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Submission date: 24-Jun-2024 11:02AM (UTC+0700)

Submission ID: 2200322568

File name: Suwandi_2022_IOP_Conf._Ser._Earth_Environ._Sci._976_012053.pdf (888.45K)

Word count: 3170

Character count: 16842

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To cite this article: S Suwandi *et al* 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **976** 012053

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Allelopathic potential of root exudates from perennial herbaceous plants against *Ganoderma boninense*

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Abstract. Allelopathic potential of root exudates from edible perennial herbaceous (*Canna indica*, *Maranta arundinacea*, and *Xanthosoma sagittifolium*) against mycelium of *Ganoderma boninense*, basal stem rot pathogen of oil palm has been studied *in vitro*. Root exudates were extracted from 10-day old aseptically growing plants generated from surface-sterilized corm or rhizome on water agar. Allelopathic activity was tested based on inhibition of *G. boninense* mycelial growth on malt extract agar supplemented with 0.1 and 1.0 $\mu\text{L mL}^{-1}$ ethyl acetate extract of root exudates. Root exudate from *X. sagittifolium* and *C. indica* was found to significantly inhibit the mycelial growth of *G. boninense* at 0.1 and 1.0 $\mu\text{L mL}^{-1}$, respectively.

Keywords: allelopathy, axenic plant culture, *Ganoderma boninense*, perennial herbaceous, root exudates.

1. Introduction

Ganoderma boninense Pat. is main pathogen of basal stem rot (BSR) that causes widespread economically losses on oil palm plantations in South East Asia. The prevalence increases as increasing replanting generation. It has been estimated that yearly growth rate of area affected by BSR from 1994 to 2009 in Malaysia was 10.3% [1]. Yield losses caused by basal stem rot are both directly due to reducing number of harvesting palm and indirectly attributable to decreasing weight of harvested fruit bunches. *Ganoderma* infection can cause a reduction of fresh fruit bunches yield by 0.04–4.34 tons/ha on 10–20 years planting respectively [2].

Numerous species of antagonistic fungi and bacteria have been extensively studied for their ability to control BSR [3–6]. However, none of the biocontrol agents have been established successfully to control BSR of oil palm in field. Population of introduced microbial antagonist such as *Trichoderma* are rapidly depleted following application [7]. In contrast to short-term protection by microbial antagonists, *G. boninense* produces a pseudosclerotia like-structure on infected tissue that resists from chemical and biological degradation [8]. *Ganoderma* spp. could survive 90% over 2 years on colonized wood in soil [9]. Repeated applications of biocontrol products are necessity to achieved protection from infection, and therefore, frequent treatments are not economically accepted for routine field practices. Long-term survival and continuously active antagonistic organisms are potentially developed to control such long-term survival strategy of BSR pathogens.

Home gardens are prominent feature of traditional tree growing practices in Indonesia that are usually undergrowth by edible non-woody plants such as edible canna (*Canna indica*), arrowroot (*Maranta arundinacea*), and taro (*Xanthosoma sagittifolium*). The herbaceous plants undergo perennial life cycle by producing rhizome and corm. The plants have an extensive root system and therefore can secrete



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metabolite containing exudates that may influence survival and growth of soil pathogens. Suppression activity of those perennial herbaceous against wood decay fungi has been studied on *Rigidoporus microporus*, a white root rot pathogen of rubber tree. Viability and growth of rhizomorph colonizing rubber wood block was suppressed after buried for 90 days in soil planted with herbaceous plants [10]. Our recent study suggested an interference of *G. boninense* mycelial colonization exposed to exudates collected from the herbaceous plants (edible canna, arrowroot and taro *Xanthosoma*) growing under non-aseptic condition [11]. However, direct exudate effects could not be determined when the exudates extracted from plant under non-aseptic condition as potentially interfering with other metabolites secreted by root colonizing-microorganisms. This study examines antagonistic activity of root exudates extracted from aseptically growing three herbaceous (edible canna, arrowroot and taro *Xanthosoma*) against *G. boninense*.

2. Materials and Methods

2.1. *Ganoderma* culture

Ganoderma boninense isolated from pileus context tissue of basidiocarp growing on basal stem rotted oil palm was used through study. Identification has been done based on DNA sequence analysis of the ITS region. Mycelium was maintained on 2% MEA and incubated in darkness at room temperature (24–27 °C) [12].

