In Vitro Evaluation of Paclobutrazol against Selected Pathogenic Soil Fungi

Ahmad Nazarudin Mohd Roseli[1]* and Mohd Farid Ahmad[2]
[1]Forestry and Environment Division,
[2]Forest Biodiversity Division,
Forest Research Institute Malaysia (FRIM),
52109 Kepong, Selangor, Malaysia.
**Email: nazarudin@frim.gov.my*

ABSTRACT

An experiment was conducted to determine the effects of paclobutrazol (PBZ) on the growth of selected pathogenic soil fungi namely *Phellinus noxius, Rigidoporus microporus, Fusarium oxysporum,* and *Ceratocystis fimbriata*. These fungi were initially isolated from different infected trees and fields, and given FRIM reference numbers. Nine concentrations of PBZ (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40 g/L) were added into petri dishes containing potato dextrose agar prior to the inoculation of the fungal isolates. Percent inhibition of mycelial growth of each fungus as a response to PBZ was determined. Results showed that the mycelial growth of *P. noxius* (FRIM613), *P. noxius* (FRIM137), and *R. microporus* (FRIM641) was greatly inhibited for up to 90% when treated with at least 0.05 g/L PBZ, while the growth of *C. fimbriata* (FRIM1227) and *F. oxysporum* (FRIM688) was retarded at a lower rate of 68% and 70%, respectively. PBZ was found able to control the growth of these fungi. Further assessments on the effectiveness of PBZ in controlling these pathogens in field condition are essential.

Keywords: Fungal growth retardant, *Phellinus noxius, Rigidoporus microporus, Fusarium oxysporum, Ceratocystis fimbriata*

INTRODUCTION

Fungal diseases cause adverse effects on plant growth both in plantations and urban areas. Previous researchers reported that many plantation species such as *Hevea brasiliensis* (Johnston, 1989), *Cicer arietinum* (Maitlo et al., 2014), *Punica granatum* (Imran Khan et al., 2017), *Elaeis guineensis* (Hefni et al., 2017), *Eurycoma longifolia* (Wan-Muhammad-Azrul, 2018), and certain ornamental trees such as *Pterocarpus indicus* (Sanderson et al., 1997), *Quercus nuttallii, Fraxinus pennsylvanica,* and *Quercus lyrata* (Baietto and Wilson, 2010) were highly susceptible to fungal infections. According to Baietto and Wilson (2010), *Q. nuttallii, F. pennsylvanica,* and *Q. lyrata* showed the highest levels of wood decay caused by *Armillaria mellea, Ganoderma lucidum,* and *Heterobasidion annosum* attacks. In Malaysia, wilt disease of *P. indicus* (angsana) is a classic example of a soil fungus infection on urban tree. Several outbreaks of the infection were reported in various parts of Peninsular Malaysia such as Johor Bharu, Kuantan, Kemaman and Chuping (Philip, 1999). Angsana trees infected by a pathogenic *Fusarium oxysporum* showed yellowing and wilting of leaves at the early stage of infection which eventually fall, leaving barren tree canopy (Figure 1A). The trees only survived about four to five weeks after the onset of the disease symptoms (Philip, 1999). Other than angsana wilt, another strain of *F. oxysporum* was often found to be associated with sudden death syndrome (SDS) of a commercial medicinal plant, *Eurycoma longifolia* (Wan-Muhammad-Azrul et al., 2018). *E. longifolia* saplings affected with this fungus usually displays dried shoots symptom (Figure 1B).

