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Antibacterial activity of extracts of three aromatic plants from Burkina Faso against rice pathogen, *Xanthomanas oryzae*

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Plant extracts can have powerful antibacterial activity and may be used as biological control against important plant pathogens. This study assessed effect of extracts of *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Mentha piperita* against two pathovars of *Xanthomonas oryzae* attacking rice. Both *in vitro* and *in vivo* approaches were used for different strains of *X. oryzae* originating from Burkina Faso. The three essential oils (EOs) have antibacterial activity *in vitro* test (agar diffusion method), but, EO from *C. citratus* at 1:5 dilution (v/v) resulted in the highest inhibition (over 30 mm of inhibition zone) against *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*. However, they reduced significantly rice seed germination and induced herbicide effects on rice leaves. On the other hand, aqueous extracts from *C. citratus* stimulated bacterial growth, while extracts from *E. camaldulensis* had an inhibitory effect (28 mm of inhibition zone at 3:10 dilution, w/v). Therefore, the characterization of active compounds will determine one or more compound(s) involved in the antibacterial activity.

Key words: *Xanthomonas oryzae*, rice, plant extracts, antibacterial activity.

INTRODUCTION

Rice (*Oryza sativa*) is the third most important cereal crop in the world following wheat and maize with an estimated production of 500 million metric tons in 2015 (www.fao.org). It is a staple food in developing countries and its consumption is steadily increasing. In Burkina Faso, the average per capita consumption of rice is about 18 kg per year. However, rice production faces several important constraints including rice diseases. Providing healthy rice seeds is a requirement to increase rice productivity in order to answer the challenge of feeding fast growing populations. Indeed, several seed-borne bacterial pathogens of rice were described, and among those, *Xanthomonas oryzae* causes important yield losses. Bacterial leaf blight (BLB) and bacterial leaf streak (BLS) are two important diseases due to *X. oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*), respectively; they significantly reduce global rice production in the tropical areas. BLB is present in tropical

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and temperate areas wherever rice is grown. The yields losses induced by BLB under irrigation scheme can reach about 20 to 80% depending on the rice variety and climatic conditions (Ou, 1985). However, BLS is mostly confined to tropical and subtropical areas in Asia, Africa and Australia (Ou, 1985) and can cause yield losses of up to 30% (Gonzalez et al., 2007). BLS was first reported in Burkina Faso in 2009 by Wonni et al. (2011). The disease is present in all major rice production areas and it is becoming a threat to rice production (Wonni et al., 2014).

Seeds are primary sources of inoculum for both bacterial diseases (Ou, 1985; Mew et al., 1993; Agarwal et al., 1994). *Xoo* and *Xoc* are quarantine pathogens in the USA and in some countries where these pathogens are endemic in order to limit the introduction of new virulent strains.

To avoid the dissemination of bacterial diseases by the means of infected seeds, Agarwal et al. (1994) suggested seeds disinfection with bactericides and heat. However, chemical treatments are expensive for the small rice producers of developing countries and harmful for environment and human health. Also, the thermotherapy needs a device that can maintain a constant temperature duration of treatment. The lack of seeds treatment against *X. oryzae*, and consequently the exchange of contaminated seeds, are likely involved in the rapid range expansion of BLB and BLS.

Compounds of some tropical aromatic plants have been reported to possess potent antimicrobial activities (Ilodibia et al., 2015; Akale et al., 2015; Singh et al., 2016; Salem et al., 2016). Notably, antibacterial activity was reported for three plants found commonly in Burkina Faso: *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Mentha pipera*.

C. citratus or lemongrass is a perennial tropical grass; it is resistant to different temperatures and can grow in all climates. The essential oil from *C. citratus*, besides having antimicrobial effects, has been shown to have anti-inflammatory, analgesic and antipyretic properties (Kpoviessi et al., 2014; Vázquez-Briones et al., 2015). *Mentha piperita* L., a medicinally important plant belonging to the family Lamiaceae and commonly known as peppermint is a hybrid of *Mentha spicata* L. and *Mentha aquatic.* The extracts of *Mentha piperita* are found to possess antibacterial, antiviral and antifungal activities (Singh et al., 2016; Ilboudo et al., 2016). The leaves of *Eucalyptus camaldulensis* are usually used for various purposes, including treatment of infections. All these plants are grown traditionally on small or large areas everywhere in Burkina Faso, so interesting candidates for biological control of bacterial diseases.

