Pertanika J. Trop. Agric. Sc. 43 (4): 457 - 466 (2020)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Botanical Extracts as Biofungicides against Fungal Pathogens of Rice

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ABSTRACT

Diseases such as blast, brown spot and sheath blight considerably affect the health and productivity of rice worldwide. Chemical fungicides have been routinely used in combating these diseases; however, a safe and environmental-friendly approach using bio-fungicides is desirable in disease management of food crop such as rice. Identification of botanical extracts with antifungal potentials would be instrumental in the development of bio-fungicides. In this study, the antifungal potentials of *Andrographis paniculata*, *Backhousia citriodora*, and *Phaleria macrocarpa* against selected rice fungal pathogens were analysed. Crude extracts obtained from leaves of these plants were diluted to 5, 10, 15, and 20% and tested against *Pyricularia oryzae*, *Exserohilum rostratum*, and *Rhizoctonia solani in vitro* using poisoned agar method. Percentage inhibition of diameter growth (PIDG) of each crude leaf extract against test pathogens was calculated. The aqueous extract of *A. paniculata* showed a significant mycelial inhibitory effect against *P. oryzae* at 20% concentration (PIDG 81.9%) as compared to other test concentrations and pathogens. On the contrary, the aqueous extract of *B. citriodora* at 15 and 20% concentrations had little influence on the mycelial growth inhibition on *P. oryzae* and *E. rostratum* with PIDG values less than 50%. In addition, *P. macrocarpa* methanol extracts at concentration of 10% and above significantly inhibited the mycelial growth of *P. oryzae*, *E. rostratum*, and *R. solani* (PIDG 100%). *Phaleria macrocarpa* leaf extract had been identified

ARTICLE INFO

Article history:
Received: 03 May 2020
Accepted: 04 August 2020
Published: 27 November 2020

DOI: https://doi.org/10.47836/pjtas.43.4.03

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ISSN: 1511-3701
e-ISSN: 2231-8542

to give the highest efficacy against all three rice pathogens *in vitro* and therefore, has the potential to be developed into a bio-fungicide as a safe alternative to synthetic fungicides for disease management of rice.

Keywords: Biopesticide, botanical extract, green technology, percentage inhibition of diameter growth (PIDG), poisoned agar method

INTRODUCTION

Diseases occur in agriculture crops cause substantial losses to farmers around the world and are mostly caused by plant pathogenic fungi (Agrios, 2004). Plant fungal pathogens such as *Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*), *Exserohilum rostratum* (teleomorph: *Setosphaeria rostrata*), and *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) cause important rice diseases such as blast, brown spot, and sheath blight, respectively. The estimated annual losses caused by rice blast range from nearly 10 to 30% globally (Skamnioti & Gurr, 2009). During rice blast epidemics, yield loss of rice can go up to 50% (Ashkani et al., 2015). Similarly, *R. solani* can cause reduction in yield by 50% when the environmental conditions are favourable (Richa et al., 2016). Meanwhile, *E. rostratum* has been identified as an emerging rice pathogen and its impact on rice productivity is still unknown (Toher et al., 2016).

Chemical fungicides have been considered as the most effective and commonly used in the management of most of the fungal diseases worldwide. For instance, probenazole, tricyclazole, azoxystrobin, isoprothiolane, and propiconazole are widely used in rice blast control (Gohel & Chauhan, 2015; Skamnioti & Gurr, 2009). Farmers are likely to choose chemical fungicides to protect their crops from fungal diseases because of its rapid effect, availability and cheaper price as compared to other methods of crop protection. However, synthetic fungicides possess detrimental attributes such as high and acute toxicity, long degradation period, accumulation in food chain and an extension of their power to destroy both useful organisms and harmful pests (Gatto et al., 2011). Moreover, long-term use of chemical fungicides may result in development of fungal resistant races and consequently, it will be more challenging to fight the disease. Therefore, it is vital to minimise the use of chemicals to maintain the sustainability of agriculture.

