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Full Length Article

Effect of Contact-Pesticide Mancozeb on the Growth, Enzyme Production, and Morphology of White-Rot Fungi Isolated from Berbak-Sembilang, Indonesia

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Abstract

Mancozeb (MZ) is a non-systemic dithiocarbamate fungicide, which is widely used as a contact fungicide to treat fungal plant diseases. The MZ poses a potential health risk for rural communities. White-rot fungi (WRF) can efficiently degrade many organic contaminants, including MZ. However, research on the effect of MZ on the growth of the fungus itself is still limited. The purpose of this study is to investigate the tolerance of the Indonesian WRF *Leiotrametes menziesii* BRB 73, *Ceriporia lacerata* BRB 81, *Phellinus noxious* BRB 11, and *Lentinus sajor-caju* BRB 12 on the MZ-containing media. The growth, enzyme activity, and morphological structure of the fungus in response to Mancozeb were studied. The four WRFs show tolerance to MZ. No statistically significant changes in fungal growth, enzyme production, and morphological structure were observed between the control and sample treatments. The growth and enzyme inhibition of all fungal isolates were less than 40 and 35%, respectively at all tested dosages of MZ. The present study emphasizes the significant potential of the Indonesian WRF discovered from the Berbak-Sembilang region, demonstrating a notable resistance to the contact pesticide Mancozeb. These fungal species have been recognized as potential candidates for the bioremediation of this pesticide.

Keywords: Contact-pesticide; Mancozeb; Pesticide-degrading enzyme; Morphological structure; White-rot fungi

Introduction

Pesticide use has been the primary method of controlling pests and diseases in crops and after harvest to ensure productivity and product quality. Overuse of pesticides, or even picking before the preharvest interval, can result in pesticide residues in the product. The extensive use of pesticides also results in the accumulation of chemical residues in the environment, posing a severe hazard to land and aquatic ecosystems. Pesticide residues endanger the environment by creating resistant pathogens that disrupt the environment and endanger human health (Rodrigues *et al*. 2019; Venkatachalapathy *et al*. 2019; Ukaogo *et al*. 2020).

Pesticides are classified into numerous categories. Systemic and non-systemic (contact) pesticides are distinguished by their mode of action (Bilal *et al*. 2019). Pesticides with systemic action are absorbed through the leaves, stems, or roots and then transported into the plant through the vascular system. In contrast, non-systemic (contact) pesticides exclusively target the external surface of fruit and vegetable plants (Rodrigues *et al*. 2019, 2021). Some types of pesticides included in contact pesticides are dichlorvos, malathion, chlorpyrifos, chlorothalonil, and mancozeb (Fatma *et al*. 2018; Carniel *et al*. 2019; Rodrigues *et al*. 2019, 2021).

Mancozeb (MZ) is a non-systemic dithiocarbamate fungicide and has been widely used in agriculture for more than 50 years as an effective disease-control tool for vegetables and fruit crops such as corn, sorghum, peanuts, potatoes, tomatoes, apples, melon, etc. (Gullino *et al*. 2010; Fatma *et al*. 2018). When MZ is exposed to water, it degrades into ethylene bisiothiocyanate sulfide (EBIS), which is then converted to ethylene bisiothiocyanate (EBI) by the action of UV radiation. EBIS and EBI are considered to interfere with metabolic processes inside the cytoplasm and mitochondria of fungal cells, resulting in suppression of spore germination by altering lipid metabolism, respiration, and adenosine triphosphate (ATP) synthesis (Geissen *et al*. 2010; Iorio *et al*. 2015).

Microbial degradation is the most significant and effective method to remove pesticides from the environment. Microorganisms can interact with substances, both chemically and physically, resulting in structural changes or complete degradation of the target molecules.

