Preliminary Study on the Tolerance of Soil Fungi to Methyl Parathion

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ABSTRACT Pesticides such as methyl parathion (MP) are widely used to prevent pests from destroying crops and reducing the harvest. However, indiscriminate use of pesticides led to accumulation of pesticides in soil and drinking water, which result in severe impacts on human's health. Mycoremediation has been suggested as an environmentally friendly and cost-effective way to remediate pesticides by breaking down the pollutants. Hence, the objective of this research is to find out which fungi species have the potential to tolerate the toxicity of MP at various increasing concentration. Eleven fungi species were screened using 10 ppm of MP on PDB. Out of the 11 species, five were found to grow and were further tested on PDB using increasing concentration of MP (10-40 ppm). Results showed three species namely, *Penicillium chrysogenum*, *Aspergillus nidulans* and *Aspergillus niger* have the highest mean dry biomass (g) in PDB with 40 ppm of MP, and thus were determined as potential fungi species to tolerate MP.

KEYWORDS: Mycoremediation; Methyl parathion; *Penicillium chrysogenum*; *Aspergillus nidulans*; *Aspergillus niger*; Environmental toxicity; Pesticide tolerance

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INTRODUCTION

In this modern era, pesticides are widely used to put pests in control and to secure high product quality (Begum *et al.*, 2017). Methyl parathion is an organophosphorus pesticide which is used to kill insects like mites, weevils, and leafhoppers (United States Environmental Protection Agency, 2003). The pesticide is highly mobile in the soil, which overdoses usage will easily cause contamination to nearby area. Methyl parathion toxicity is primarily associated with the inhibition of cholinesterase activity and with deleterious effects on the nervous system. Acute toxicity test from single oral dose showed that MP is highly toxic to other non-targeted insect and terrestrial species. In human, sub-lethal dosage of MP can cause anorexia, reproduction deformity or even mortality. The pesticide is the most hazardous organophosphate insecticide allowed in the United States for food supply (Shimazu *et al.*, 2001).

The persistency of the MP and the potential hazards on human raised public concern of the presence of the pesticide in the environment. There are a number of ways to clean up or reduce the pesticides in soil, such as volatilization, incineration and chemical treatments. However, the overall cleaning methods aforementioned are expensive and inefficient. Thus, the alternative approach of removing MP using fungi, or mycoremediation technique has been proposed, since fungi are highly versatile and capable to survive in stressed environment *(Ong et al., 2017; Thenmozhi et al.*, 2013). The fungi possesses several enzymatic systems involving glucose oxidase, catalase, lactanase, cytochrome P450 monooxygenase and ligninolytic enzymes, which are useful in breaking down pesticides and other xenobiotics (Marinho *et al.*, 2011; Usharani & Muthukumar, 2013).

The extracellular enzymes are produce by fungi to break down complex organic compounds as energy sources, from the surrounding area. In addition, extensive growth of hyphae in soil contributed to the aggressive growth of fungi in large quantity, making fungi a suitable candidate in degrading contaminants in polluted soil (Stanley & Immanuel, 2015). The main objective of this

study was to evaluate different fungi species tolerance level towards MP toxicity. The results obtained could be used to determine the potential of fungi for mycoremediation of MP.

METHODOLOGY

Samples collection

Fungi were collected from surface soil (5 cm deep) from three locations within a metal scrapping facility in Shah Alam, Klang, Selangor, Malaysia (geo-coordinate: 3.028138, 101.479779). The collected soil samples were then diluted with sterilized water to 10^{-3} and 10^{-5} (w/v) and mixed with rose bengal agar (RBA) provided by OXOID. Colonies formed were sub-cultured onto potato dextrose agar (PDA) obtained from OXOID to obtain pure and young cultures. The identification was done thorough molecular approach with primer ITS1 (5´TCC GTA GGT GAA CCT TGC GG 3´) and ITS4 (5´TCC TCC GCT TAT TGA TAT GC 3´). Then, the PCR product was purified and sequenced by Genomics BioSci & Tech, Malaysia. The obtained DNA sequence was studied using Basic Local Alignment Search Tool (BLAST). A total of 11 species namely *Penicillium chrysogenum*, *Trichoderma erinaceum*, *Aspergillus nidulans*, *Fusarium oxysporum*, *Trichoderma longibrachiatum*, *Penicillium simplicissimum*, *Aspergillus flavus*, *Aspergillus niger*, *Hypocrea Koningii*, *Aspergillus ustus*, and *Gongronella butleri* were isolated from soil samples.

Tolerance study

Fungi were screened on 10 ppm of MP in potato dextrose broth (PDA), to find out which species were able to tolerate MP, based on the growth diameter of colonies and the distribution pattern. Fungal species with good growth (more than 3 cm) and well distributed were used in tolerance study.

