Petri dishes were incubated at 28 °C under ultraviolet light for 12 hours, alternated with 12 hours of darkness (Mathur and Kongsdal, 2003). The concentrations of aqueous extracts tested were 5, 10 and 15%, or respectively 5, 10 and 15 g of powder from plants macerated in 100 ml of distilled water. Mycelial growth was measured at three day intervals starting from the third day up to the tenth day after incubation. Mycelial growth measurement was done according to two diameters of the Petri dish. Two controls were used, namely a "water" control (WW) and a "fungicide" control (WF) (leaves treated with benomyl).

Field experimental setup. In the fields, the solution obtained from the plant extracts was sprayed foliarly as soon as the first fungal disease symptoms appeared, using a sprayer at concentrations of 10%, namely 100 g of powder for 1000 ml of water; and 15%, namely 150 g of powder for 1000 ml of water. A total of 26 treatments were administered.

The experiment was set up in a randomised complete block design, with three replications. Each plot consisted of 6 rows, with 0.15 m

TABLE I. Plant extracts used in the study of bio-fungicdes in rice

Plant species	Plant part used	Active compounds
Lippia multiflora	Leaf	Polyphenol, flavonoid, alcaloid, thymol, carvacrol
Cymbopogon citratus	Leaf	Citral, mycene, geraniol, folic acid
Eucalyptus camaldulensis	Leaf	Eucalyptol, flavonoid, resin, tannin
Citrus limon	Leaf	Bioflavonoid, pectin, carotenoid, coumarin
Azadirachta indica	Leaf	Azadirachtin, oleic acid , stearic, palmitic acid, linoleic, myristic

Photo 1. Dry leaves of *Cymbopogon citratus.*
Source: Soura, 2018.

Photo 2. Dry leaves of *Eucalyptus camaldulensis*.
Source: Soura, 2018.

Photo 3. Dry leaves of *Lippia multiflora.*
Source: Soura, 2018.

Photo 4. Dry leaves of d'*Azadirachta indica*.
Source: Soura, 2018.

Photo 5. Dry leaves of *Citrus limon.*
Source: Soura, 2018.

within rows and, 0.30 m between rows with inter-plot separations of 1 m.

Data collection. Data collected included efficacy of plant extracts on mycelial growth and sporulation, the diameter of the fungus colony developed in the Petri dishes measured in cm using a circometer, from the 3rd day until the 10th day incubation. Efficacy of treatments for leaf incidence, on the development of the epidemic and on the panicular incidence of cercosporiosis were assessed every 7 days from onset of early symptoms to seed maturity on the IRRI-2002 9-point scale, whereby the scoring was *viz.* (1) no symptoms, (2) less than 1% diseased leaf area, (3) 1-3% diseased leaf area, (4) 4-10% diseased leaf area, (5) 11-15% diseased leaf area, (6) 16-25% diseased leaf area, (7) 26-50% diseased leaf area, (8) 51-75% diseased leaf area, and (9) 76-100% diseased leaf area.

Statistical analysis. The data collected were entered in an Excel Spreadsheet, before being subjected to analysis of variance using R statistical Software. Significant of means were separated using the SNK test at 5% level of significance.

RESULTS

Mycelial growth and sporulation of Cercospora oryzae. Figure 1 shows the effect of different treatments on mycelial growth and sporulation of *C. oryzae*. The various plant extract treatments had a suppressive effect on the rate of mycelial growth and net sporulation of the fungi. Three days after inoculation, the water control (WW) and all the 5% treatments, except that of the fungicide control (WF); showed slight growth. On the fifth day, mycelial development occcured in treatments without sporulation T1 (*L. multiflora* at 5%), T4 (*C. citratus* at 5%), T5 (*C. citratus* at 10%),