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Several types of microorganisms, such as algae: *Anabaena azotica* (Zhang *et al*. 2012), *Chlorella* sp., and *Scenedesmus* sp. (Matamoros and Rodriguez 2016), bacteria: *Microbacterium* sp. (Cabrera *et al*. 2010), *Klebsiella* (Singh and Singh 2014), *Pseudomonas aeruginosa* (Khan *et al*. 2023) and fungi: *Aspergillus* sp. (Hultberg and Bodin 2018), *Phanerochaete chrysosporium* (Wu *et al*. 2013), *Ganoderma lucidum* (Kaur *et al.* 2016), and *Lentinus* (Serbent *et al.* 2020) are known to be capable producing enzymes belonging to the Pesticide-degrading enzymes (PDE) group. This group of enzymes includes monooxygenase, oxidoreductase, dehalogenase, phosphotriesterase, esterase, dioxygenase, laccase, manganese peroxidase, lignin peroxidase (Sarker *et al*. 2021), and polyphenol oxidase (Gouma *et al*. 2019).

Bacteria and fungi are the primary transformers and pesticide degraders in microbial ecosystems (Pinto *et al*. 2020). Several studies have investigated the degradation process of MZ using microorganisms, both bacteria and fungi. Bacteria such as *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*, and *Enterobacter cloacae* effectively degrade MZ residues in tobacco leaves (ChunHua *et al*. 2017). *Aspergillus niger* and *Aspergillus flavus* isolated from pesticide-contaminated water degrade MZ up to 23% and more than 50%, respectively (Aimeur *et al*. 2016). However, the response of the microorganism to pesticide exposure has not been well studied. Only Serbent *et al.* (2020) reported the daily fungal growth rate, laccase production, and mycelial structure of *Lentinus crinitus* EF 58 after several days of 2,4-D herbicide exposure.

In previous research, new fungi *Leiotrametes menziesii* BRB 73, *Ceriporia lacerata* BRB 81, *Phellinus noxious* BRB 11, and *Lentinus sajor-caju* BRB 12, isolated from Berbak-Sembilang National Park, Jambi, Indonesia showed a good ability to decolorize textile dyes of anthraquinone (AB129, RBBR), mono azo (AO7), and diazo (RB5) (Nurhayat *et al.* 2022). Screening of white-rot fungi (WRF) for pesticide resistance is useful for identifying potential candidates for active bioremediation applications. Moreover, research on the effect of MZ on the growth of the fungus itself is still limited. In this study, four species of WRF were examined for their ability to grow on an agar plate culture system with the pesticide MZ. The growth, enzyme activity, and morphological structure of the fungi in response to MZ were examined. Therefore, this research aims to evaluate the potential use of Indonesian WRF, *L. menziesii* BRB 73, *C. lacerata* BRB 81, *P. noxious* BRB 11, and *L. sajor-caju* BRB 12 as biodegradation agents of MZ by assessing their tolerance and growth in an agar-plate culture system with this fungicide.

Materials and Methods

Experimental materials and design

Experimental material: The research activity was carried out at the Integrated Laboratory of Bioproducts (iLaB), National Research and Innovation Agency (BRIN), Indonesia, during January–August 2023. The white rot fungi (WRF), *L. menziesii* BRB 73 (NCBI GenBank, accession No. MT804553), *C. lacerata* BRB 81 (NCBI GenBank, accession No. MT804554), *P. noxious* BRB 11 (NCBI GenBank, accession No. MT804574), and *L. sajor-caju* BRB 12 (NCBI GenBank, accession No. OR050821), were isolated from Berbak-Sembilang National Park, Jambi, Indonesia (Nurhayat *et al.* 2022). Potato dextrose agar (PDA) was purchased from Merck (Germany). The contact pesticide, Tridex WP80 (Mancozeb (MZ) 80%), was purchased from an Indonesian pesticide retail market. Gallic acid as an indicator of enzyme production was supplied by Sigma-Aldrich (St. Louis, MO, USA). Lactophenol cotton blue for fungal staining was provided by Himedia (Mumbai, India).

Experimental design: The research design used a completely randomized design (CRD) with 5 treatments and 3 repetitions. Treatments given were MZ 0 mg/L (MZ0, as a control), MZ 100 mg/L (MZ100), MZ 500 mg/L (MZ500), MZ 1000 mg/L (MZ1000), and MZ 2000 mg/L (MZ2000). The treatments were applied to four isolates of white-rot fungi (WRF), *L. menziesii* BRB 73, *C. lacerata* BRB 81, *P. noxious* BRB 11, and *L. sajor-caju* for 7 days. The effects of the treatment on fungal growth, enzyme production, and morphological structure were evaluated.