For tolerance study, young colonies from PDA were transferred to the respective PDB with a range of MP (10 ppm, 20 ppm, 30 ppm, and 40 ppm) and incubated for 7 days. A control set without any MP was run simultaneously. After incubation, the culture was filtered using filter paper and dried in oven at 60°C to obtain constant dry weight. The dry biomass of fungi was recorded. The experiment was carried out in triplicates.

Statistical analysis was carried out using SPSS version 22.0 for the analysis of variance (ANOVA), to determine the significant (95% level of confidence) growth of fungal biomass from tolerance study.

RESULTS AND DISCUSSION

Initially, a total of 11 fungi species were screened using 10 ppm of MP in PDA showed five species exhibiting good growth rate on the PDA with colonies exhibiting diameter > 3 cm, namely *P. chrysogenum*, *T. erinaceum*, *T. longibrachiatum*, *A. nidulans* and *A. niger*. Figure 1 shows the biomass obtained for each of the five fungal species cultured in 10 ppm, 20 ppm, 30 ppm and 40 ppm of MP, respectively. The potential of the 5 fungi species tolerating MP were evaluated (Table 1). Generally, various mechanisms in fungi including alteration of their enzymatic antioxidant activity which acts as a toxicity tolerance mechanism (Ong et al., 2015).

P. chrysogenum was reported to have the ability to breakdown monocyclic aromatic hydro carbons, benzene, toluene, ethyl benzene and xylene, phenol compounds (Singh et al., 2013). In addition to it, Klimek *et al.* (2001), reported that *P. chrysogenum* had the ability to break down herbicide glyphosate as a source of nitrogen. This shows that *P. chrysogenum* possesses certain

mechanism to breakdown nitrogen group in MP. Moreover, it was also reported that *P. chrysogenum* have the ability to produce carboxyl esterase enzyme, which catalyzing the cleavage and formation of ester bonds that are widely distributed in animals, plants and microorganisms (Bornscheuer, 2002). The cleavage of phosphate-ester bond in MP can leads to its degradation (Pattanayak *et al.*, 2018).

There is no reported study for *T. erinaceum* and *T. longibrachiatum* in remediating MP. However, some other species under same genus was reported to have the ability to degrade MP. According to Fang *et al.* (2008), chlorpyrifos, an organophosphate pesticide could be degraded by *Trichoderma* sp. Baarschers and Heitland (1986), who found that the fungus *T. viride* could hydrolyse both compounds to 3-methyl-4-nitrophenol which was then further degraded by co-metabolic reactions which indicating the possibility to break down methyl group in the pesticides.