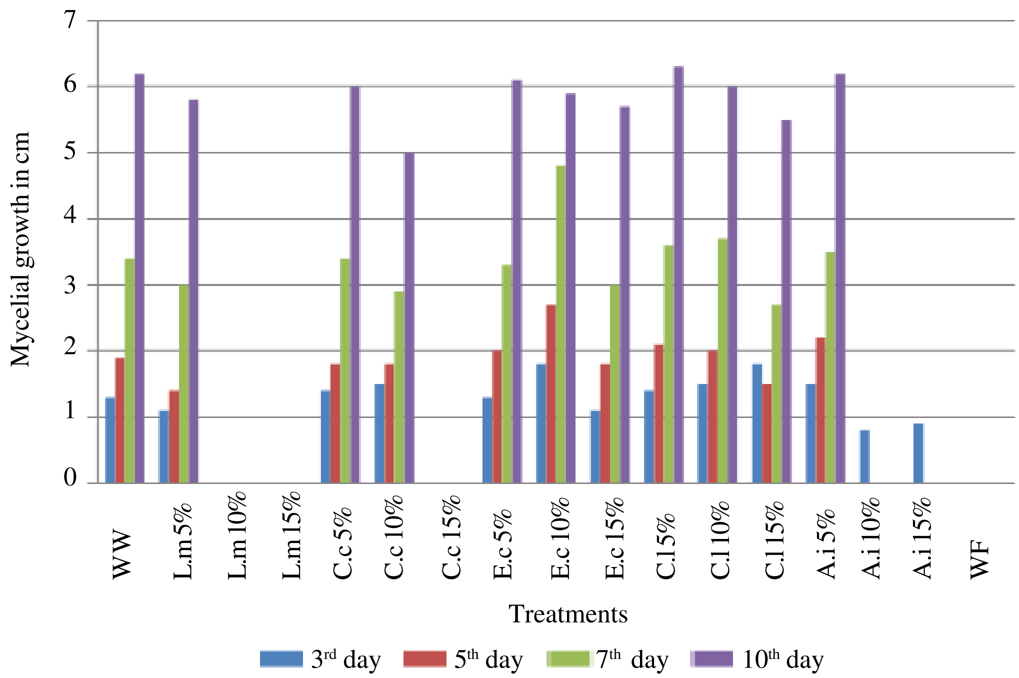

Figure 1. Effect of treatments on mycelial growth and sporulation of *Cercospora oryzae*. WW = Water control, L.m = *Lippia multiflora*, C.c = *Cymbopogon citratus*, *Eucalyptus camaldulensis*, C.l = *Citrus limon*, A.i = *Azadirachata indica*, W.F = Fungicide control.

T7 (*E. camaldulensis* 5%), T10 (*C. limon* 5%), T11 (*C. limon* 10%) and T13 (*A. indica* 5%). From day 3 to day 10 after incubation, some extracts totally inhibited growth of the fungus; thus precluding sporulation. These included the aqueous extracts of *Lippia multiflora* at the concentrations of 10 and 15%; *Azadirachata indica* at both concentrations; and *Cymbopogon citratus* at the concentration of 15%.

Incidence of leaf spot. Figure 2 shows the incidence of cercosporiosis in rice fields in Docomey. The incidence decreased starting from days 56 after sowing; but this varied from plant species extract to another. This decrease in disease incidence was temporal, but stabilised at the end of the plant development cycle.

Lippia multiflora recorded a decrease in disease intensity from day 56 to 77 after sowing (DAS), for the two concentrations used; as well as with the fungicide control, WF. Treatment T17 (*L. muliflora* 15%) obtained better results compared to T16 treatment (*L. muliflora* 10%). T17 treatment recorded decreases in the incidence ranging from 2.21 to 0.87% from day 56 to 77; while that of T16 treatment led to decreases in the incidence of the disease by 2.72 to 6.83% from the 56th to the 77th DAS.

Cymbopogon citratus had no significant effect on disease incidence for the two treatments (T18 and T19). But T19 treatment (*C. citratus* 15%) performed better compared to the T18 treatment (*C. citratus* 10%). T19 treatment recorded disease incidence results ranging from 3.48 to 6.11% from the 56th to the 77th DAS. T18 treatment led to reductions in the incidence of the disease from 3.58 to 6.55% from 56 to 77th DAS.

Eucalyptus camaldulensis recorded a decrease in the incidence of the disease from 56th to 77th DAS for the two treatments used. T21 (*E. camaldulensis* at 15%) showed better results compared to T20 (*E. camaldulensis* at 10%). T21 recorded decreases in the incidence ranging from 2.15 to 1.98% from 56th to 77th DAS; while the T20 resulted in decreases in the incidence of 2.78 to 2.34% from 56th to 77th DAS.