Effect of MZ on fungal growth and enzyme production

The effect of contact-pesticide on fungal growth and enzyme production was conducted following the method of Serbent *et al.* (2020). The contact-pesticides, MZ solution (MZ100, MZ500, MZ1000 and MZ2000), were sterilized by filtration through a 0.22-µm syringe filter and applied to a final volume of 100 µL on the surface of PDA medium containing 0.05% (w/v) gallic acid in a Petri dish (Ø 90 mm). The pesticide solution was spread with a sterilized Drigalski spatula. An eight-millimeter mycelial disk of each fungus (7 days old) was inoculated on the center of the agar plates with and without MZ (control, MZ0). The dishes were incubated in an incubator at 27±3°C for 5–7 days (the time when the control covered the whole surface of the growth medium). The diameter of the central mycelium was then measured in a single position. Enzyme production was indicated by substrate oxidation, resulting in a halo formation. The diameter of the halo formation was also assessed for 5–7 days.

Effect of MZ on the morphological structure of fungi

The mycelia of each fungus were examined under an optical light microscope to confirm visible changes in morphology when the fungi grew in the presence of the contact pesticide. A 7-day-old of 5 mm diameter of mycelium each fungus was inoculated into a sterile cover glass placed over the PDA medium in a Petri dish following Putra *et al.* (2023).

After two days of incubation, 100 µL of MZ solution (MZ100, MZ500, MZ1000 and MZ2000) was dripped into the mycelial disk. The cultures were then incubated in an incubator at 27±3°C for 5 days. After incubation, the mycelial disk was placed on a microscope slide, protected by a cover slip, and the slides were stained with lactophenol cotton blue and examined under an optical microscope (Olympus, BX63). Counting and measuring the width of hyphae, and assessing cytoplasmic density, clamp connection, and cell wall were performed without contact pesticides (control, MZ0) and with contact pesticides (MZ100, MZ500, MZ1000 and MZ2000).

Growth rate, enzyme production rate, and inhibition analysis

Daily growth rate (DGR) and enzyme production rate (DER) were estimated by subtracting the mycelium and halo diameter formed at the end of incubation from the beginning of incubation, as described by Serbent *et al.* (2020).

$$\text{DGR/DER (mm/day)} = \frac{\text{Db} - \text{Da}}{\text{tb} - \text{ta}}$$

Where Db is the diameter of the mycelium/halo formation at the end of the incubation time, Da is the diameter of the mycelium disk/halo formation at the initial time (8 mm) and tb–ta is the incubation time interval (in days). The percentage of growth and enzyme production inhibition was calculated using an equation according to Abadi *et al*. (2022).

$$\text{Inhibition (\%)} = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \times 100$$

Where Dc is the diameter mycelium/halo of fungal control (mm), Dt is the diameter mycelium/halo of fungal treatment (mm).

Statistical analysis

The assay was performed in three replicates. Data were subjected to a one-way analysis of variance (ANOVA) and the means were compared using Tukey's test at the 5% level.

Results

Effect of MZ on fungal growth

The mycelium diameter of four fungi is shown in Fig. 1A and Table 1. Mycelial growth of *L. menziesii* BRB 73, *C. lacerata* BRB 81, *P. noxious* BRB 11, and *L. sajor-caju* BRB 12 was detected in all concentrations of MZ. The mycelium diameter is lower as the quantity of MZ increases. The results of one-way ANOVA and Tukey test compared means of daily mycelium diameters showed no significant ($P>0.05$) differences between the control and the addition of MZ at a concentration of 100 mg/L for four fungi. The mycelium diameter of *L. menziesii* BRB 73 and *C. lacerata* BRB 81 was significantly different from the control at MZ, concentration between 500–2000 mg/L, while the mycelium diameter of *P. noxious* BRB 11 was significantly different at higher concentrations of MZ, namely 1000–2000 mg/L. However, there were no significant differences in the growth of *L. sajor-caju* BRB 12 between the control and all treatments. MZ inhibited the growth of the four fungal isolates by 0–8.52, 0–18.52, 6.94–20 and 8.33–37.96% at doses of 100, 500, 1000 and 2000 mg/L, respectively (Fig. 1B).