Ceratocystis fimbriata, Phellinus noxius and *Rigidoporus microporus* are also commonly reported as the causal agent of plant mortality (Brooks, 2002; Poojary and Mugeraya, 2012; Sahashi et al., 2014). *Ceratocystis fimbriata* causing wilt disease and death in many plants species such as *H. brasiliensis* (Silveira et al., 1994), *Eucalyptus* sp. (Roux et al., 2000), *Coffea* sp. (Marin et al., 2003),

Mangifera indica (van Wyk et al., 2007), and *Acacia mangium* (Tarigan et al., 2010). The disease is lethal and it can be recognised by the yellowing and sudden wilting of leaves and defoliation that often leads to tree death (Figure 1C). Cross section of the infected stem exhibited brown discolouration in the outer xylem from roots to the main trunk (Somasekhara et al., 2000). *P. noxius* and *R. microporus* are pan-tropical root rot fungi that were often reported causing serious mortality on trees especially in *H. brasiliensis* plantations (Holliday, 1980). These destructive pathogens usually spread from tree to tree via root systems and kill the vascular tissue of the infected trees (Brooks, 2002), showing symptoms such as wilting, leaf chlorosis and necrosis, and defoliation (Poojary and Mugeraya, 2012). In more advanced stages, the infected tree canopy often manifested heavy defoliation (Figure 1D). Both root disease fungi are also known to be pathogenic and destructive to various trees in the tropics (Mohd Farid et al., 2009; Sahashi et al., 2014). Control of the root rot fungi using commercial fungicides is often costly, ineffective, and labour intensive. Similarly, a biological approach using antagonistic *Trichoderma* was also found to be less effective, especially in the field trial as compared to the laboratory (Jayasuriya and Thennakoon, 2007).

Concern on the economic importance of root fungi infection, studies on control and mitigation of the pathogens still continue either in laboratory or in the field. Studies have shown the potential of paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol] in controlling plant pathogens. It is extensively reported to have plant growth regulating properties, protecting plants from biotic and abiotic stresses (Fletcher et al., 2000; Jacobs and Berg, 2000). Commercially, it is used to control plant height, thus favouring a more compact appearance, produce more intense green foliage, and increase stress tolerance (Bañón et al., 2005; Ahmad Nazarudin, 2012; Matsoukis et al., 2014; Ahmad Nazarudin et al., 2015). PBZ was also reported to successfully delay the growth of *Botrytis cinerea* causing blight disease in a landscape plant, *Chamelaucium uncinatum* (Martínez et al., 2007). Likewise, application of PBZ on *Zea mays* infected by dwarf mosaic virus was also effective as 56% of the plants recovered after treatment with 200 mg/L (Mohamed, 2010).

Application of PBZ in combating pathogens was thought to be an option beside other chemicals approach. Thus, a preliminary fungicidal screening aimed to determine the efficacy of PBZ against four selected fungi, *P. noxius, R. microporus, F. oxysporum*, and *C. fimbriata* was carried out at a laboratory scale. The findings from this study might be beneficial for future investigation on the potential of PBZ towards controlling growth of certain fungal species.

MATERIALS AND METHODS

Preparation of fungal isolate

The experiment was carried out at the Pathology Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Selangor. A total of four pathogenic cultured soil fungi i.e., *P. noxius, R. microporus, F. oxysporum*, and *C. fimbriata* were selected. All fungi were initially isolated from their host trees at different fields, and given FRIM reference numbers (isolate IDs) (Table 1). In the laboratory, they were cultured at 25±2°C for 7 days.

Figure 1. *Fusarium* wilt disease infection on road side angsana tree (A); sudden death syndrome showing dried shoots of *E. longifolia* (B); wilting young foliage of *A. mangium* tree in plantation caused by *Ceratocystis* wilt disease (C); and heavy defoliation of *H. brasiliensis* due to root rot disease (D).

Table 1. Detail information of the selected fungi.