Citrus limon had no significant effect on the disease incidence for the two doses used. T23 (*C. limon* 15%) showed better results

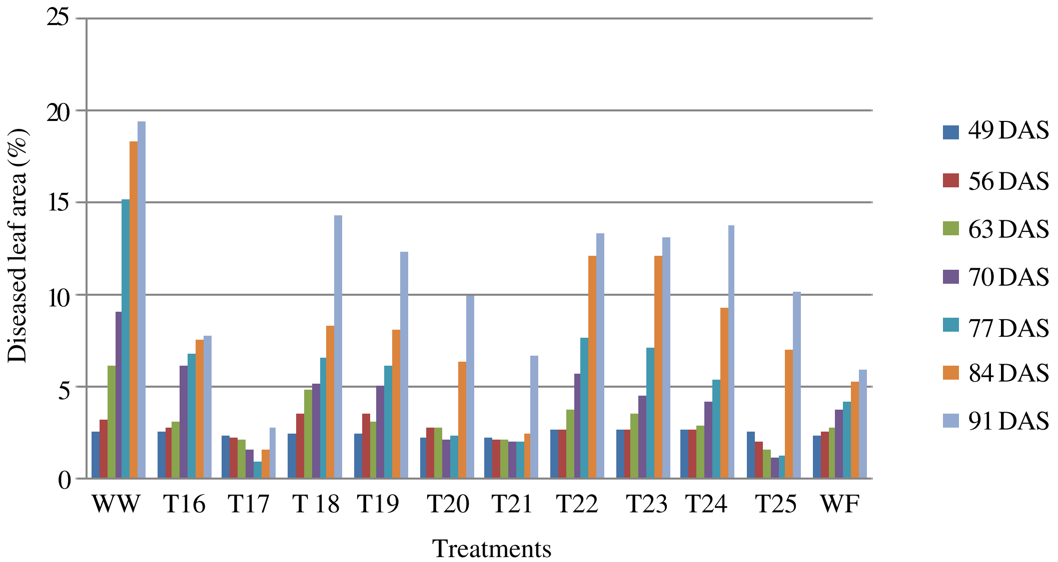

Figure 2. Post-treatment evolution of the incidence *cercosporiosis*. DAS = Day After Sowing; T = Treatment; TE = Water control; TF = Fungicide control.

compared to T22 (*C. limon* 10%). T23 recorded disease incidence ranging from 2.68 to 7.08%, from 56th to 77th DAS; while T22 led to a decrease in the disease incidence of 2.70 to 7.62% from 56th to 77th DAS.

Azadirachta indica also recorded a decrease in disease incidence from 56th to 77th DAS for the two extract concentrations. T25 (*A. indica* 15%) performed better than T24 (*A. indica* 10%). T25 decreased the disease incidence from 2.06 to 1.25% from 56th to 77th DAS; while T24 decreased the disease incidence of 2.63 to 5.33% from 56th to 77th DAS.

We observed a strong and increasing evolution of the foliar incidence of the disease over time, on the control plots treated with WW distilled water, which ranged from 2.54% at 49th DAS to 19.34% at the 91 DAS. For the plots treated with the second fungicide control, the results showed a slow progression of the disease from 2.5 to 5.9%, from 49 to 91 DAS.

Evolution after treatments of the epidemic of leaf spot of rice

Rate of progression of the epidemic after treatment. Figure 3 shows the rates of cercosporiosis epidemic progression (r) in Docomey during the second 2019 season. Weekly progression rates are represented by r1 (from 56 to 63 DAS), r2 (from 63 to 70 DAS), r3 (from 70 to 77 DAS), r4 (from 77 to 84 DAS), and r5 (from 84 to 91 DAS).

The rate of progression of the epidemic (r1) was low with treatments T17, T21, T25 or r1= 0.004- 0.009, and high with treatments T19 or r1 = 0.010 and T23 or r1 = 0.109.

