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In vitro Management of *Bipolaris oryzae* the Causal Pathogen of Brown Spot of Rice by Plant Extracts

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Abstract: The antifungal potential of aqueous roots extracts of *Securidaca longepedonculata* and essential oil from leaves and flowering buds of *Lippia multiflora* against *Bipolaris oryzae*, causal agent of brown spot of rice was evaluated. Conidia of *B. oryzae* (Breda de Haan) Shoem, isolated on naturally infected leaves of the variety FKR19. The efficacy of the extracts to inhibit fungal growth was tested using well diffusion method onto PDA (Potato Dextrose Agar) growth medium in Petri plates. Results were record from the 5rd day until complete growth in control plates. The fresh root extracts of *Securidaca longepedonculata* (10% and 15%) significantly reduced the radial growth of the fungus with 42.49% and 72.56% efficiencies rates respectively. On the other hand, the dried extracts of the roots of the same plant at 15% and 20% concentrations with an efficiency of 4.81% and 14.44% respectively did not significantly reduced the radial growth of *B oryzae*. Essential oil of *Lippia multiflora* at 0.01% concentration significantly reduced the growth of the fungus to 62.64%, and the concentration of 0.1% completely inhibited its growth with 100% efficiency. The present study reveals that there is an alternative to chemicals through the use of local plant extracts that have antifungal properties capable of protecting the rice plant against possible attack of *Bipolaris oryzae*.

Keywords: *Bipolaris oryzae*, radial growth, antifungal potential, *Lippia multiflora*, *Securidaca longepedonculata*

Introduction

Rice (*Oryza sativa* L.) is the most cultivated cereal in the world (about 150 million hectares). It is the staple food of more than half of humanity (CIRAD-GRET, 2002). Insignificant in the early 1960s, rice consumption has now reached more than 200,000 tons in Burkina Faso and is growing at a rate of 5.6% per year, a rate higher than that of population growth (MAHRH, 2005). In Burkina Faso, among crops cultivated, cereals occupy the 4th place with a production of 325.138 tons from a cultivated area of 142.715 ha in 2015 (DGESS, 2015). The country's rice needs are rapidly increasing with population growth, especially in urban areas (Traore *et al*., 2001). The cultivation of rice is confronted with a number of biotic factors (fungal, bacterial or viral diseases) among which fungal diseases play a major role. In Burkina Faso, losses due to fungal infections ranges from 20 to 80% (CNRA, 2012). A number of these known fungal diseases are seed borne with some such as brown spot having a wide geographical distribution. The brown spot disease caused by *Bipolaris oryzae* is a reported disease in all rice producing countries of Asia, America and Africa (Agarwal *et al*., 1994). In Burkina Faso for example, it has been reported to be responsible for up to about 40% reduction in seedling emergence caused (Ouedraogo, 2001). This eventually leads to low plant density resulting in low yield. In the Kou valley and in Karfiguela, two major rice producing areas in Burkina Faso, yield losses of 10.3% and 16.6% respectively have been attributed to *B. oryzae* (Ouedraogo, 2008).

A number of control strategies have been developed and employed in an attempt to reduce the impact of

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B. oryzae in rice production in Burkina Faso, notably among them is the use of chemicals which is currently remains the most widely used method against this disease (Nebie *et al*., 2002).

However, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Today, there are strict regulations on chemical pesticide use. Additionally, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases (Kagale *et al*. 2004; Pal *et al*. 2006). Among these alternatives are those referred to as plant extracts.