Use of naturally-occurring antifungal compounds in herbal plants has been regarded as one of the best alternatives to synthetic fungicides (Dissanayake & Jayasinghe, 2013; Kumar et al., 2017). Secondary metabolites such as phenols, flavonoids, and phenolic glycosides are produced abundantly in herbal plants and many of them possess antifungal activity. For many years, researchers have documented the antimicrobial properties and activities of plant oils and extracts. For instance, bioactivities of phytochemicals of *Andrographis paniculata*, a medicinal plant from the family Acanthaceae, including

andrographolide, isoandrographolide, neoandrographolide, 14-deoxy-11, 12-didehydroandrographolide, flavonoids, quinic acid derivatives, and xanthones have been well-reviewed by Ganapumane and Nagella (2020). Besides, four essential oils of *Backhousia citriodora*, an Australian shrub from the family Myrtaceae, appeared to be effective antimicrobial agents when tested against a wide range of pathogenic fungi and bacteria (Wilkinson et al., 2003). Likewise, the antimicrobial activity of essential oil of *B. citriodora* and citral, the major constituent of its essential oil against an array of bacteria, fungi and yeast have been also reported (Hayes & Markovic, 2002). On the other hand, the fruit extract containing flavonoid compounds such as kaempferol, myricetin, naringin, quercetin, and rutin of *P. macrocarpa*, a medicinal plant from the family Thymelaceae, exhibited varied antimicrobial activity against test bacteria and fungi (Hendra et al., 2011).

However, the above mentioned studies were conducted to evaluate the efficacy of these herbal extracts on human pathogens. It is hypothesized that these antimicrobial compounds would exert similar effects on plant pathogens. Percentage inhibition of diameter growth (PIDG) served as an indicator for antifungal activity of *Brucea javanica* extracts against several *Candida* species as compared to positive control (Nordin et al., 2013). Similarly, the antifungal effect of *Asteriscus imbricatus* extracts against *Botrytis cinerea* was compared using PIDG values (Senhaji et al., 2013). Therefore, in this study, we evaluated the antifungal potential of *A. paniculata*, *B. citriodora*, and *P. macrocarpa* against *P. oryzae*, *E. rostratum*, and *R. solani*, the three major fungal pathogens of rice *in vitro* using PIDG method.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves of *A. paniculata* were collected from Herb Garden, University Agriculture Park, Universiti Putra Malaysia (UPM), Serdang, Selangor. Meanwhile, the leaves of *B. citriodora* and *P. macrocarpa* were collected from Department of Agriculture, Serdang, Selangor. The experiments were carried out in Mycology Laboratory, Department of Plant Protection, Faculty of Agriculture, UPM, Serdang, Selangor. *Phaleria macrocarpa* leaf samples were thoroughly washed and air-dried at room temperature (26 ± 2°C) according to Venkateswarlu et al. (2013). Meanwhile, *A. paniculata* and *B. citriodora* were dried at 50-60°C upon a thorough washing according to Buchaillot et al. (2009) with modifications. The samples were then separately ground to fine uniform texture using grinder (Retsch Model SK 100) and stored at room temperature (26 ± 2°C) until use.

Fungal Cultures

Fungal stock cultures were obtained from the Culture Collection Unit, Department of Plant Protection, Faculty of Agriculture, UPM, Serdang, Selangor. Fungal species

that were used in this study were *P. oryzae*, *E. rostratum*, and *R. solani*. The culture of each fungal species was sub-cultured and maintained on potato dextrose agar (PDA) and kept in the culture chamber at 26 ± 2°C.

Preparation of Plant Crude Extracts

Fifty (50) grams of ground leaves of *A. paniculata* and *B. citriodora* were separately soaked in 300 ml distilled water and stirred at 120 rpm for 24 h using an orbital shaker as described by Venkateswarlu et al. (2013). Then, each mixture was filtered using Whatman No-1 filter paper and the solvent was evaporated using a rotary evaporator, BUCHI Model R215W. The dried extract was collected in an air-tight container and stored at 4°C. The same method was used in preparation of crude extract of *P. macrocarpa*; however distilled water was replaced with 300 ml methanol as described by Aras et al. (2016).

Screening of Antifungal Activity

The antifungal test was carried out by testing four concentrations of extract (5%, 10%, 15%, and 20%) as compared to control (0%). The stock solutions of the crude extract of *A. paniculata* and *B. citriodora* were separately prepared by diluting the crude extract of each plant with distilled water at the ratio of 1:1 (w/v). Meanwhile, the stock solution of *P. macrocarpa* crude extract was prepared by diluting the crude extract with acetone at the ratio of 1:10 (w/v) as described by Mahlo et al. (2016). Further serial dilution was done to achieve test concentrations. Petri dishes containing 15 ml of poisoned medium were used. Then, a respective fungal plug (0.4 cm diameter) was placed at the centre of containing each plant extract at the defined concentrations. The antifungal activity of *A. paniculata*, *B. citriodora*, and *P. macrocarpa* extracts were separately tested against *P. oryzae*, *E. rostratum*, and *R. solani*. The plates were incubated at room temperature (26 ± 2°C) until the mycelial growth in control plates for certain fungal species had reached the edge of the plates. The colonial diameter was measured daily and PIDG values were calculated using Equation [1] as described by Lee et al. (2018):

$$PIDG(\%) = \frac{D1 - D2}{D1} \times 100 \qquad \text{Eq.1}$$

where;
D1 = Average increase in mycelial growth in control plates
D2 = Average increase in mycelial growth in treatment plates