From tillering to panicle initiation (56 DAS), the epidemic progression rates (r2 and r3) were 0.000 for treatments T17, T21, T25 and WF. T17 which had the weakest disease attack with 0.87% diseased leaf area (DLA) at the 77th DAS, had an epidemic progression rate

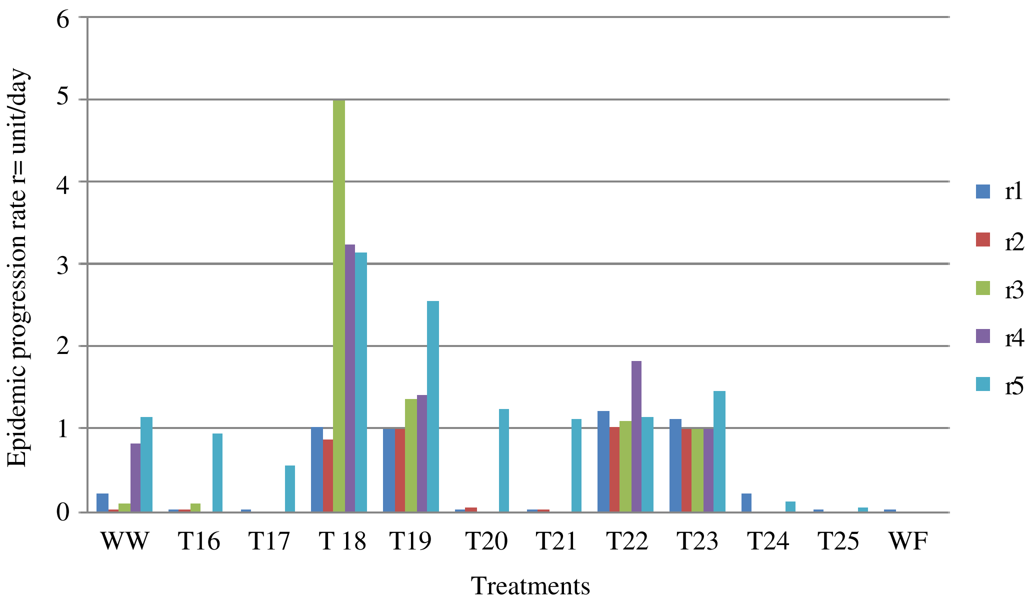

Figure 3. Variation after treatments in the rate of progression of the leaf spot outbreak. T = Treatment; TE = Water control, TF = Fungicide control; r = Regression rate.

(r2) of 0.000. This reflects absence of disease progression. From the start of the swelling panicle stage to heading (between 56 and 91 DAS), the progression rates r4 and r5, were variable for T16 (0.954%) and T20 (1.234%) in r4 and zero for T21 and WF in r4. When we consider the average rate of disease progression over the entire observation period (average r), we see that for three treatments T17, T21, T25 and TF, the rate was less than 0.35. This confirms the classification of the materials controlling cercosporiosis. For T19, this rate varied from 2.024 to 1.028. Most of the extracts of 10% concentration; with the exception of T16 and T20, were unable to supress the epidemic and, therefore, of the disease.

Incidence of panicular cercosporiosis. Figures 4 and 5 show the change in panicle incidence of cercosporiosis disease in the field; and the rate of progression of the panicle epidemic after treatment, respectively. This was evidence of a continuous regression of the cercosporiosis epidemic from the beginning of heading to the pasty grain stage of rice, that is from the beginning of heading to the milky grain stage (between 10 and 17 day after heading (DAH)). All plant extracts concentrations resulted in a decrease the level of the disease. Ten days after heading (DAH), no treatment indicated more than 20% of the panicles attacked. Treatments T17 (*L. Multiflora* at 15%), T21 (*C. Citratus* at 15%) and T25 (*A. Indica* at 15%) and WF demonstrated good efficacy of the extracts on the panicle, with incidences varying, respectively from 13.96 to 10.25%. From the milky grain to the pasty grain stage (between 24 and 31 DAH), treatments led to a decrease in the incidence of the disease to a maximum value of 19.87% of diseased panicles for T23(*C. Limon* at 15%). Treatments T16, T18, T20, T22, T24 were ineffectiveness against *C. oryzae* leaf attack. T17, T25 and WF were attacked by cercosporiosis, with an incidence of less than 13% of diseased panicles. The WW (distilled water control) treatment had an incidence ranging from 10.15 to 21.63, and was clearly greater than other treatments. The