The present study aimed at assessing antibacterial activities of extracts from three aromatic plants (*C. citratus*, *E. camaldulensis* and *M. pipera)* on *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* strains *in vitro* and to assess their potential antibacterial effect on rice seed germination and rice plant development.

MATERIALS AND METHODS

Bacterial cultures

The authors used two strains of *X. oryzae* originating from Burkina Faso: BAI3 (*X. oryzae* pv. *oryzae*) isolated from rice plants with BLB in 2003 by Gonzalez et al. (2007) and BAI105 (*X. oryzae* pv. *oryzicola*) isolated from BLS symptoms in 2012 by Wonni et al. (2014). These strains belong to major group representing the genetic diversity in West Africa (Poulin et al., 2015). These strains from INERA-IRD collection were stored at -80°C and recovered on Peptone, Sucrose, Agar (PSA) medium for use.

Vegetal material source

The leaves of *C. citratus*, *E. camaldulensis* and *M. piperita* were collected in the station of environmental and agricultural research of Farako-Ba located 10 km from the city of Bobo Dioulasso (11°9'56.97''N; 4°18'19.80''W) in Burkina Faso. After collection, all the others tests were achieved in the laboratory.

Preparation of aqueous extracts for phytochemicals analysis

Leaves were dried at 40°C and milled to obtain a fine powder using a vegetable blender and then stored in airtight bottles. 100 g of fined powder of each plant was suspended in 100 ml of distilled water and kept under room temperature for 24 h. Then, it was filtrated through muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected under vacuum using a millipore membrane of 0.20 µm. The aqueous extract obtained was stored at 4°C for further use. Dilutions of 1:10; 2:10 and 3:10 were used for antibacterial activity tests.

Preparation of essential oils for phytochemicals tests

Essential oils (EOs) from *C. citratus*, *E. camaldulensis* and *M. piperita* were obtained from Phytofla laboratory located in Banfora (10°38'26.09''N; 4°45'33.15''W), a town located in the west of Burkina Faso. These EOs were extracted from leaves by the hydrodistillation method. The emulsion of EOs and 0.1% water-agar solution were prepared following Remmal et al. (1993) method. Each EOs was mixed at the ratio of 1:5, 1:10 and 1:20 (v/v).

In vitro antibacterial assay

The modified method of Nguefack et al. (2005) was used to study the antibacterial activity of plants extracts. 15 µl of bacterial suspension concentrated at 10^8 CFU was inoculated in 4.5 ml of PSA liquid medium at 75%. The mix was homogenized and poured in solid PSA medium containing plates.

A volume of 10 µl of each ratio of emulsion oils and aqueous extract respectively was placed equidistantly onto *Xoo* and *Xoc* spread medium followed by incubation at 28°C. Water-agar was used as the control treatment. The inhibition zones were measured after 96 h of incubation. Each treatment was replicated three times, with three plates per replication. Three independent assays were performed with similar results.

In vivo antibacterial assay

The EOs from *C. citratus* and *E. camaldulensis* respectively found

Table 1. Mean diameter (mm ±SE) of inhibition zone of *Xanthomonas oryzae* strains with essential oils from *Cymbopogon citratus, Eucalyptus camaldulensis* and *Mentha piperita*.

Essential oils	Mean diameter of inhibition area					
	Ratio 1:5		Ratio 1:10		Ratio 1:20	
	Xoo	*Xoc*	*Xoo*	*Xoc*	*Xoo*	*Xoc*
C. citratus	31±1[b]	34.33±0.00[c]	26±0.76[b]	29.55±3.50[b]	18±1[b]	13.34±0.58[c]
E. camaldulensis	13.78±1.7[a]	12.67±1.34[b]	9.67±0.88[a]	9.44±0.20[a]	8±0.00[a]	8.67±0.34[b]
M. piperita	12.78±0.39[a]	10.22±1.17[a]	8.67±0.00[a]	7.67±0.58[a]	8.11±0.19[a]	6.56±0.20[a]

Values followed by different letters are significantly different (P< 0.05) based on Duncan's multiple range test.

to be highly and moderately effective in suppressing the growth of *Xoo* and *Xoc* strains *in vitro*, were tested for their antibacterial activity on seed germination and their effects on rice leaves.