Experimental Design and Statistical Analysis

The *in vitro* screenings of antifungal potential of all test plants were conducted in complete randomized design (CRD) with 5 treatments (0%, 5%, 10%, 15%, and 20%). There were 6 replicates for each treatment. Statistical analysis was conducted using SAS® software (SAS Institute, North Carolina State University, USA, Version 9.4, 2012) and comparison of means using least significant difference (LSD) at 5% probability level.

RESULTS AND DISCUSSION

Extract Yield and Antifungal Activity of *Andrographis paniculata* and *Backhousia citriodora*

About 5 g extract yield was obtained from 150 g of each plant. Each of the test fungal pathogen had different incubation period as follows: 12 days after inoculation (DAI) for *P. oryzae*, 5 DAI for *E. rostratum*, and *R. solani*, respectively. The effectiveness of antifungal activity of aqueous extract of *A. paniculata* differed among the test fungal pathogens (Figure 1). Aqueous extracts of *A. paniculata* exhibited different mycelial growth inhibitory activities on *P. oryzae* and *E. rostratum* as compared to *R. solani*. Figure 1 reveals that there had been a gradual rise in mycelial inhibitory activity on *P. oryzae* and *E. rostratum* as the concentration of aqueous extract of *A. paniculata* increased from 0% to 20%. This corroborates with findings of Olufolaji et al. (2015) on the increasing antifungal potency of several plant extracts with increasing concentration in the order against *P. oryzae*. Among all test concentrations, aqueous extracts of *A. paniculata* at 20% concentration significantly inhibited the mycelial growth of *P. oryzae* (PIDG 81.9%) while less inhibition was demonstrated on *E. rostratum* (PIDG 41.3%). It has been reported that both ethanol and aqueous extracts of *A. paniculata* were inactive against *P. oryzae* (Hu et al., 2001), which is contrary to the high inhibitory effect demonstrated in this study. Rajalakshmi et al. (2012) had reported different antifungal efficacy of *A. paniculata* crude extract when tested on five fungal species was attributable to phytochemical constituents of the plant. According to Singha et al. (2003), the significant antimicrobial activity of the *A. paniculata* aqueous extract may be due to the combined effect of the isolated arabinogalactan proteins and andrographolides. Besides, Nidiry et al.

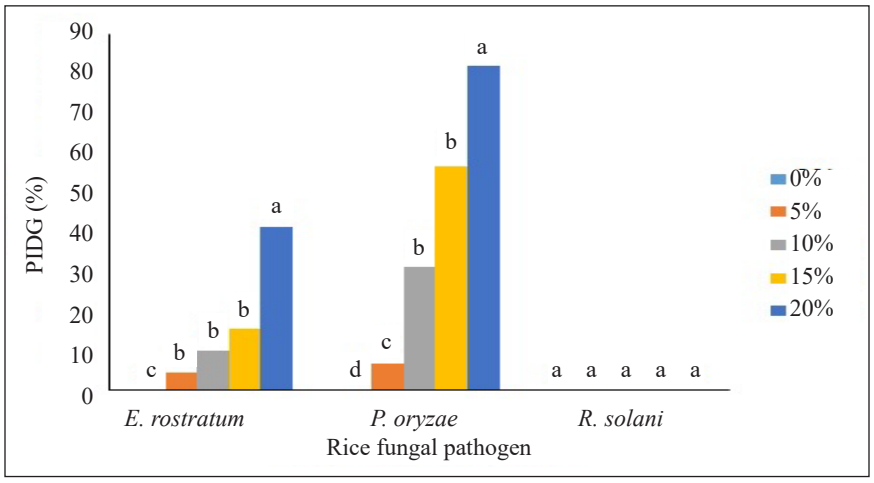

Figure 1. Percentage of inhibition of diameter growth (PIDG) of *Andrographis paniculata* against selected plant fungal pathogens. Measurement made at 5 days after inoculation (DAI) for *Exserohilum rostratum*, 12 DAI for *Pyricularia oryzae*, and 5 DAI for *Rhizoctonia solani*. Values are the means of 6 replicates. Means with the same letter are not significantly different at P = 0.05

