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Original Article

Powdered bamboo shoot extracts on the growth of anthracnose (*colletotrichum gloeosporioides*)

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ABSTRACT

Bamboo shoots are known to contain antifungicidal properties. This contains moderate dietary fiber, essential amino acids and other anti-inflammatory agents. The antimicrobial activity of crude methanolic extracts of thorn (*Bambusa blumeana Schultes f.*), golden (*Schizostachyum brachycladum*) and giant (*Dendrocalamus asper*) bamboos were investigated in this study. Equivalent amounts of each powdered sample of the bamboo shoot powder were soaked in methanol solvent and were filtered and evaporated. The extracts were spread over potato dextrose agar (PDA) medium under aseptic condition and incubated at 37^0C. A completely randomized design (CRD), with three replications, was used in this experiment. The result showed that all bamboo shoot extracts showed highly significant anti-fungal activity resulting in zero growth (0) mm from 24 and 72 hours or equivalent to 100% inhibition as compared to the control that has observed fungal growth on 48 and 72 hours at 6.5±0.5SD mm and 8.63±0.71SD mm, respectively. Thus, the result showed that *Bambusa blumeana Schultes f.*, *Schizostachyum brachycladum,* and *Dendrocalamus asper* extracts have potential anti-fungal compounds. Hence, bamboo shoots are effective in combatting anthracnose and its major economic loss in several crops.

KEYWORDS: *bamboo methanolic extracts, inhibition zone, postharvest pathogen, anti-fungal*

1 INTRODUCTION

Bamboo is a large woody grass that belongs to the family *Poaceae*. It is a tremendously diverse plant that can adapt to any extreme climatic and soil conditions. About 90 genera and 1200 species of bamboo are found in the world (FAO, 2007). Bamboo shoots contain high protein but less fat, dietary fiber, essential amino acids, selenium, potassium, and minerals for healthy hearts. It has been utilized in Korea for traditional treatment to relieve hypertension, sweating, and paralysis. It has been established that bamboo extract has antioxidant activities and anti-inflammatory effects (Jung *et al*., 2005).

Fungal diseases cause extensive yield loss in important crops, thus becoming one of the major challenges in the production and post-production of agricultural crops. Most postharvest pathogens cause decay and postharvest losses, and one of the destructive plant pathogens distributed worldwide is anthracnose which causes major economic losses in several crops. Thus, it is a re-emerging disease problem for growers worldwide (Sakar, 2016). It is considered one of the most problematic and economically harmful plant diseases on various hosts, from trees to grasses. In immature fruits, the disease may not be readily observed as it remains in the quiescent stage. However, after harvest, symptoms of the disease become readily observable (Muirhead & Gratitude, 1986; Dodd *et al*., 1997). Synthetic fungicides are a primary means to control postharvest diseases (Eckert, 1990 and 1991). They are either used alone, combined in the mixtures, or applied separately in sequence (Ismail & Zhang, 2004). However, several fungicides have been removed from the market due to possible toxicological risks. In addition, repeated use of certain systemic fungicides in packinghouses has led to the appearance of fungicide-resistant pathogens.

Due to health concerns and environmental degradation brought about by synthetic fungicides in the agriculture sector for more than a decade, many alternatives to chemical control have been investigated in reducing postharvest diseases of fruits and vegetables. These include the utilization of biocontrol agents, irradiation and other physical treatments, natural antimicrobial substances, and organic and inorganic compounds [Janisiewicz and Korsten, 2002; Nigro *et al*., 2002; Ippolito and Nigro, 2003; Palou *et al*., 2002]. A vital alternative to fungicides promising to manage postharvest diseases of fruits in a wide range of crops is plant extracts. Bamboo powder derived from the shoots

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of *Bambusoidae,* has become a promising alternative treatment due to its natural characteristics, anti-fungal activity, and elicitation of defense responses in plant tissue (Terry and Joyce, 2004). Research results have indicated that bamboo powder can inhibit *Sclerotium rofsii* in melon *Cucumismelo var. amanta* (Darma *et al.,* 2006). According to Wang and Ng (2003), dendrocin, a distinctive anti-fungal protein, is found in bamboo shoots. Dendrocin exerts anti-fungal activity to *Botrytis cinerea, Fusarium oxysporum* and *M. arachidicola*.

However, information in using extracts of the bamboo shoot as an agent against fungus is still scarce and has limited literature. Therefore, this study was conducted to investigate the natural anti-fungal properties of bamboo shoots native to the Philippines by measuring the mycelial growth of each treatment on different duration.

2 MATERIALS AND METHODS

Extraction of Plant Material

Bambusa shoots were collected from Sirao farm, Sirao, Cebu City. The collected Bambusa shoots were washed with distilled water to remove dust particles. The shoots were crushed and air-dried on a plastic tray at ambient temperature for three days, then dried in an oven at 50℃ for 6 days to remove residual moisture. Thorn, golden, and giant Bambusa shoots powder were separated and produced by crushing into fine powder form in a blender. Each 200g ground sample of different bamboo species was placed in an empty container and soaked in 1.2L methanol solvent. Each container was sealed with aluminum foil and parafilm to prevent evaporation. The suspension solutions were left to stand for seven days and then filtered (filter paper 90mm) and evaporated by vacuum using a rotary evaporator. The extracts were used as an anti-fungal agent by using 0.10 g/L on the potato dextrose agar (PDA) media.