Effect of EOs on rice seeds germination

The seeds were treated with an emulsion of EOs from *C. citratus* and *E. camaldulensis,* and with agar-water at the following ratios: 1:5, 1:10, 1:20 and 1:25. 100 µl of each EO mixture was applied on 400 rice seeds according to Adegoke and Odela (1996) method. The treated seeds were placed at room temperature for 24 h before germination test. One control with water-agar solution without EOs was used. Then, the seeds treated were tested using the blotter papers method. Four replicates of 100 seeds for each EO were rolled in blotter papers and placed in polyethylene bags then incubated at 28-30°C under a cycle of 12 h light/12 h darkness. The rate of seeds germination was assessed at 3, 5 and 7 days after incubation. The experiment was repeated twice.

Effect of EOs on rice leaves

The effect of EOs on leaves was tested for efficient concentration of *C. citratus* and *E. camaldulensis* at the concentration found to have antibacterial effect *in vitro*. Three EOs emulsions at the ratio of 1:5; 1:10 and 1:20 were sprayed on both sides of each leaf from thirty days old plants. Five leaves were treated with each EOs and three replicates were done. Data were collected on the leaves 48 h after treatment.

Statistical analysis

The data on diameter of inhibition area and the germination rate of rice seeds treated, induced by the essential oils were assessed by analysis of variance (ANOVA), and treatment means were compared by Duncan's multiple range test. Statistical significance was set at P<0.05 and the analyses were performed with IBM SPSS Statistics Base 20 software.

RESULTS

Effect of plant extract *in vitro*

Efficiency of essential oils

All tested EOs exhibited considerable antibacterial activity against both pathovars of *X. oryzae* (Table 1). EOs from *C. citratus* was the most effective in inhibition

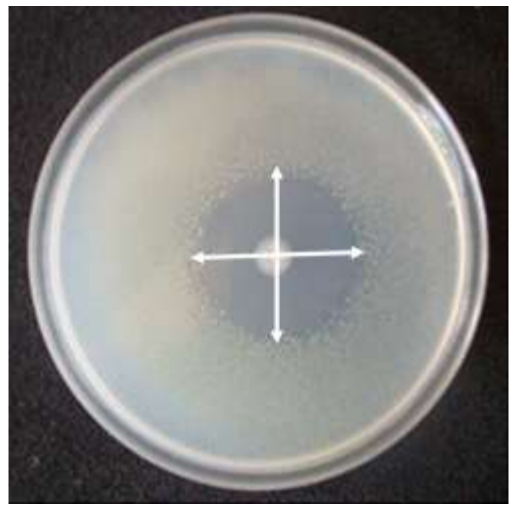

Figure 1. Inhibition zone (34 mm of diameter) of the growth of *X. oryzae* pv. *oryzae* induced by essential oil from *C. citratus* at the ratio 1:5*.*

of *Xoo* and *Xoc* growth than *E. camaldulensis* and *M. piperita*. The inhibition area of *Xoc* at 1:5 ratio was to 34 mm for OE from *C. citratus* (Figure 1) against 12.67 and 10.22 mm OEs from *E. camaldulensis* and *M. piperita* respectively.

Efficiency of aqueous extracts

Bacterial growth was affected by the aqueous extract of *E. camaldulensis* only with 19.67 and 15 mm mean diameter of inhibition zone, respectively for *Xoc* and *Xoo* after 5 days incubation at 1:10 dilution (data not shown). The inhibition effect increases with the high concentration of aqueous extracts. In comparison with the control, aqueous extract from *C. citratus* stimulates the growth of *X. oryae* strains at the level of the deposit point of the drop of aqueous extracts (Figure 2).