Several authors have reported on the impact of plant extracts in the control of phytopathogenic fungi on seeds and those that evolve during the vegetative cycle of the crop. Koïta *et al*. (2010) confirmed the *in vitro* efficacy of three local plants (*Lippia multiflora, Securidata longepedonculata* and *Ziziphus mucronata*) in the inhibition of spore germination of *Cercospora arachidicola* and *Puccinia arachidis*, two species responsible for fungal diseases of peanuts. The aqueous extract of *Yucca schidigera* is known to inhibit completely the mycelial growth of *Colletotrichum capsici* and *Rhizoctonia solani* on cowpea (Soalla, 2011). It has also been reported that aqueous extracts of lemon grass (*Cymbopogon citratus*) and neem (*Azadirachta indica*) have the ability to inhibit the growth of *Phoma sorghina* in sorghum seeds even better than Calthio C fungicide (Bonzi *et al.* 2012). Aqueous extracts of Acacia gourmaensis A. Chev. and Eclipta alba (L.) Hassk. are known to inhibit the infection of sorghum and pearl millet seeds by *P. sorghina* and *F. moniliforme* by 27 and 52% respectively (ZIDA *et al*. 2008).

Kabore *et al*. (2007) reported that aqueous extracts of *A. indica, S. longepedonculata* and *P. oleracea* had inhibitory effect on *Bipolaris oryzae, Curvularia lunata, Fusarium moniliforme* and *Phoma sorghina.* Reduced seed contamination by 61.2%, 46.7%, 59.7% and 58.6% respectively while improving their germination quality. Other investigations have shown that the aqueous extract of *Eclipta alba* at concentrations of 25% and 30% greatly reduced the infection rate of rice seeds by *B. oryzae* and *F. moniliforme* (Tiendrebeogo, 2011). This shows the growing importance of plant extracts as safe alternatives to synthetic chemicals. It is from this perspective that this study has been conducted. Its objective is to test *in vitro* the efficacy of *Lippia multiflora* and *Securidaca longepedonculata* extracts against *Bipolaris oryzae*, causal agent of brown spot of rice.

Materials and methods

Fungal isolates

Conidia of *B. oryzae* (Breda de Haan) Shoem, isolated on naturally infected leaves of the variety FKR19, were used for this study. The infected leaves were collected in Koubri, a rural community located 25 km south of Ouagadougou, in a farmer's field that had not received antifungal treatment. Conidia were grown on Potato Dextrose Agar (PDA) growth medium.

Plant material

Experimental plant materials were secondary roots of *S. longepedunculata* which were obtained from old individuals. Samples were taken from ten plants and were obtained from the same locality from which fungal isolates were obtained (Koubri). Once collected, the root bark was removed, one portion was stored in the refrigerator at 4 ° C and the other dried in the shade at room temperature (25 ° C - 30 ° C) for 15 days. The leaves and flowering buds of *Lippia multiflora* were collected in the same locality as *S. longepedunculata.*
The botanical certification of these two species was carried out by the Botanical Laboratory of University Ouaga I Joseph Ki-Zerbo.

Preparation of PDA

39g of PDA was weighed into 1 L distilled water in a conical flask, plugged with cotton wool, properly wrapped with aluminium foil, and sterilized at 121°C for 15 minutes. The medium was allowed to cool to about 45°C then dispensed aseptically into sterile glass Petri-dishes in a laminar flow hood and allowed to solidify.

Isolation and obtaining pure culture of *Bipolaris oryzae*

B.oryzae was isolated from rice foliar showing disease symptoms using the blotter technique (Mathur and Kongsdal, 2003). After five days of incubation at 22 ° C with an alternation of 12 h of light / dark, the fungus was transferred onto PDA in Petri plates. Several transplants were performed on PDA in order to obtain a pure culture that will be used for the implants of radial growth.

Preparation of extracts

The freshly collected bark of the roots of *S. longepedonculata* were washed with water, disinfected by immersion in a 2% of sodium hypochlorite solution for 30 min, and thoroughly rinsed with distilled water. The collected roots were

divided into two batches: one used in the fresh state and the other dried. The fresh material was lightly crushed, weighed and incorporated in sterile PDA. Two concentrations 10% (T2) and 15% (T3) were used to test the efficacy of fresh aqueous extracts of S. *longepedonculata* on *B.oryzae*.

Part of the collected bark of the roots were dried at room temperature in the dark and ground to a fine powder using a laboratory scale mill. The resulting powder was incorporated at the concentration of 15% (T4) and 20% (T5) in sterilized PDA. The different concentrations used for this work was based on previous work done by Kabore *et al*. (2007).