Anti-fungal Activity of Bamboo Shoots

0.10g/L of bamboo shoots extract, as used by Igbinosa *et al.,* (2009). Amycelial plug of 6mm in diameter was taken from a 7-day old culture fungus using inoculating loop/ needle. Then, the culture was seeded in the center of each PDA plate. A 550 µl (microliter) of the culture was spread over each PDA plate by shaking the plate under an aseptic condition in laminar airflow. The inoculated plates were incubated for 72 hours at 37℃. The diameter of mycelial growth of fungus (colony diameter) on each PDA plated was measured using a ruler. The anti-fungal action of the extract was calculated using the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Colony diameter of (control - treatment)}}{\text{Colony diameter of control}} \times 100$$

Experimental design and statistical analysis Treatments

A completely randomized design (CRD), with three replications with 10 samples, was used in this experiment. Data on mycelial growth were analyzed using SPSS (Statistical Package for the Social Sciences) on analysis of variance, and a further test was done using Tukey's test at $p<0.05$ to test for differences between treatment means.

3 RESULTS AND DISCUSSION

In this figure, all the added fungicide treatments, thorn bamboo extract, golden bamboo extract, and giant bamboo extract, were all effective on the growth of anthracnose. This has zero growth (0) mm from 24 and 72 hours or equivalent to 100% inhibition as compared to the control where fungal growth was observed after 48 and 72 hours at 6.5±0.5SD mm and 8.63±0.71SD mm, respectively (Figure 1). This result was supported by Liao *et al.*, (2021) in the article where the extract of moso bamboo leaf showed good anti-fungal activity to *Phytophthora capsic* and *Fusarium graminearum* with a 100% and 75.12% inhibition, respectively. This was also supported with the result of Darma *et al.,* (2016), where a significant decrease was observed in disease incidence (up to 77%) occurred in (*Bacillus subtilis*) BMB26 - treated melon leaves. This was inoculated with *S. rolfsii* after four days post-inoculation in which it was concluded that *Bacillus subtilis* BMB26 has a potential application as a bio-control agent against phytopathogenic fungi.

Table 1. Development of mycelial growth of fungus during the 3-day inoculation on different treatments of bamboo

	24 hrs	48 hrs	72 hrs
Control	0	6.5±0.5SD	8. 63±0.71SD
Thorn BE	0	0	0
Golden BE	0	0	0
Giant BE	0	0	0

Chemicals including tannins, steroids, phenols, glycosides, flavonoids, carbohydrates, and proteins were identified and were responsible for the antimicrobial activity (Wu et al., 2012). This was reported by Othman *et al.,* (2019), where it stated that polyphenols are one of the most numerous and diverse group of secondary metabolites and their antioxidant properties provide the basis for antimicrobial effects while alkaloids provided the underlying structure for the development of several

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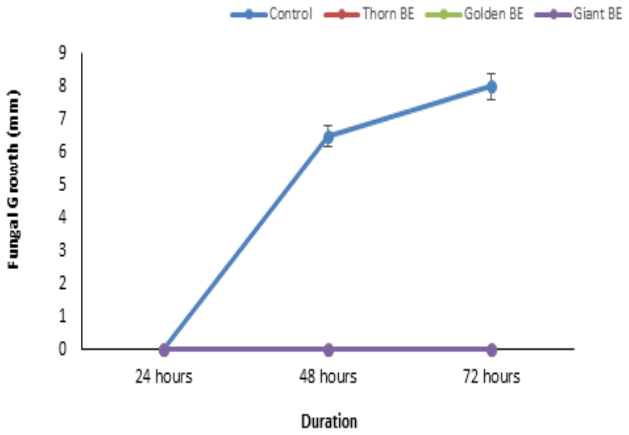

Figure 1. Development of mycelial growth of fungus during the 3-day inoculation on potato dextrose agar containing different treatments

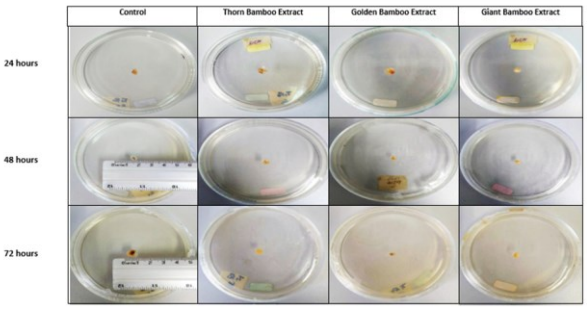

Figure 2. Zone of growth inhibition (mm) of fungus after incubation for 24, 48 and 72 hrs (top to bottom) at 37ºC on potato dextrose agar medium

antibiotics with a diverse range of action. Moreover, in the study of Rathod et al., (2011), it was found out that the different parts of bamboo contain silica, cholin, betain, cynogenetic glycosides, albuminoids, oxalic acid, reducing sugar, resins, waxes, benzoic acid, various amino acid, proteolytic enzyme, nuclease, urease.

Preliminary studies demonstrated that diblock amino acid copolymers, containing cationic and hydrophobic blocks could show substantial antimicrobial activity (Bevilacqua *et al*., 2015). It was also mentioned in the study of Jastrzębowska and Gabriel, (2015) that threonine, amino acids found in bamboo seeds, has a role as an intermediate of isoleucine synthesis aspartate pathway, in which its biosynthesis has been studied extensively as a source of potential anti-fungal targets.

4 CONCLUSIONS

All the powdered bamboo shoot extracts are effective as anti-fungal which inhibit the growth of anthracnose. This resulted in zero growth (0) mm from 24 and 72 hours or equivalent to 100% inhibition compared to the control after 48 and 72 hours at 6.5±0.5SD mm, 8.63±0.71SD mm, respectively.

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