The growth rate of the four fungi was reduced as the concentration of MZ got higher, by the diameter of the fungal mycelia (Table 2). The control of *L. menziesii* BRB 73 and *C. lacerata* BRB 81 had the same DGR value of 11.71 mm/day. Meanwhile, *P. noxious* BRB 11 and *L. sajor-caju* BRB 12 showed a higher DGR value of 16.40 mm/day. The DGR value for each fungus was significantly different from the control when MZ was added at concentrations between 500 and 2000 mg/L. *L. menziesii* BRB 73 grew at the slowest rate, 6.38 mm/day, with the addition of 2000 mg/L MZ.

Effect of MZ on enzyme production

The halo diameter of four fungi is shown in Fig. 2A. Bavendamm (gallic acid oxidation) response was positive in all our experiments, as indicated by the formation of a dark brown halo around the fungal colony (Table 3), indicating gallic acid degradation by oxidative enzymes. Similar to mycelial growth, halo diameter decreased with increasing pesticide concentration. The results of one-way ANOVA and the Tukey test compared means of halo formation showed that the addition of MZ at a concentration of 100 mg/L did not affect the halo formation of four fungi. The halo diameter of *L. menziesii* BRB 73 and *C. lacerata* BRB 81 appeared to decrease when MZ was added at doses ranging from 500 mg/L to 2000 mg/L. The halo diameter of *C. lacerata* BRB 81 at a concentration of 2000 mg/L of MZ had the smallest diameter (60 mm only). However, the decrease in halo diameter did not appear to be significant for *P. noxious* BRB 11 and *L. sajor-caju* BRB 12 even at a concentration of 2000 mg/L.

The MZ inhibited the enzyme production by the four fungal isolates by 0–13.89, 1.85–13.33 and 3.70–33.33% at doses of 500, 1000 and 2000 mg/L, respectively (Fig. 2B). Suppression of enzyme production did not occur with the addition of MZ at 100 mg/L. The enzyme production rate of the four fungi dropped as the concentration of MZ got higher, in accordance with the halo diameter (Table 4). The control of *L. menziesii* BRB 73 and *C. lacerata* BRB 81 had the same DER value of 11.71 mm/day. Meanwhile, *P. noxious* BRB 11 and *L. sajor-caju* BRB 12 showed a higher DER value of 16.40 mm/day. The DER value for each fungus was significantly different from the control when MZ was added at concentrations between 500 and 2000 mg/L. The lowest DER value (7.43 mm/day) was obtained by *C. lacerata* BRB 81 at 2000 mg/L of MZ concentration.

Table 1: Growth of WRF in medium with and without Mancozeb

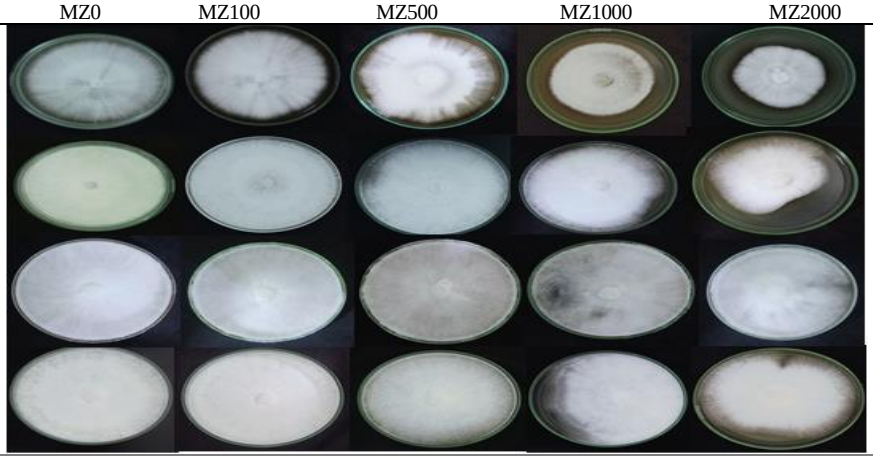
Fungi	Mycelia				
	MZ0	MZ100	MZ500	MZ1000	MZ2000
L. menziesii BRB 73					
C. lacerata BRB 81					
P. noxious BRB 11					
L. sajor-caju BRB 12					