The essential oils of *L. multiflora* obtained by hydrodistillation was incorporated in PDA at 0.1% (T6) and 0.01% (T7) concentrations. All the six treatments were compared with two controls, ie a negative control PDA without any extract (T1) and a fungicide (TOPSIN-M with Methyl-thiophanate as active ingredient) applied at 5 g / l as a positive control (T8).

Antifungal activity screening

The efficacy of the extracts to inhibit fungal growth was tested using well diffusion method (Khyade *et al*. 2009)). Mycelial disc of about 0.5 cm in diameter obtained from a 5-day old culture was inoculated in the centre of each Petri plate containing the mixture (PDA + botanical extract). With each preparation, a dose of 0.4 g of sodium azide was added in 1 liter of medium to limit bacterial contaminations. Five replicates of each treatment were used in a completely randomized design. The Petri plates were then incubated at 22 ± 2°C in the dark in an incubator (THERMOSI, SR 3000) and results were record from the 5 days after inoculation (DAI) until 28 DAI. Growth rate was determined by considering the average of the measurement of the two perpendicular diameters of the colony and subtracting 0.5cm which corresponds to the diameter of the sampled washer of initial inoculum.

The efficiency percentage of the extracts was determined by the formula proposed by Greche and Hajjani (2000).

$$E\,(\%) = 100[DMT - DME]/DMT$$

DMT represents the average diameter of the untreated control (absolute control) and DME, the average diameter of the treatment with the plant extract.

Data management and statistical analysis

Data were analyzed using a one way analysis of variance (ANOVA).Values were mean of ± SE (n=3). Duncan's multiple range test was applied as post hoc test at p=0.05. To ensure homogeneity of variances and normality of the distribution of each variable, data recorded as percentages were arcsine transformed before the ANOVA was carried out. Analysis of data was done using XLSTAT statistical software version 7.5.2.

Results and discussion

Effectiveness of fresh and dried extracts of *S. longepedunculata* on the *B.oryzae* mycelium growth

The effect of fresh and dried extracts of *S. longepedonculata* roots on the radial growth of the *B. oryzae* fungus is shown in Fig. 1. The Anova data showed that the effect of the different treatments, that is T2 (fresh extract at 10%), T4 (dried extract at 15%) and T5 (dried extract at 20%) on average diameter of fungal colony were statistically no different from negative control (T1). These treatments recorded 8 cm, 8.5cm and 8.5 cm of diameter respectively. Among the plant extracts however, the maximum inhibitory effect was induced by fresh extract at 15% (T3) with 5.2 cm of diameter. In general, the highest level of inhibition (2.3 cm) of mycelial growth was obtained with the Methyl-thiophanate (T8).

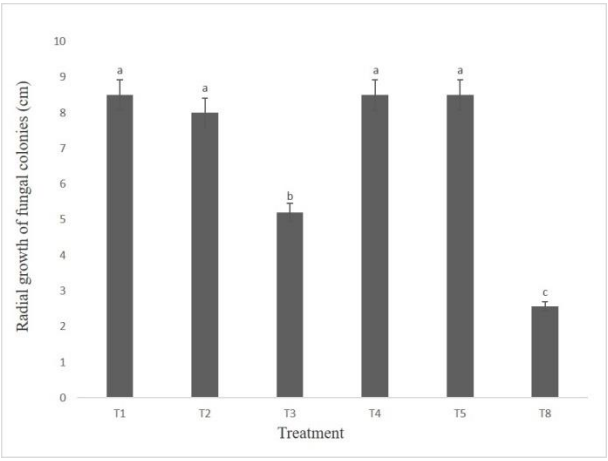

Figure1. Mycelial growth inhibition as a function of S. longepedunculata fresh and dried extracts. The extracts were obtained from S. longepedunculata and applied on *Bypolaris oryzae* as mentioned in the text. Radial growth of fungal mycelium was measured (cm) after 5rd day until 28rd day at 22°C±2. Data are the average of ten measurements in three individual experiments. Columns with unlike letters differ significantly according to Duncan's multiple range test at $P \leq 0.05$. T1 : negative control ;T2 : *S. longepedonculata* fresh extract (10%) ; T3 : *S. longepedonculata* fresh extract (15%) ; T4 : *S. longepedonculata* dried extract (15%) ; T5 : *S. longepedonculata* dried extract (20%); T8 : positive control fungicide (0,5%).