Isolate ID	Fungi	Host tree	Location	Disease
FRIM137	*Phellinus noxius*	*Acacia mangium*	Kalimantan, Indonesia	Brown root
FRIM613	*Phellinus noxius*	*Tectona grandis*	Sabak Bernam, Selangor	Brown root
FRIM641	*Rigidoporus microporus*	*Hevea brasiliensis*	Batu Anam, Johor	White root
FRIM688	*Fusarium oxysporum*	*Eurycoma longifolia*	KESEDAR, Gua Musang, Kelantan	Sudden death syndrome
FRIM1227	*Ceratocystis fimbriata*	*Eurycoma longifolia*	Felda Jengka, Pahang	Sudden death syndrome

Preparation of treatments and growing medium

Cultar® formulation containing 250 g a.i. PBZ/L was used in this study. Nine PBZ concentrations i.e., 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40 g/L were prepared. Each concentration of PBZ was then transferred into 250 mL Duran bottle containing liquid potato dextrose agar (PDA) as growing medium and stirred evenly for 3 to 5 min. Subsequently, 25 mL of the mixture was poured into sterilised Petri dishes and left harden at room temperature. A 5 mm mycelial disc of seven-day-old fungi was excised from the margin of the culture by using a surface sterile cork borer, placed at the centre of the Petri dish, and incubated at 25±2°C for 10 days. PDA medium without addition of PBZ served as control. The treatments were replicated thrice and assigned in a randomised complete block design (RCBD).

Data collection

The radial growth (cm) of each fungus was measured in four directions and averaged, on a daily basis. The measurement was carried out for ten days. Percent inhibition of the pathogens was then calculated by using a formula described by Vincent (1947).

Percent inhibition (I) = (C-T)/C x 100 % ---------- (Vincent, 1947)

Where,

C = Radial growth of fungus in control

T = Radial growth of fungus in treatment

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and the Tukey's Studentized Range (HSD) Test was used to compare treatment means. The significant differences of the means were identified at $p< 0.05$.

RESULTS AND DISCUSSION

This study showed that all untreated control fungi demonstrated a drastic growth increment starting from day 3 towards the end of the experiment except for isolate FRIM1227. At day 10, the untreated control of FRIM613, FRIM137, FRIM641 and FRIM688 demonstrated a rapid growth of more than 3 cm compared to FRIM1227. The expansion of these isolates has increased more than two folds than the untreated

FRIM1227. It was also noted that the growth of these fungi was greatly suppressed at increasing concentrations of PBZ (Figure 2). PBZ-treated fungi namely FRIM613, FRIM137, and FRIM641 had a relatively slower growth increment when compared to FRIM1227 and FRIM688.

At 10 days after treatment, significant differences in terms of growth were recorded between the untreated control and PBZ-treated fungi (Table 2). PBZ has effectively inhibited the growth of FRIM137, FRIM613, FRIM641 and FRIM1227 isolates, showing less than 1 cm growth at ten days after treatment as compared to FRIM688. These results suggested that FRIM688 was more robust towards different PBZ concentrations as compared to other pathogens (Table 2). The growth of all fungi was significantly inhibited, however, their sensitivity towards fungicidal effects of PBZ varied. In other words, the suitable dosages (inhibiting dose) required to restrain the growth were relatively differed, depending on the aggressiveness of each fungus. For instance, the inhibiting dose was 40 ppm PBZ for *Macrophomina phaseoli*, while it was 50 ppm PBZ for *F. oxysporum* (Bolu and Cimen, 2006). Previous study by Jacobs and Berg (2000) concluded that the mycelial growth of eight plant pathogens namely *Armillaria gallica*, *Botryosphaeria dothidea*, *Ceratocystis fadacearum*, *Fusarium roseum*, *Ophiostoma novo-ulmi*, *Sirococcus clavigignenti-juglandacearum*, *Sphaeropsis sapinea* and *Verticillium dahliae* was also inhibited by PBZ treatment. The unique chemical formation of PBZ that allows it to bind to an iron atom in the enzymes crucial for gibberellins biosynthesis in plants could also have the ability to bind to the enzymes necessary for steroids production in fungi as well as those that are responsible for destruction of abscisic acid in plants (Mohamed, 2010). The fungicidal action of triazoles is usually attributed to their interfering with the biosynthesis of sterols, which are necessary components of fungus cell membranes (Mohamed, 2010).