(2015) had identified andrographolide as one of the antifungal compounds present in the methanol extract of *A. paniculata*, which resulted in spore germination inhibition of *Alternaria solani*. To date, the antifungal activities of *A. paniculata* leaf extract have been predominantly reported on phytopathogenic *Fusarium* species (Neela et al., 2014; Nidiry et al., 2015; Yasmin et al., 2008). This is the first report on evaluation of antifungal potential of *A. paniculata* aqueous leaf extract on *E. rostratum*. However, a further study is needed to identify the antifungal compounds present in *A. paniculata* leaves.

On the other hand, the PIDG values lower than 50% showed that the aqueous extract of *B. citriodora* did not exert any effective inhibitory activities on the mycelial growth of test organisms (Figure 2). Leaf paste of *B. citriodora* showed antibacterial activity against seven bacterial strains (Wilkinson et al., 2003). Ineffective mycelial inhibition of *P. oryzae* and *E. rostratum* by the aqueous extract of *B. citriodora* at the highest test concentration suggests low abundance of active compounds in the crude extract. Another possible reason is that the active compounds were not efficiently extracted using water suggesting organic solvents may perform better for *B. citriodora* extraction. Nevertheless, significant increase in PIDG values from extracts with concentrations of 15-20% indicating that the inhibitory activity of the aqueous extract of *B. citriodora* on *P. oryzae* and *E. rostratum* was concentration-dependent (Figure 2). Therefore, a further screening of the antifungal potential of crude leaf extract of *B. citriodora* at higher concentrations is needed to validate these plausible reasons. To date, no reports of antifungal activity of *B. citriodora* against plant pathogens were found in the literature. Present experiment suggests that *B. citriodora* may serve as good

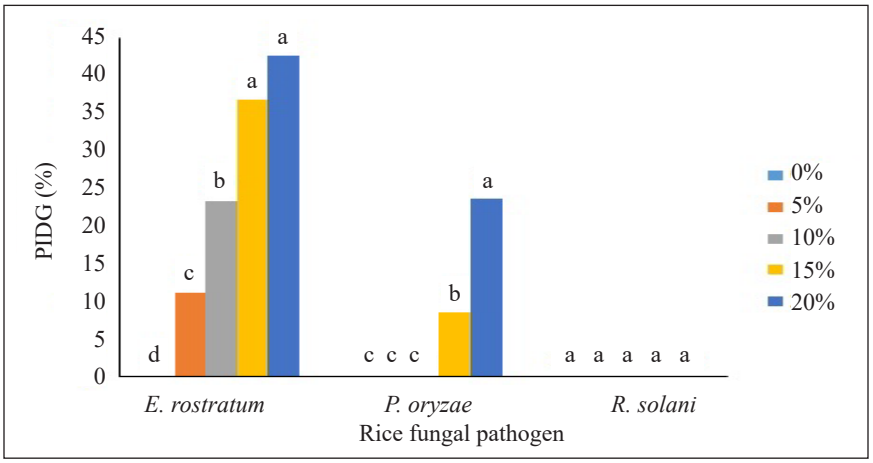

Figure 2. Percentage of inhibition of diameter growth (PIDG) of *Backhousia citriodora* against selected plant fungal pathogens. Measurement made at 5 days after inoculation (DAI) for *Exserohilum rostratum*, 12 DAI for *Pyricularia oryzae*, and 5 DAI for *Rhizoctonia solani*. Values are the means of 6 replicates. Means with the same letter are not significantly different at P = 0.05

natural resource of bioactive compounds for controlling *P. oryzae* and *E. rostratum*. Nevertheless, determination of suitable concentration of crude leaf extract of this herbal plant for inhibition of mycelial growth of *P. oryzae* and *E. rostratum* is crucial.

From Figures 1 and 2, it can be clearly seen that aqueous extracts of both *A. paniculata* and *B. citriodora* were ineffective in inhibiting the mycelial growth of *R. solani*. According to Kurucheve et al. (1997), the variation in the inhibitory effect of plant extracts is caused by qualitative and quantitative differences in antifungal properties.