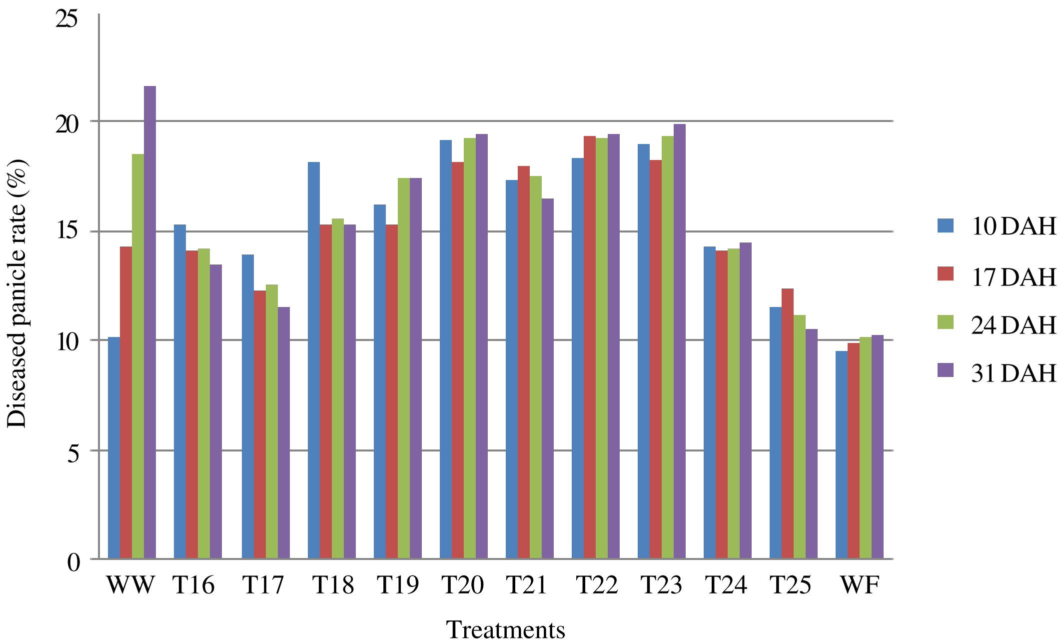

Figure 4. Incidence of panicle cercosporiosis after treatments. DAH = Day After Heading; T = Treatment; WW = Water control; WF = Fungicide control.

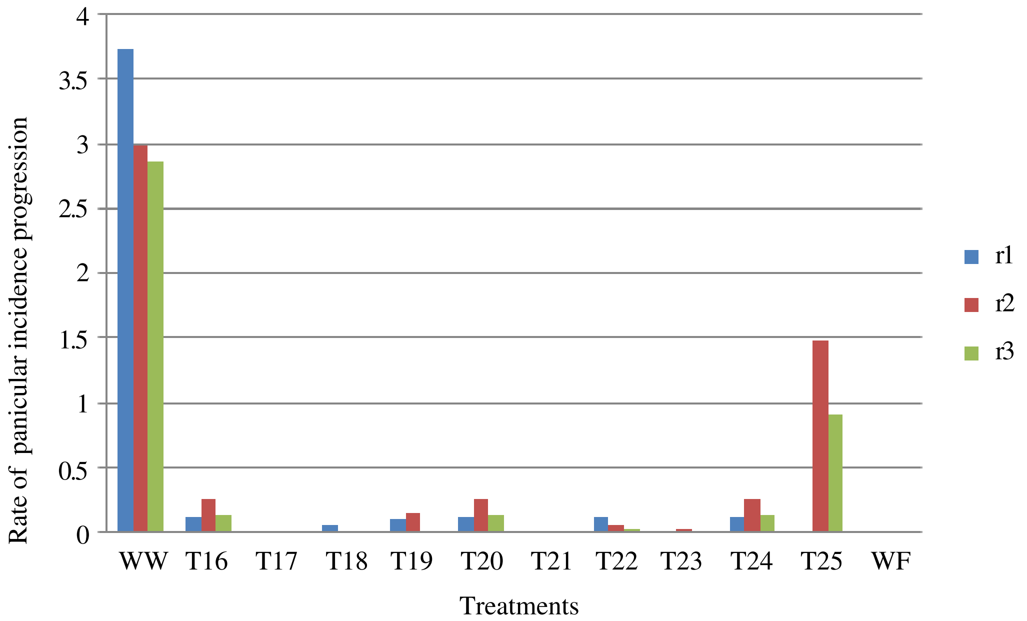

Figure 5. Rate of progression of panicular Cercosporiosis after treatments. T = Treatment; WW = Water control; WF = Fungicide control.

epidemic's progression rates ranged from 0.000 to 3.732 over the period covered by the observations. The progression of the disease was greatly delayed by T17 and T25 at the start of heading (r1 = 0.05 and 0.007). In contrast, it was explicit for other treatments such as T22 and T24, with progression rates of 0.057 and 0.127, respectively. The three treatments T17, T21 and T25, showed ability to slow down progression of the disease at the start of heading (r1); however, at the end of the cycle (24 DAH), three treatments (T17, T21 and T25) were found to be the most effective in slowing down the epidemic (r^2) of the disease.