In the present study, the growth of all pathogenic fungi was retarded following PBZ treatments (Table 3). More than 90% growth retardation was observed in FRIM613, FRIM137, and FRIM641 compared to FRIM1227 and FRIM688. These results suggested that PBZ has the fungicidal effect which inhibits the growth of fungi in this study.

CONCLUSIONS

PBZ showed fungicidal effects that inhibited the radial growth of the selected fungi. However, the intensity of growth retardation varied amongst species. The growth of three fungi i.e., FRIM613 (*P. noxius*), FRIM137 (*P. noxius*), and FRIM641 (*R. microporus*) was highly inhibited by approximately 90% after being treated with at least 0.05 g/L PBZ. The efficacy of PBZ against the selected fungi in descending order was FRIM613 (*P. noxius*), FRIM137 (*P. noxius*), FRIM641 (*R. microporus*), FRIM1227 (*C. fimbriata*) and FRIM688 (*F. oxysporum*). PBZ is believed to have the potential to be the alternative in controlling plant diseases caused by these fungi. However, more studies are needed to determine the optimum concentration of PBZ that could be applied against the fungi.

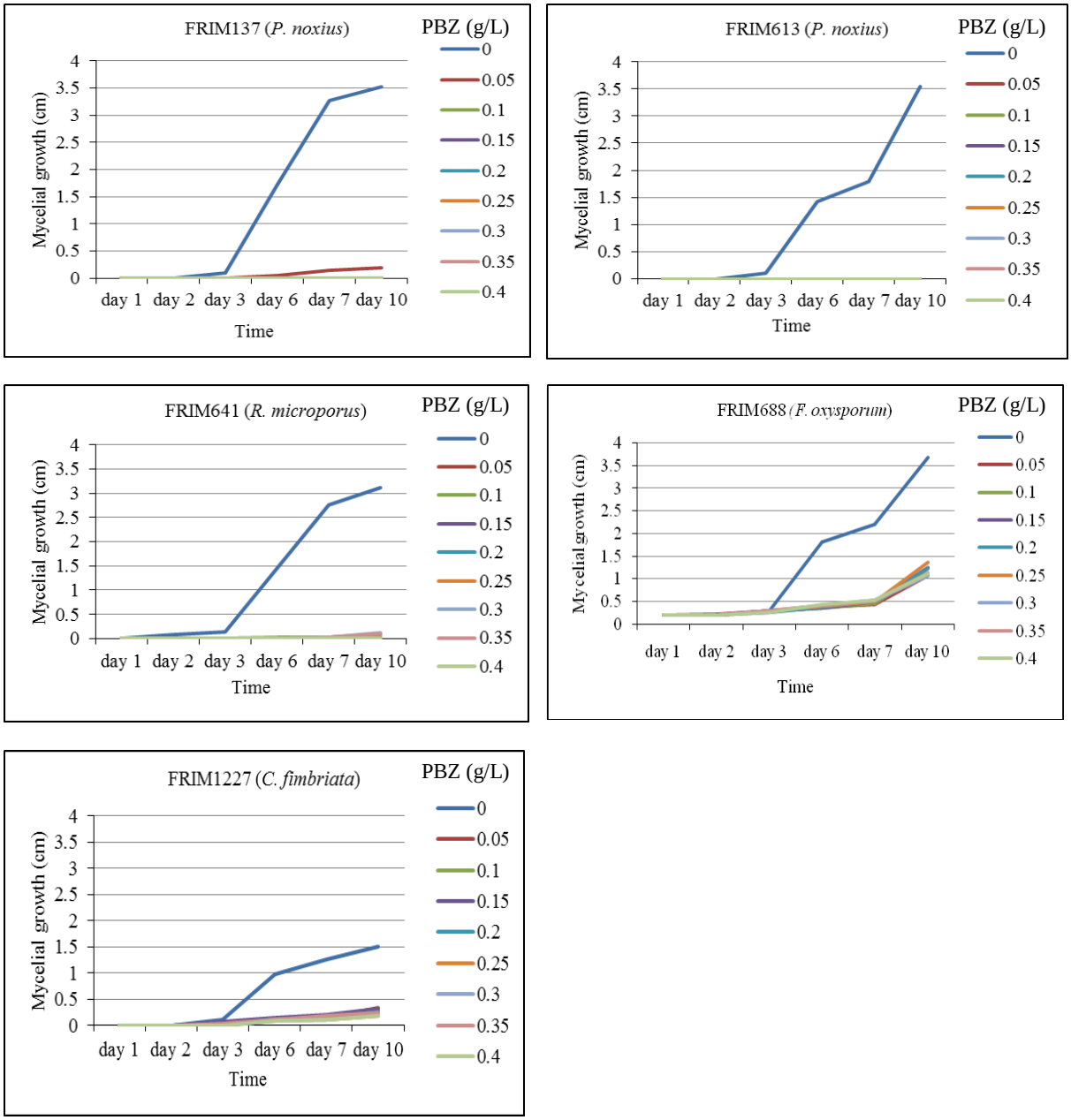

Figure 2. Growth of selected fungi as influenced by different concentrations of PBZ.

Table 2. Mycelia growth of selected fungi at 10 days after treatment with different concentrations of PBZ.

PBZ (g/L)	Mycelial growth (cm)									
	FRIM137		FRIM613		FRIM641		FRIM688		FRIM1227	