In vivo activity of essential oils

Effect of EOs on rice leaves

The three efficient concentrations of EOs from *C. citratus*

Figure 2. Stimulation of the growth of *Xanthomonas oryzae* pv. *oryzicola* strain in deposit area of aqueous extracts of *Cymbopogon citratus.*

Figure 3. Effect of essential oils of *Cymbopogon citratus* and *Eucalyptus camaldulensis* on rice leaves.

and *E. camaldulensis* used to treat rice leaves *in vitro* induce partial burns or complete desiccation of the leaves (Figure 3).

Effect of EOs on rice seeds germination

The rate of seeds germination varied according to EOs and the incubation duration. The seeds treated with Eos from *E. camaldulensis* and *C. citratus* at the ratio of 1:5 and 1:10 reduced significantly seed germination (≤70%) as compared to the control. However, at the concentration of 1:20, the percentage of germination was similar to the control (Figure 4).

DISCUSSION

EOs from *C. citratus* was found to be highly effective in inhibiting the growth of two pathovars of *X. oryzae*. Moderate activity was recorded from Eos of *E. camaldulensis* and M. piperita. The data showed that efficiency of each EOs was similar for both pathovars of *X. oryzae*. Considering aqueous leaf extracts, it was found that only *E. camaldulensis* inhibited *Xoo* and *Xoc* growth, while *C. citratus* stimulated bacterial growth as compared to the control.

The efficiency of extracts from the three aromatic plants on phytopathogenic bacteria was previously reported (Nguefack et al., 2005; Paret et al., 2010; Lucas et al., 2012). In addition, several plant extracts are known to possess antibacterial activity. Govindappa et al. (2011) have reported antibacterial activity of aqueous extract of *Adathoda vasica* on *Xoo*. Nguefack et al. (2005) demonstrated the efficiency of EOs from *Ocimum gratissimum* and *Thymus vulgaris* on *Xoc* NCPPB 1632 R1 strain. In contrast, they found that *Xoo* NCPPB 2446R11 strain was not sensitive to EO from *C. citratus* at 1:10 dilution which is not in conformity with the present study results.

The antibacterial activity results from the chemical composition of each essential oil. EOs from *C. citratus* contains 80% of citral and *E. camaldulensis* contains 77% of 1,8-cinéol which give them antibacterial properties (Mehani and Ladjei, 2012; Vázquez-Briones et al., 2015). Singh et al. (2016) reported the strong antibacterial effect of *M. piperita*. It contain active ingredients such as flavonoids, polymerized polyphenols, carotenes, tannins, etc; known to play antibacterial activity (Sokovic et al., 2009).

The results showed that seeds treatment with the EOs from *C. citratus* and *E. camaldulensis* reduces seeds germination rate, except when applied at a low concentration. Also, these EOs cause various symptoms ranging from burns to drying, when applied on the leaves. The secondary metabolites contained in the EOs of these two aromatic plants could explain the reduction of seeds germination rate, burns and leaves drying that was observed following the treatment and may confer them allelopathic and herbicide effects. Indeed, Gargouri et al. (2014) reported that *C. citratus* and *E. camaldulensis* exhibited inhibitory effects on associated vegetation. It is known also that the use of citrals-rich EOs induces the inhibition of weed seeds germination. For instance, the citrals, major component of the EO from *C. citratus* showed phytotoxicity effects against *Sinapis arvensis* and *Phalaris canariensis* even at low concentration.

During this study, the antibacterial activity of the EOs from *C. citratus*, *E. camaldulensis* and *M. piperita* against both pathovars of *X. oryzae* was demonstrated. These plants are an alternative to the use of pesticides and have the advantage of protecting human health and the environment. However, these results reveal that the effective concentrations of these Eos reduce seeds