2.2. Aseptic plant-growth condition and root exudate extraction

Indian shot (*Canna indica*) rhizome, arrowroot (*Maranta arundinacea*) rhizome, and taro (*Xanthosoma sagittifolium*) corm were collected from home garden in South Sumatera. Aseptic root exudates were extracted from aseptically growing plants generated from surface-sterilized corm or rhizome on 3% water agar following procedure described by Schalchli et al. [13] with slight modifications. Corm and rhizome were washed and gently scrubbed under running tap water to remove outer peels, adhering roots and soils. Cleaned corm and rhizome were then surface sterilized by soaking for 5 min in 0.25 g L⁻¹ benomyl and then followed by 5 min dipping using 0.75% hydrogen peroxide for 5 min. Corm or rhizome were then planted on 3% water agar in a transparent glass jar (11.5 cm diam. and 12.5 cm height) and placed in a growth room (24–27 °C, 45–58% RH, 1500 lux). Sprouting corms or rhizomes with vigorous root and shoot growth that free from contamination were selected for exudate extraction. After 10 days of incubation, all plant parts were removed from water agar. Water agar was macerated with 1× volume ethyl acetate and incubated for 24 hours. Macerated solution was then filtered and concentrated under reduced pressure using a rotary evaporator at 40–60 °C. Ethyl acetate extracts were transferred to amber glass vials and stored at –20 °C until used.

2.3. Fungicidal activity assays

Ethyl acetate extracts of herbaceous root exudates (*C. indica*, *M. arundinacea*, and *X. sagittifolium*) were evaluated in two concentration, 0.1 and 1.0 µL of extract per mL of culture medium. MEA (2%) in 9 mm diameter Petri plate was used for culture medium and extracts were added after autoclaving. MEA plate containing 0.1 and 1.0 µL/mL ethyl acetate were used as control. Assay was performed in five replicative plates and arranged in a randomized complete design. Mycelial disks (5 mm diameter) taken out from 5-day-old *G. boninense* culture were planted in the centre of plates prepared with tested extracts. Fungi were incubated at 27 °C until the control reached the edge of the plate. Fungal growth was measured daily based on colony diameter and data were expressed as mycelial growth rate (mm day⁻¹) derived from slope of linear regression between colony diameter (y axis) and days of incubation (x axis) [14].

2.4. Data analyses

Colony diameter and its growth rates between different concentrations were analysed using one-way analyses of variance and compared using Tukey's honestly significant test. Analyses were applied using the packages Rcmdr and Agricolae for R version 3.6.1 (R Foundation for Statistical Computing).

3. Results

Marked inhibition of *G. boninense* mycelial growth was observed on MEA supplemented with *X. sagittifolium* root exudate at both applied concentrations (0.1 and 1.0 $\mu\text{L mL}^{-1}$). *G. boninense* produced smaller colony with dense irregular aerial mycelium. Inhibition of colony growth was also found in *C. indica* root exudate treatment, but colony diameter was greater than that of *X. sagittifolium* root exudate treatment. Root exudate of *M. arundinacea* showed a slight growth inhibition on colony growth of *G. boninense*. Mycelial growth of tested fungus was not affected on MEA containing both ethyl acetate concentrations (0.1 and 1.0 $\mu\text{L mL}^{-1}$) by producing a uniform pattern of aerial mycelium (Figure 1).

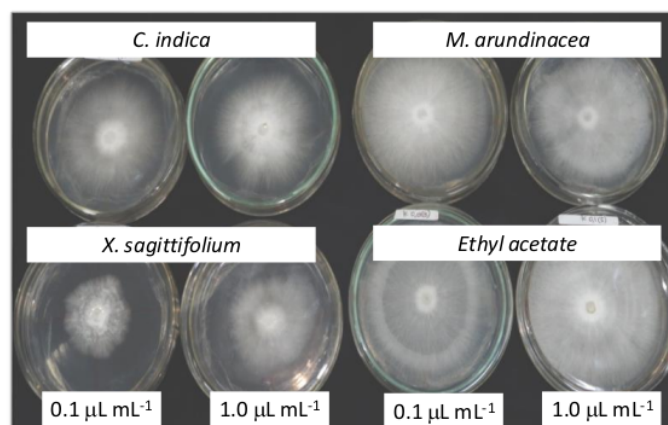


Figure 1. Colony of *Ganoderma boninense* on malt extract agar supplemented with ethyl acetate extracts of herbaceous plant root exudates. Root exudates were extracted from 10-day old aseptically growing plants generated from surface-sterilized corm or rhizome on water agar.