Extract Yield and Antifungal Activity of *Phaleria macrocarpa*

Around 5 g of extract was obtained from 100 g of *P. macrocarpa* leaf powder. The incubation period of each test pathogen was as follows: 7 days after inoculation (DAI) for *E. rostratum*, 11 DAI for *P. oryzae*, and 5 DAI for *R. solani*. As presented in Figure 3, methanol extracts of *P. macrocarpa* were effective against *P. oryzae*, *E. rostratum*, and *R. solani* at test concentrations of 10% and above (PIDG 100%) as compared to control plates. The presence of an assortment of chemical compounds with antifungal and antibacterial properties in *P. macrocarpa* as reported by Altaf et al. (2013) might have contributed to mycelial inhibition of the test pathogens in this study. For instance, phorbolesters in *P. macrocarpa* seeds inhibited growth of certain fungi including *Aspergillus niger*, *Fusarium oxysporum*, *Ganoderma lucidum*, and *Mucor indicus* (Altaf et al., 2013). Furthermore, Cordell et al. (2001) had reported that flavanoids were the responsible compound for the antifungal activities in higher plants. However, it is important to identify the phytochemicals with antifungal properties of *P. macrocarpa* leaves in order to develop bio-fungicide

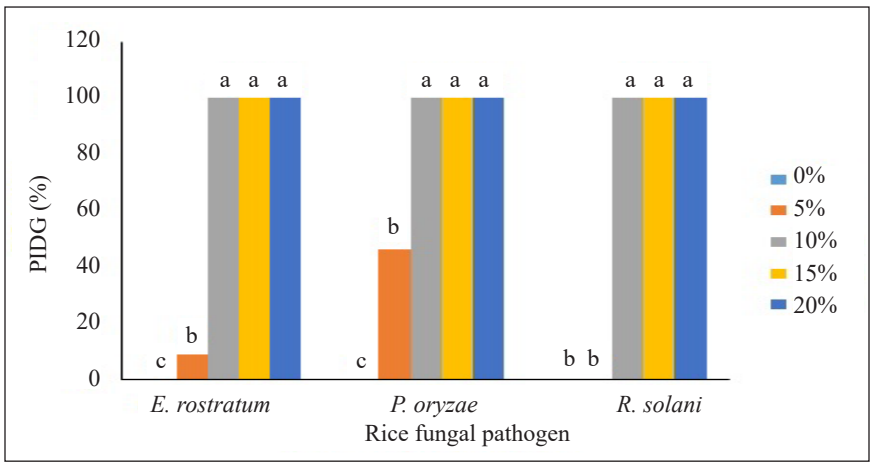

Figure 3. Percentage of inhibition of diameter growth (PIDG) of *Phaleria macrocarpa* against selected plant fungal pathogens. Measurement made at 11 days after inoculation (DAI) for *Pyricularia oryzae*, 7 DAI for *Exserohilum rostratum*, and 5 DAI for *Rhizoctonia solani*. Values are the means of 6 replicates. Means with the same letter are not significantly different at P = 0.05

as an alternative to chemical fungicides. On the other hand, 10% concentration and above had the highest inhibitory activity on the mycelial growth of all test pathogens and these treatments were significantly different from that of 5% concentrations. This suggests that 10% concentration of *P. macrocarpa* methanol extracts is sufficient to inhibit the mycelial growth of *P. oryzae*, *E. rostratum*, and *R. solani in vitro*. To our best knowledge, this is the first report on the antifungal potential of *P. macrocarpa* leaf extracts against fungal pathogens of rice.

CONCLUSION

The antifungal potential of *Andrographis paniculata*, *Backhousia citriodora*, and *Phaleria macrocarpa* against three selected rice fungal pathogens has been determined in this study. Among these herbal plants, *P. macrocarpa* leaf extract had the highest potential to inhibit the mycelial growth of *Pyricularia oryzae*, *Exserohilum rostratum*, and *Rhizoctonia solani* at concentration of 10%. Meanwhile, *A. paniculata* was proven effective against *P. oryzae* at the highest test concentration (20%). The results revealed that leaf extracts of *A. paniculata* and *P. macrocarpa* had high potential to be used as sources of biofungicides in management of rice diseases mainly rice blast. Further studies are required to identify the bioactive compounds of these two herbs and to determine their mechanism of antifungal activities.

ACKNOWLEDGEMENT

The authors thank the Herb Garden, University Agriculture Park, Universiti Putra Malaysia (UPM), Serdang, Selangor and Department of Agriculture, Serdang, Selangor for providing the plant materials. We also acknowledge the Department of Plant Protection, Faculty of Agriculture, UPM for providing research materials in this study.

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