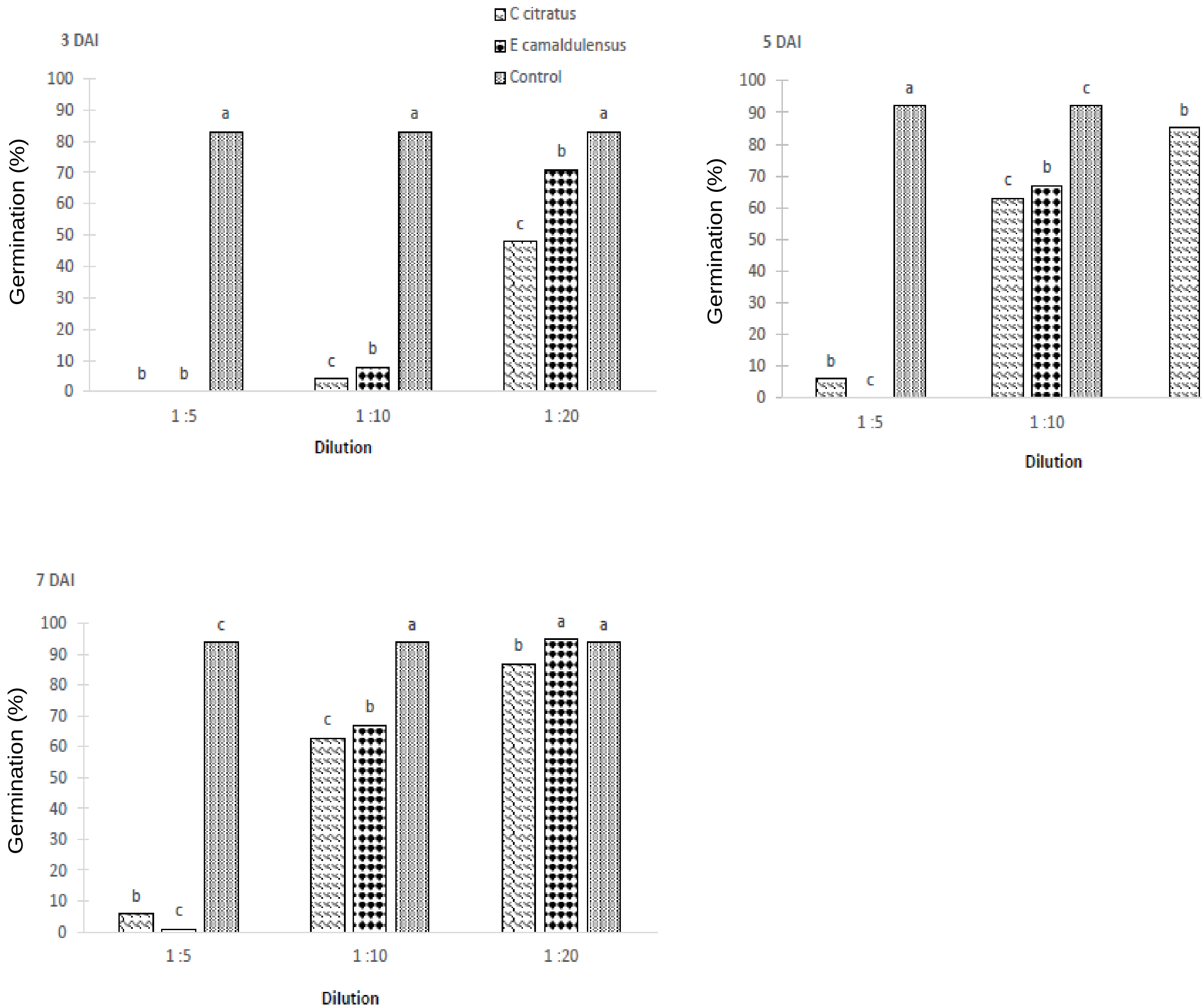

Figure 4. Germination rate of Azecuna seeds treated with essential oil from *Eucalyptus camaldulensis* and *Cymbopogon citratus.* The germination rate was evaluated at 3, 5 and 7 days after incubation (DAI). Different letters denote a significant difference between essential oil at 1:5, 1:10 and 1:20 dilution.

germination and induce herbicide effects on rice leaves.

This study shows the identification of molecules responsible for the antibacterial activity and their purification in order to test their effects on seeds contaminated with *Xoo* and *Xoc*. Also, the components of aqueous extracts from *E. camaldulensis* that stimulates *Xo* strains growth could be used to enrich medium culture.

Conflict of Interests

The authors have not declared any conflict of interests.

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