	0-day	10-day	0-day	10-day	0-day	10-day	0-day	10-day	0-day	10-day
0	0.200 a	3.708 a	0.200 a	3.667 a	0.200 a	3.308 a	0.200 a	3.692 a	0.200 a	1.708 a
0.05	0.200 a	0.467 b	0.200 a	0.200 b	0.200 a	0.325 b	0.200 a	1.242 b	0.200 a	0.533 b
0.10	0.200 a	0.200 c	0.200 a	0.200 b	0.200 a	0.300 b	0.200 a	1.258 b	0.200 a	0.500 b
0.15	0.200 a	0.200 c	0.200 a	0.200 b	0.200 a	0.283 b	0.200 a	1.225 b	0.200 a	0.508 b
0.20	0.200 a	0.200 c	0.200 a	0.200 b	0.200 a	0.233 b	0.200 a	1.375 b	0.200 a	0.458 b
0.25	0.200 a	0.200 c	0.200 a	0.200 b	0.200 a	0.217 b	0.200 a	1.075 b	0.200 a	0.392 b
0.30	0.200 a	0.200 c	0.200 a	0.200 b	0.200 a	0.200 b	0.200 a	1.142 b	0.200 a	0.417 b
0.35	0.200 a	0.200 c	0.200 a	0.200 b	0.200 a	0.200 b	0.200 a	1.767 b	0.200 a	0.450 b
0.40	0.200 a	0.200 c	0.200 a	0.200 b	0.200 a	0.200 b	0.200 a	1.117 b	0.200 a	0.383 b

Means followed by the same letter(s) within column do not differ ($p<0.05$) by Tukey's Studentized Range (HSD) Test

Table 3. Efficacy of PBZ against selected fungal pathogens.

Fungi	Per cent inhibition (%) of selected fungi							
	0.05 g/L	0.10 g/L	0.15 g/L	0.20 g/L	0.25 g/L	0.30 g/L	0.35 g/L	0.40 g/L
FRIM613	94.67	94.67	94.67	94.67	94.67	94.67	94.67	94.67
FRIM137	90.00	94.63	94.63	94.63	94.63	94.63	94.63	94.63
FRIM641	93.95	91.44	93.95	93.95	93.45	90.18	90.93	93.95
FRIM1227	68.78	70.73	70.24	73.17	77.07	75.61	73.66	77.56
FRIM688	70.59	66.29	65.84	66.74	62.67	70.81	69.01	52.04

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