The performance of the treatments was also judged by the rate of effectiveness over the days of treatment. On the 5th day after incubation T1, T2, T3 and T8 had induced efficiency rate of 0%, 73.33%,

94.04%, and 100% respectively (Fig.2). These values decreased to 5.88% and 48% efficiency for T2 and T3 respectively at 28 DAI. The fungicide (T8) was the most effective at the end of the experimental period with an efficiency rate of 83.76%. Among the plant extracts, T3 (15%) was proved to be the most effective treatment in the control of *B. oryzae* mycelium development. For this treatment the efficiency rates ranged from 83.88% to 48.23% at 10 DAI to 28 DAI respectively. T2 maintained an efficiency greater than 50% up to 10 DAI but subsequently decreased in efficiency rate to 5.8% at 28 DAI.

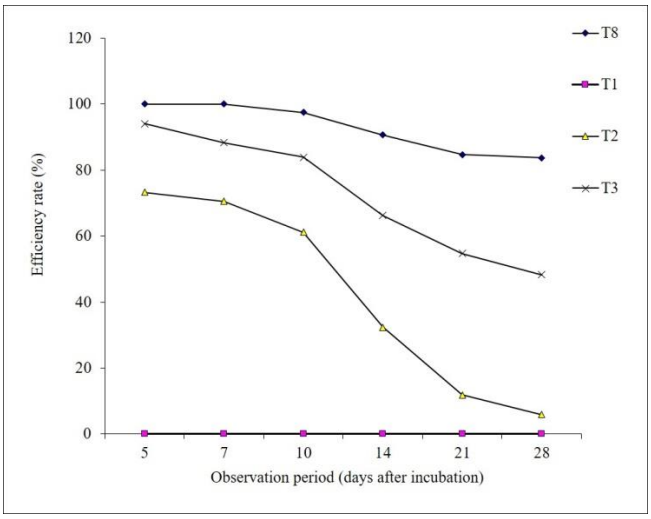

Figure 2. Efficiency of fresh extracts of *Securidaca longepedonculata* roots on the inhibition of the radial growth of *Bipolaris oryzae*.
T1: negative control ;T2 : *S. longepedonculata* fresh extract (10%) ; T3 : *S. longepedonculata* fresh extract (15%) ; T8 : positive control fungicide (0,5%).

With the dried bark extracts, at 5 DAI the treatments showed low efficacy of 15.51% and 39.08% for T4 and T5 respectively (Fig.3). From 10 to 21 DAI, the efficiency rate of the plant extracts further decreased resulting in optimal radial growth of the fungus. It was observed that at the end of the experimental period none of the dried back extracts was able to control the growth of *B. oryzae.*

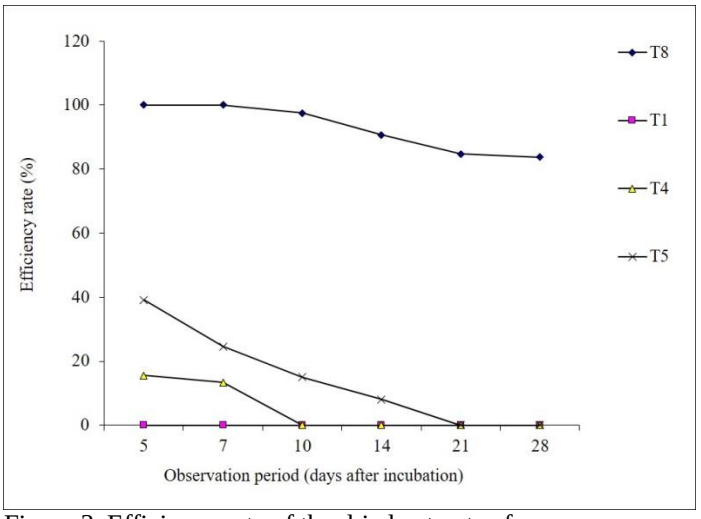

Figure 3. Efficiency rate of the dried extracts of *Securidaca longepedonculata* roots on the inhibition of the radial growth of *Bipolaris oryzae*.
T1 : negative control ; T4 : *S. longepedonculata* dried extract (15%) ; T5 : *S. longepedonculata* dried extract (20%); T8 : positive control fungicide (0,5%).