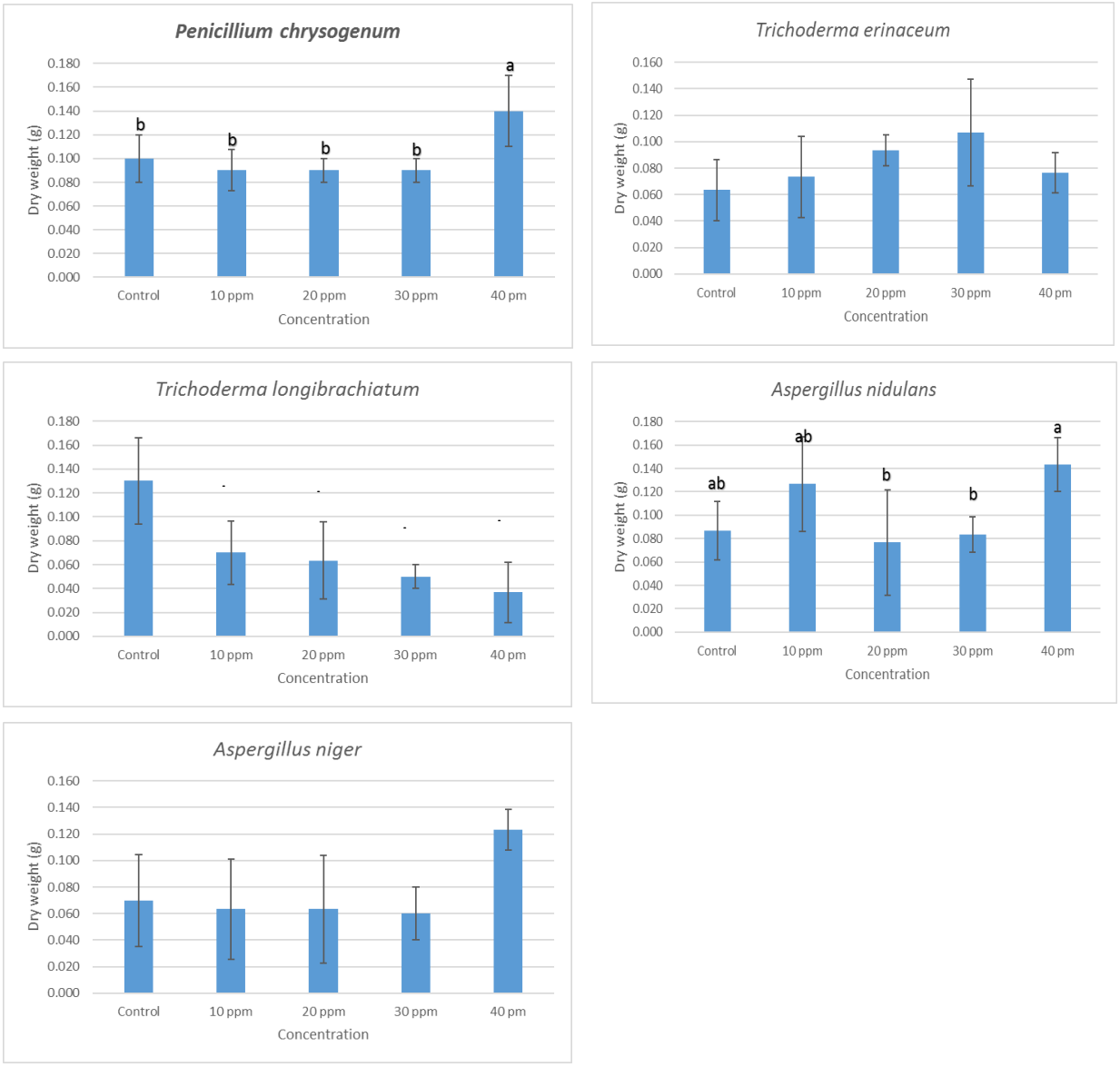

Figure 1. Dry weight (mean ± standard deviation) for selected fungi after incubation with methyl parathion for one week. Alphabet (a, b) in each column indicates different significance mean value (LSD test, $p < 0.05$). No alphabet indicates no significant difference

Table 1. The potential of fungi tolerance to MP

Species	Highest tolerance	Potential	Justification
P. chrysogenum	40 ppm	High	High tolerance, high growth rate
T. erinaceum	40 ppm	Low	High tolerance, low growth rate
T. longibrachiatum	40 ppm	Low	Clear growth inhibition
A. nidulans	40 ppm	High	High tolerance, high growth rate
A. niger	40 ppm	High	High tolerance, high growth rate

Another study by Matsumura and Boush (1968) showed that a strain of *T. viride* isolated from soil heavily contaminated with various insecticides, has the ability to degrade several organophosphorus, carbamate, and chlorinated hydrocarbon insecticides; probably through an oxidative system. This study clearly demonstrated that degradation of pesticides can be accelerated by employing fungi from the Trichoderma genus which can be effectively utilized as soil cleanser (Senthilkumar *et al*., 2011). *T. harzianum* was also reported as one of the potential fungus to mineralize organic P component in insecticides (Omar, 1998). *T. harzianum* Rifai 1295–22 (T-22) has been reported to solubilize phosphate *in vitro* by Altomare *et al*. (1999). Thus, it can be hypothesized that both *T. erinaceum* and *T. longibrachiatum* might be practicing similar mechanism in the present of MP, as other *Trichoderma* sp when exposed to insecticide.

A. nidulans (I-8), a filamentous fungi, which according to Maheswari & Murugesan (2009) can absorb 84.35% arsenic from contaminated soil. The ability of *A. nidulans* to tolerate MP up to 40 ppm might be due to the bioadsorption mechanism of *A. nidulans*, preventing the pesticide from entering the cell causing toxicity. Although there are not many studies on this species, but it is possible that *A. nidulans* share some similar enzymatic mechanism in degrading MP, as in *A. niger*, which is more well reported.

A. niger (I-26) was reported to secrete a wide range of enzymes, including lipase, esterase and protease (Höfelmann *et al*., 1985). This ability enables the fungi to have a wide range of enzymatic activities which includes the breakdown of complex chemical compound including MP which is a type of organophosphates. This is further shown by Liu *et al.* (2001), whereby *A. niger* was reported to efficiently degrade several pesticides including organophosphates. Another study shows that *A. terreus* had the greatest potential to mineralize organic phosphate followed by *A. tamarii, A. niger, T. harzianum* and *P. brevicompactum* (Omar, 1998). In addition to it, *A. niger* was reported to tolerate the toxicity of MP up to 60 mg/L (Marinho *et al*., 2011).

CONCLUSIONS

It was determined *Penicillium chrysogenum* (I-1), *Aspergillus nidulans* (I-8) and *Aspergillus niger* (I-26) as potential fungi species to remediate MP because these species were able to tolerate the highest concentration of MP tested at 40 ppm. The highest mean dry mass obtained at 40 ppm MP, were 0.140 g, 0.143 g, and 0.123 g, respectively. Mycoremediation is still novel to society, and further studies need to be done to find out the relationship of the distinctive mechanism and characteristics of each fungi species to remediate MP.

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