Growth inhibition was started to observe for 3 days after incubation as measured by a significantly smaller colony for *X. sagittifolium* root exudate compared to that of ethyl acetate control. All treated exudates, except exudate of *M. arundinacea* caused colony growth retardation at 5 days after incubation. Following incubation for 6 and 7 days, inhibition on colony diameter was observed on treatment with all concentration of *X. sagittifolium* exudate and *C. indica* at 1.0 $\mu\text{L mL}^{-1}$, but no substantial inhibition on treatment with exudate of *M. arundinacea* (Figure 2).

Effects of root exudates on growth of *G. boninense* was also demonstrated based on growth rate of colony. *G. boninense* colony showed a reduced growth rate on MEA supplemented with root exudates of *X. sagittifolium* and *C. indica* compared to those on ethyl acetate-supplemented medium. *X. sagittifolium* inhibited growth rate of fungal colony at both applied concentrations (0.1 and 1.0 $\mu\text{L mL}^{-1}$) whereas *C. indica* suppressed fungal growth when applied at a higher concentration (1.0 $\mu\text{L mL}^{-1}$). Root exudates of *X. sagittifolium* suppressed growth of *G. boninense* by 32.2 and 30.1% at concentration 0.1 and 1.0 $\mu\text{L mL}^{-1}$, respectively, while *C. indica* inhibited fungal growth by 28.1 % at concentration 1.0 $\mu\text{L mL}^{-1}$ relative to growth rate on control ethyl acetate-supplemented medium. Supplementation of medium with 0.1 $\mu\text{L mL}^{-1}$ *M. arundinacea* resulted in 17.6% growth retardation, but statistically was not significant (Figure 3).

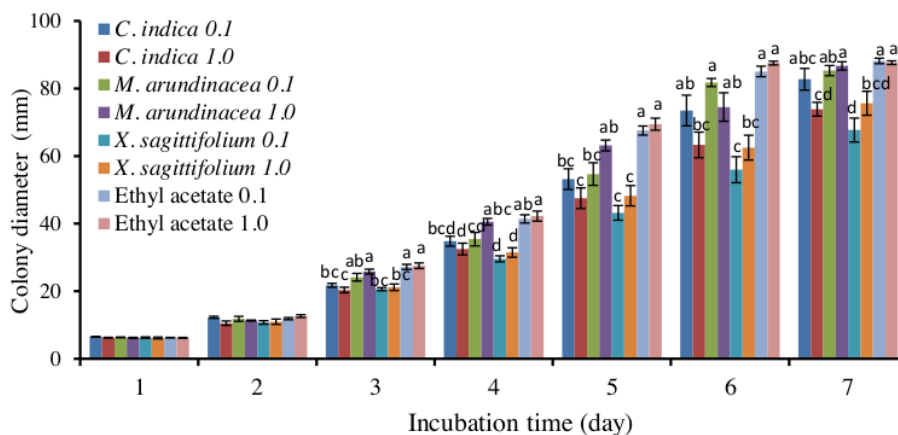


Figure 2. Development of *Ganoderma boninense* colony on malt extract agar supplemented with ethyl acetate extracts (expressed in $\mu\text{L mL}^{-1}$) of herbaceous plant root exudates. Bars are means \pm SEM of five replicate plates. For each day of incubation time, bars with different letters are significantly different ($p < 0.05$) according to the Tukey HSD test.

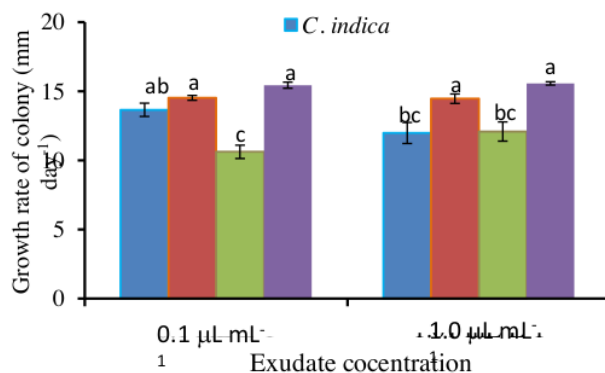


Figure 3. Growth rate of *Ganoderma boninense* colony on malt extract agar supplemented with ethyl acetate extracts (expressed in $\mu\text{L mL}^{-1}$) of herbaceous plant root exudates. Error bars represent standard error of mean from five replicate plates. Bars are means \pm SEM of 5 replicate plates; bars with different letters are significantly different ($p < 0.05$) according to the Tukey HSD test.