Antifungal activity of essential oil of *Lippia multiflora* on the radial growth of *B. oryzae*

Essential oils from of *L. multiflora* (T6 (0.1%) and T7 (0.01%)) showed a high level of antimicrobial effect against the fungus strain (Fig 4). T6 (0.1%) was the most effective in inhibiting the radial growth of the fungus. Although the radial growth of this treatment was not statistically significant from the fungicide treatment it is of interest to note that recorded a lower value than that of the control fungicide (2.37 cm) (Fig. 4).

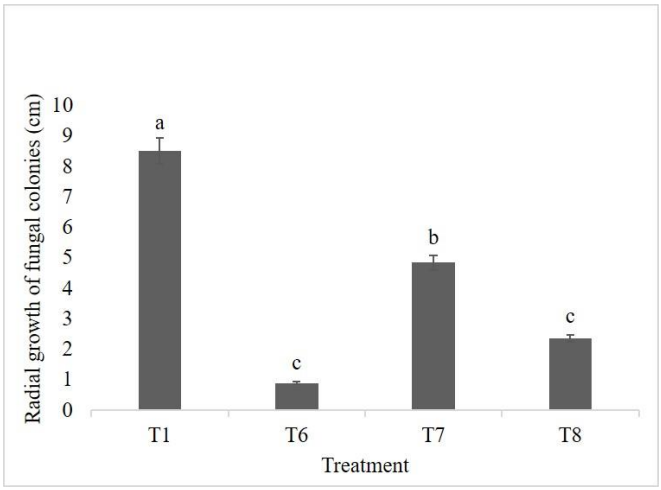

Figure 4. Mycelial growth inhibition as a function of essential oil of *Lippia multiflora* concentrations. The essential oil of *L. multiflora* obtained by hydrodistillation. Radial growth of fungal mycelium was measured (cm) after 5rd day until 28rd day at 22°C±2. Data are the average of ten measurements in

three individual experiments. Columns with unlike letters differ significantly according to Duncan's multiple range test at $P \leq 0.05$.
T1 : negative control; T6 : essential oil (0.1%) ; T7 : essential oil (0.01%); T8 : positive control fungicide (0,5%).

Over the experimental period, it was observed that T6 was extremely efficient (100%) in controlling the growth of the fungus. This treatment was more efficient than the control fungicide which showed and efficiency of 83% 28 DAI (Fig. 5). T7 was however, less efficient, showing an efficiency rate of only 27% at 28 DAI.

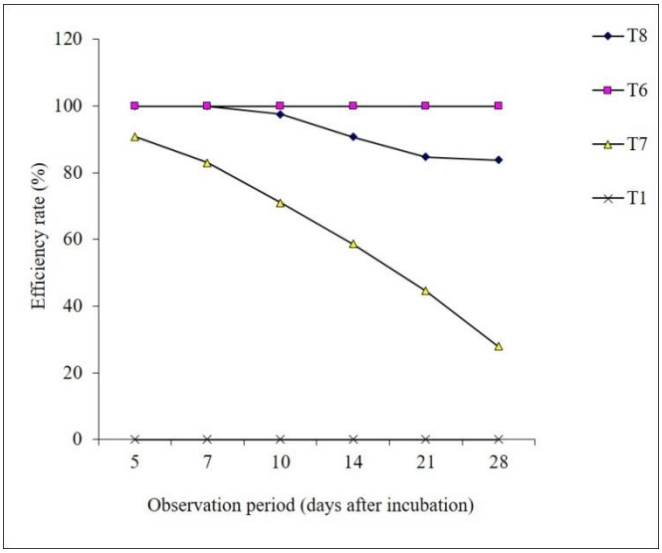

Figure 5. Efficiency rate of essential oil of *Lippia multiflora* on the inhibition of the radial growth of *Bipolaris oryzae*.
T1 : negative control ; T6 : essential oil (0.1%) ; T7 : essential oil (0.01%); T8 : positive control fungicide (0,5%).