Table 2: Daily fungal growth rate (DGR) of white rot fungi in agar medium with and without Mancozeb

Fungi	Fungal daily growth rate (DGR) (mm/day)				
	MZ0	MZ100	MZ500	MZ1000	MZ2000
L. menziesii BRB 73	11.71±0.00^{c}	10.62±0.32^{bc}	9.33±0.21^{b}	9.14±1.25^{b}	6.38±0.21^{a}
C. lacerata BRB 81	11.71±0.00^{c}	11.71±0.00^{c}	10.64±0.00^{b}	10.64±0.00^{b}	7.25±0.05^{a}
P. noxious BRB 11	16.40±0.00^{c}	16.40±0.00^{c}	11.71±0.00^{b}	10.67±0.33^{a}	9.88±0.65^{a}
L. sajor-caju BRB 12	16.40±0.00^{b}	16.40±0.00^{b}	11.12±0.63^{a}	10.82±0.62^{a}	9.99±1.19^{a}

Note: The mean value followed by the same letter is not significantly different according to the Tukey (HSD) test at the 0.05 significance level

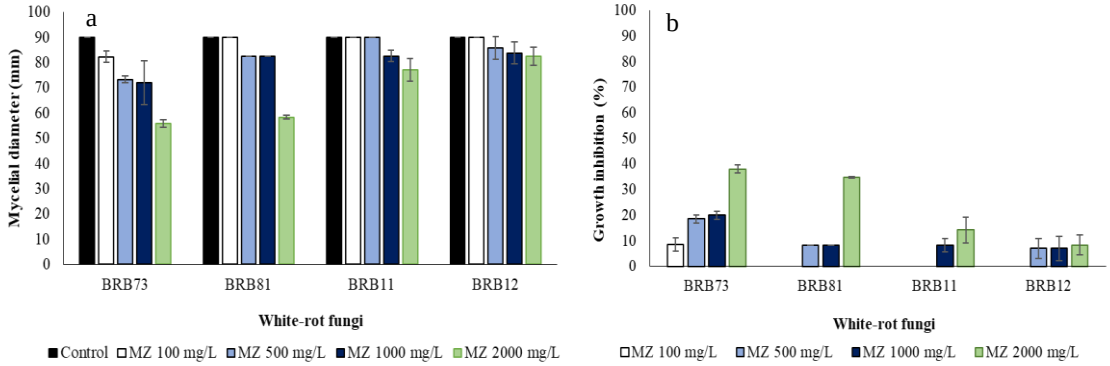


Fig. 1: Mycelia diameter (a) and growth inhibition (b) of WRF in agar medium treated with increased MZ concentrations

Effect of MZ on the morphological structure of fungi

The morphological characteristic of four fungi with and without MZ was analyzed by optical microscopy. The clamp connection (Fig. 3) was detected at all treatments, including the control. However, the cytoplasmic density of each fungus, reduced as MZ concentration increased (Table 5 and Fig. 4A–C). In general, the diameter of the hyphae decreased as MZ concentration increased, except for *P. noxious* BRB 11, which appeared to enlarge compared to the control. Some of the hyphae treated with 2000 mg/L MZ pose thicker cell walls compared to control and other treatments (Fig. 4D). However, at certain doses of MZ, the hyphae diameter of the four fungi did not differ significantly from that of the control (Fig. 5).

Discussion

Among the WRF tested, *L. sajor-caju* BRB 12 was the fungus with the highest growth rate even when exposed to MZ up to 2000 mg/L. Several *Lentinus* strains have also been reported to grow in the range of 10–16 mm/day when cultured on PDA, including *L. crinitus* EF 58 (13.83 mm/day), *L. sajor-caju* (Fr.) Fr. (13 mm/day), and *L. cladopus* Lév. (15 mm/day) (Serbent *et al.* 2020). However, the highest growth rate of 18 mm/day was achieved by *L. squarrosulus* Mont. (Sharma and Atri 2013).