DISCUSSION

Results of this study highlight the importance of damage of Cercosporiosis in the rice-growing fields in the Docomey Valley; and the potency of various plant extracts in controlling it. *In vitro*, most of the plant extracts significantly influenced mycelial growth and sporulation of the *C. oryzae*. The effectiveness of aqueous extracts on the development of the microorganism varied with concentrations. The aqueous extracts of *Lippia multiflora*, *Eucalyptus camaldulensis* and *Azadirachata indica* entirely inhibited mycelial growth and sporulation of *C. oryzae*. The effectiveness of these extracts could be explained by their antifungal properties, which likely suppressed mycelial production and sporulation of the fungus (Koita *et al*., 2012.).

The aqueous extracts of *Cymbopodon citratus* and *Citrus limon* at 5 and 10%, stimulated mycelial growth of *C. oryzae*. Their inefficiencies could be explained by the low concentrations used or by the resistance capacities of the microorganism concerned. The stimulation of mycelial growth could be explained by the fact that often the extracts act as sources of nutrients when the active compounds are in low doses, which is in agreement with the observation by Tiendrebeogo *et al*. (2017). On the other hand, this result could be attributed to the fact that thermal factors denatured the antifungal properties of plant extracts (Amadioha, 2000).

In addition, extracts from herbs used were effective against other fungi; this was the case with aqueous extracts of *C. Citratus* which effectively controlled mycelial production of *Bipolaris oryzae* (Tiendrebeogo *et al*., 2017); and *L. multiflora*; both of which control the mycelial growth of peanut rust (Koita *et al*., 2012). Likewise, Kabore *et al*. (2007) reported the efficacy of *A. indica*, *P. oleracea* and *Securidaca longepedonculata* on *F. moniliforme*, *C. lunata*, *Phoma sorghina*. Bonzi (2005) found that *C. occidentalis* seed extracts effectively limited mycelial development of sporulation of several species of fungi.

In the field, damage induced by the disease varied among treatments; particularly with *Cymbopogon citratus*, *Citrus limon* and *Eucalyptus camaldulensis*. Treatments carried out with *Lippia multiflora* and *Azadirachta indica* at 15%, controlled rice cercosporiosis, as well as blast significantly. These results are in agreement with those of Tiendrebeogo *et al*. (2017), who tested the effect of *Cymbopogon citratus* against helminthosporiosis in rice. The present study results confirm those of Dabire (2001) that the population of parasitic fungi of millet, sorghum and rice seeds can be controlled using aqueous extracts of *Eucalyptus camaldulensis*, *balanitis* and *Cymbopogon citratus*. In general, the 10% concentration of all aqueous treatments, except that of *Lippia multiflora,* did not significantly control the cercosporiosis population. *Lippia multiflora* showed better antifungal activity than *Citrus limon*. Two treatments for *Lippia multiflora* or *Eucalyptus camaldulensis* or *Azadirachta indica* are required once the first symptoms appear to control the disease.

These results are similar to those of Serghat *et al*. (2004) who demonstrated that benomyl showed *in vitro* more or less significant fungitoxic activity on the three stages of development of isolates of *Pyricularia grisea* and *Helmintosporium oryzae*: mycelial growth, sporulation, and the germination of spores. The low incidence of the disease in the field on the leaves confirms the ability of the three plant species to inhibit the development of leaf spots. Extracts of *L. multiflora*, *A. indica* and *E. camaldulensis* effective against *C. oryzae* were equally effective against *P. oryzae* and *H. oryzae* in the field.

After administration of the treatments, the development of other pathogenic fungi such as blast, curvulariasis and helminthosporiosis commonly present, could not be observed. This implies that the extract of *Lippia multiflora* also inhibits the development of these pathogens. Finally, it is recommended to verify the synergistic effect of the aqueous extracts of the plants used.

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