Discussion

The study was carried out to access the effectiveness of botanical extracts and the possibility of using it to control the pathogenic fungus responsible for brown spot of rice in the field. Two fresh extracts of *S. longepedonculata* (T2 (10%) and T3 (15%)) were able to reduce the radial growth of *B. oryzae*. These fresh extracts are known to have antifungal activity capable of slowing the growth of the pathogenic fungi (Pousset (2004)). For the dried extracts of *S. longepedonculata* roots, none of the treatments showed antifungal activity.

This work has shown that fresh extracts parts of the test botanicals are more effective than extracts from dried parts. This may be due to the fact that the inhibitory molecules needed for inhibition of fungal growth may be more active in the fresh extracts of *S. longepedonculata* roots and less active in the dried extracts due to degradation or volatilization during drying. Drying and moist heat of the autoclave are factors that could inhibit or destroy the active ingredients of the dried root inhibitory molecules. These results are comparable to those of Dabire (2004) which showed that the application of aqueous extracts of *S. longepedonculata* on millet seeds leads to a significant decrease in their infection with *Phoma sorghina*. Similarly, Koita *et al*. (2010) showed that fresh extracts of *S. longepedonculata* (25%) induced a 100% inhibition of *Cercospora arachidicola* and *Phaeoisariopsis personata*, causals agents of early and late leaf spot of groundnut respectively. Inhibitory activity of *S. longepedonculata* against *B. oryzae* and other fungal pathogens such as *Curvularia lunata*, *Fusarium moniliforme* and *Phoma sorghina* has previously been reported by Kaboré *et al*. (2007) .

With regard to *Lippia multiflora* essential oil, the two different concentrations (0.1% and 0.01%) had very significant effects on the inhibition of mycelial growth of the fungus suggesting strong activity levels of the inhibitory molecules in the oil. The data also showed that not only are the inhibitory molecules highly active in the oil, but their activity or efficiency increases with increasing concentration. It is also of interest to note that at 0.1% concentration, the essential oil was more effective than the methylthiophanate. These results are comparable to those of Montel (2004) who showed that the essential oil of *Lippia multiflora* completely inhibited the growth of *Alternaria longissima* at a concentration of 1.5%, *B. oryzae* and *Fusarium sp* at concentration of 1%. Koita (2005) had also shown that the essential oil of *L. multiflora* at concentrations of 1%, 1.5%, 2% could inhibit the growth of fungi (*Bipolaris oryzae*, *Curvularia lunata*, *Fusarium moniliforme*, *Phoma sorghina*) to 100% as well as reducing seed contamination by fungi between 28 to 81%. It was also able to improve seed germination capacity by 22%, which proves that it had no phytotoxic effect on germination. Similarly, the work of Kintega (2014), on the fight against *Fusarium oxysporium*, the agent responsible for the damping-off and onion bulb rot, revealed a fungicidal activity with a 100% effectiveness of *Lippia multiflora* (0.1ml / l) on the radial growth of this fungus.

Conclusion

This laboratory study has confirmed that all the botanical extracts used have varied antifungal action on the growth of *Bipolaris oryzae*, a causal agent of brown spot disease of rice. The essential oil of *Lippia multiflora* (1ml/ l) showed more efficiency than the conventional chemical (Methylthiophanate). Botanical extracts that have exhibited promising

antifungal activities can be used by growers in treating rice seed against *Bipolaris oryzae*. These botanical extracts could be bio-fungicides capable of substituting conventional pathogen control chemicals. However, it would be necessary to carry out further studies on the application of these extracts in the field.

Competing interests

Authors have declared that no competing interests exist.

Authors' contributions:

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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