The results of this study indicated that WRF were resistant to MZ. The percentage of growth inhibition for all fungal isolates was less than 40% at all tested doses of MZ (Fig. 1B). Meanwhile, none of the four WRFs tested were

Table 3: Enzyme production of WRF in medium with and without Mancozeb

Fungi	Halo formation				
	MZ0	MZ100	MZ500	MZ1000	MZ2000
L. menziesii BRB 73					
C. lacerata BRB 81					
P. noxious BRB 11					
L. sajor-caju BRB 12					

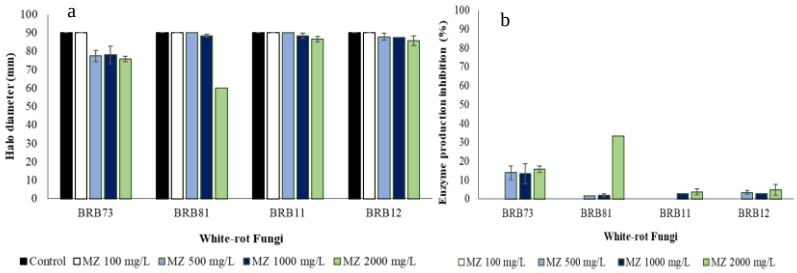


Fig. 2: Halo diameter (a) and enzyme production inhibition (b) of WRF in agar medium under increased MZ concentrations

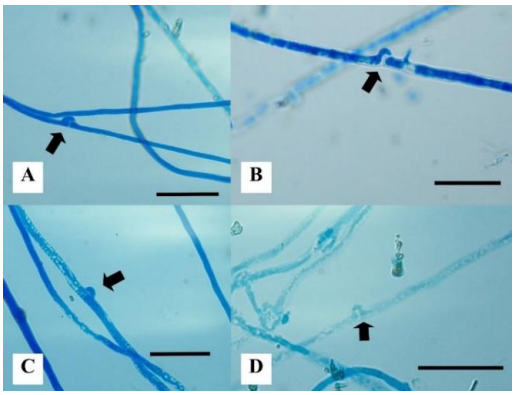


Fig. 3: WRF clamp connection (arrow) observed under increased MZ concentrations

completely suppressed in their enzyme production by MZ. The enzyme inhibition percentages for the four fungi were less than 35% at all tested MZ doses (Fig. 2B). There are still limited scientific reports that have been studied on the ability of WRF on biodegradation of MZ compound. The previous studies that report on biodegradation are mostly using filamentous fungi or bacteria. Abadi *et al.* (2022) reported a 35% inhibition in the growth of the filamentous fungus *Trichoderma harzianum* TD1 at a concentration of 150 mg/L of MZ. Mohiddin and Khan (2013) observed that the presence of MZ at concentrations of 755 mg/L and 625 mg/L inhibited the growth of *T. harzianum* and *T. virens*, respectively. Furthermore, *Bacillus subtilis* and *Pseudomonas fluorescens* growth was suppressed at MZ concentrations of 1000 and 2000 mg/L, respectively.

This study demonstrates that white-rot fungi are more

Table 4: Daily enzyme production rate (DER) of WRF in agar medium with and without Mancozeb

Fungi	Daily enzyme production rate (DER) (mm/day)				
	MZ0	MZ100	MZ500	MZ1000	MZ2000
L. menziesii BRB 73	11.71±0.00^{b}	10.62±0.32^{b}	9.33±0.45^{a}	10.00±0.69^{a}	9.69±0.21^{a}
C. lacerata BRB 81	11.71±0.00^{c}	11.71±0.00^{c}	11.50±0.00^{b}	11.48±0.11^{b}	7.43±0.00^{a}
P. noxious BRB 11	16.40±0.00^{c}	16.40±0.00^{c}	11.71±0.00^{b}	11.48±0.21^{ab}	11.24±0.21^{a}
L. sajor-caju BRB 12	16.40±0.00^{b}	16.40±0.00^{b}	11.42±0.27^{a}	11.36±0.00^{a}	10.05±1.82^{a}

Note: The mean value followed by the same letter is not significantly different according to the Tukey (HSD) test at the 0.05 significance level