4. Discussion

In this study, we demonstrated the antifungal activity or allelopathic inhibition of herbaceous plant root exudates that were extracted from an axenic culture system on *G. boninense*, basal stem rot pathogen of oil palm. All three extracted root exudates had ability to suppress mycelial growth of *G. boninense* with *X. sagittifolium* that exhibited the strongest antifungal activity. Little information was available on direct fungitoxicity of axenic culture-derived root exudates of *X. sagittifolium*, *C. indica*, and *M. arundinacea* or even from other species of the three plant genera. Most fungitoxicity assays used rhizome extracts rather than root exudates to evaluate antifungal activity of those herbaceous plants. Water extract from *X. sagittifolium* corm has been demonstrated to have a weak antifungal activity against *Fusarium* sp. [15]. Strongly antifungal activity of *X. sagittifolium* has been reported from aqueous extract of *X. sagittifolium* leaves against human fungal pathogens *Candida albicans*, *Trichophyton rubrum* and

Cryptococcus neoformans [16]. Extracts from rhizome of *C. indica* also have been revealed to have an antimicrobial activity. Essential oils with terpene/terpenoid constituents extracted from *C. indica* rhizome has shown to have a moderate inhibitory activity against the Gram (+) bacteria, *Staphylococcus aureus* and *Bacillus subtilis* [17]. Further antimicrobial assay by George [18] suggested the moderate antifungal activity of those essential oils from *C. indica* rhizome against *Aspergillus niger*, *Candida albicans*, *Fusarium oxysporum*, *Rhodotorula* sp., and *Trichoderma viride*. Organic extracts from rhizome of *M. arundinacea* have been analyzed to contain 49 phytochemical compounds belong to flavonoids, alkaloids, tannins, glycosides, steroids, phenols and saponins [19], but have not been known to have antifungal activity.

The chemical nature of allelochemicals present in the root exudates was not determined. However, the literature described trypsin inhibitors present as allelochemicals in root exudates of *X. sagittifolium* [20] that may function to suppress the growth of root rot fungi. A trypsin inhibitor secreted by roots of the medicinal herb, *Pseudostellaria heterophylla* could inhibit growth of the phytopathogens, *Colletotrichum gloeosporioides* and *Fusarium oxysporum* [21]. *C. indica* roots secreted a high concentration of allelochemical organic acids and have been reported to play in inhibition against the blue-green algae *Microcystis aeruginosa* [22–23]. Hydroalcoholic extracts of *C. indica* roots and rhizomes have been detected to contain 3'-hydroxytrimethoprim and 3,7-epoxycaryophyllan-6-one [24] are usually fungitoxic.

Allelopathic and fungicidal activities of root exudates against *G. boninense* as described *in vitro* in this study may also operate in field soil. In a field soil trial, burying of rubber wood block colonized by *G. boninense* into roots of taro plants had induced in 2.1 times higher the wood decomposition [25]. Allelopathic activities had been considered to be associated with reduction in rhizomorph viability and growth of *R. microporus* colonizing rubber wood blocks buried closely to *X. sagittifolium*, *C. indica*, or *M. arundinacea* [10]. Further study is needed to determined antifungal activity of those perennial herbaceous under soil infiltration assay. For example, amongst aqueous rhizome extracts of *Curcuma domestica* (turmeric), *C. xanthorrhiza* (wild ginger), *Zingiber officinale* (ginger), *Alpinia galanga* (galangale), and *Sansevieria trifasciata* (snake plant) that had *in vitro* antifungal activities, only *C. domestica* and *A. galangal* exhibited inhibition activity under soil infiltration assay [26]. Once positively determined in soil assay, organic rhizome extracts of *C. domestica* was able to slightly reduce disease caused by *R. microporus* in a pot assay [27].

5. Conclusion

Root exudates derived from axenically growing taro *Xanthosoma* and edible canna exhibited allelopathic inhibition on mycelial growth of *G. boninense*.

Acknowledgments

This research was financially supported by the Directorate of Research and Community Service, Ministry of Research and Technology/National Research and Innovation Agency of the Republic of Indonesia through the Basic Research Program 2021 (No.150/SP2H/LT/DRPM/2021).

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