Table 5: Comparison of clamp connection and cytoplasmic density of white rot fungi with and without Mancozeb

Fungi	Clamp connection					Cytoplasmic density				
	MZ0	MZ100	MZ500	MZ1000	MZ2000	MZ0	MZ100	MZ500	MZ1000	MZ2000
L. menziesii BRB 73	yes	yes	yes	yes	yes	+++++	++++	+++	+++	++
C. lacerata BRB 81	yes	yes	yes	yes	yes	+++++	++++	+++	+++	++
P. noxious BRB 11	yes	yes	yes	yes	yes	+++++	++++	+++	+++	++
L. sajor-caju BRB 12	yes	yes	yes	yes	yes	+++++	++++	+++	+++	++

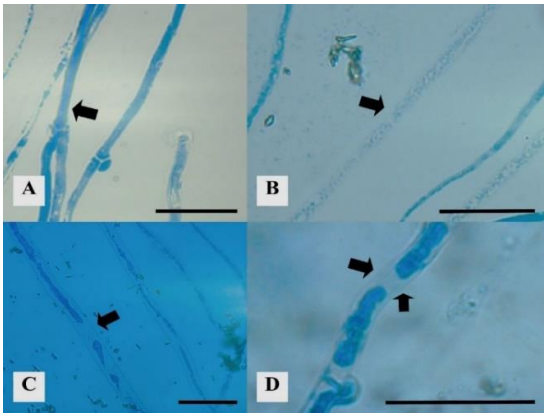


Fig. 4: WRF cytoplasm density features (arrow) under MZ100 (A), MZ500 (B) and MZ100 (C) treatments. Cell wall response at MZ2000 (D) indicated by two arrows. Bars: 20 µm

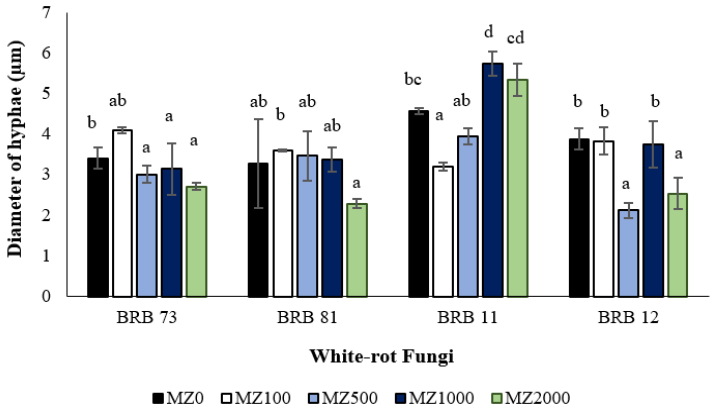


Fig. 5: Diameter of WRF hyphae under increased concentrations of MZ. The mean values followed by the same letter are non-significantly different (P > 0.05)

tolerant to MZ than filamentous fungi or bacteria. The resistance that WRF have in responding to the presence of hazardous substances in the environment, such as pesticides, can be caused by the enzymes they produce. Naman *et al.* (2022) described several pathways for the removal of pesticide residue by microorganisms. First, pesticides are adsorbed onto polysaccharides in the cell walls of microorganisms. Second, because pesticide residues are high in nitrogen, carbon, and phosphorus, microbes use them as nutrition. Finally, microorganisms produce PDE, which are responsible for biological degradation.

The WRF are known to produce enzymes that belong

to the PDE group. The enzymes include monooxygenase, oxidoreductase, dehalogenase, phosphotriesterase, esterase, dioxygenase, laccase, manganese peroxidase, lignin peroxidase (Sarker *et al.* 2021) and polyphenol oxidase (Gouma *et al.* 2019). *Pycnoporus coccineus* is resistant to contact pesticides, chlorpyrifos. This is because *P. coccineus* produces large amounts of polyphenol oxidase, which degrades pesticide mixtures even under low nutritional conditions (Gouma *et al.* 2019). *L. crinitus* is tolerant to 2,4-D herbicide at concentrations of 5–670 g/L (Serbent *et al.* 2020). The spectrum of proteins secreted by *L. crinitus* consists of CAZymes (active carbohydrate enzymes), oxidase/reductase, protease, lipase/esterase, laccase, and manganese peroxidase (Serbent *et al.* 2020). Most of the enzymes produced by *L. crinitus* belong to the PDE group and are therefore capable of degrading pesticides.

Tolerance of fungi to toxic exposure results in morphological changes at the cellular level. These morphological changes have direct impact on the growth rate and enzyme synthesis. In this study, there were no significant morphological changes in the presence of the clamp connection on fungi with and without MZ exposure (Fig. 3 and Table 5). This indicates that exposure to MZ did not affect the sexual production of WRF. Basidiomycete fungi rely on a specific type of hyphal fusion that results in the formation of small hyphal bridges surrounding the septa known as clamp connections, which are critical for allowing nuclear migration during mitotic development and maintaining a tightly controlled dikaryotic state within each cell (Fischer and Glass 2019). Clamp connections were absent in numerous species of Basidiomycetes, *L. crinitus* EF58, with and without exposure to 2, 4-D herbicide. The absence of clamps in *L. crinitus* culture is not a result of exposure to 2, 4-D. This pattern in *L. crinitus* EF 58 strain might be due to the fact that the isolate has been re-cultured many times (Serbent *et al*. 2020).

Changes in cytoplasmic density are also considered a fungal response to toxic environmental conditions. In this study, the cytoplasmic density of each fungus decreased as MZ concentration increased (Fig. 4, Table 5). Cytoplasmic density fluctuates during the cell cycle among different cell types as a function of age, diseases, and nutritional stress (Molines *et al.* 2022). The changes in density can potentially affect the amounts of macromolecules and the physical properties of the cytoplasm, including viscosity levels. Consequently, these changes may have significant implications for several cellular functions, such as protein association, phase transition, and enzyme movement (Molines *et al.* 2022).

In addition to clamp connections and cytoplasmic density, variations in hyphae diameter are a reaction to environmental stress. The MZ exposure to four WRF tested had a different effect on hyphae diameter. In this study, the hyphal diameter of four WRF tested without MZ exposure was approximately 3.28–4.58 µm. The increase in MZ concentration resulted in a decrease in the diameter of the hyphae. Some of the hyphae treated with MZ pose thicker cells as a response to the stress condition. However, at specific dosages of MZ, the hyphae diameter of the four fungi did not differ significantly from the control (Fig. 5). Generally, hyphae have different diameters ranging from 1 to 30 µm, depending on the specific species and the prevailing growth conditions (Islam *et al.* 2018).

The results of growth, enzyme production rates, and morphological changes show that the WRF tested are relatively tolerant to this contact pesticide, MZ. These fungi are identified as promising candidates for bioremediation of this pesticide. The previous study by Cupul *et al*. (2014) showed eight species of WRF strains that were still able to grow, and their enzymes were still active by the increasing of atrazine (herbicide) concentration. Recent studies have shown that tolerance is not closely related to degradation capacity. However, this needs to be further studied.

Conclusion

Berbak-Sembilang Indonesian WRF *L. menziesii* BRB 73, *C. lacerata* BRB 81, *P. noxious* BRB 11, and *L. sajor-caju* BRB 12 were tolerant to MZ. There are no significant morphological changes in the fungal structure (the presence of clamp connection and the hypha diameter). Decrease in cytoplasmic density caused by increased doses of MZ has a direct impact on the decreased growth rate and enzyme synthesis in each fungus. However, MZ did not completely inhibit the growth and enzyme production of any of the four WRFs tested. Indonesian WRF from Berbak-Sembilang region showed tolerance to the MZ, which are identified as promising candidates for bioremediation of this pesticide.

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Author Contributions

SHA designed the study, performed research, analyzed data, interpreted the results, and wrote the initial manuscript. ODN performed research, collected data, and interpreted the results. DZ performed research, collected data, and reviewed the manuscript. IPP interpreted the results, reviewed and edited the manuscript. DHYY interpreted the results, reviewed, edited the manuscript, and supervised all the experiments.

Conflicts of Interest

The authors confirm that they have no known financial or interpersonal conflicts that could have appeared to influence the research presented in this paper.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper

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