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Original Article

Isolation of atrazine-tolerant fungi from soil

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ABSTRACT

Atrazine is one of the most widely used herbicides to control broadleaf and grassy weeds in many crops around the world. However, its indiscriminate use will lead to the accumulation of atrazine in soil and drinking water, resulting in severe impacts on human health. Hence, it is crucial to develop a better remediation strategy to remove atrazine from the environment. In this research, mycoremediation using fungi, which is both an environmental friendly and a cost-effective way was evaluated to remediate the atrazine. The objective was to determine which fungi species have the potential to tolerate the toxicity of atrazine at various increasing concentration. Eleven fungi species were screened using 10 mg/L of atrazine on potato dextrose agar (PDA). Out of the 11 species, five were found to have greater biomass and were further tested on potato dextrose brooth (PDB) using increasing concentration of atrazine (up to 20 mg/L). Results showed that both *Trichoderma erinaceum* and *Aspergillus nidulans* recorded the highest mean dry biomass when exposed to the highest atrazine concentration tested at 20 mg/L, which is 0.117 g and 0.073 g, respectively. This shows both *T. erinaceum* and *A. nidulans* can tolerate atrazine up to 20 mg/L, thus having the highest potential to remediate atrazine.

KEYWORDS: mycoremediation, atrazine, pesticide tolerance.

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INTRODUCTION

Atrazine is a broad-leaf weed-control herbicide which is widely used in agriculture to reduce crop losses effectively due to unwanted weed interference to farmers. It kills the weeds by targeting the chloroplast and stopping photosynthesis. The frequent application of atrazine to the soil will lead to runoff and atrazine ends up accumulating in lakes and streams. Atrazine contamination has become a growing public concern because atrazine is one of the most commonly detected herbicides in soil and groundwater, severely affecting the organisms living in the benthic zone [1].

Atrazine has a slow rate of natural degradation, and therefore it is always detected in soil, sediments and groundwater at concentrations well above the permitted limits. Atrazine in the water is measured in parts per billion (µg/L). United States of America has established the maximum contaminant level in the water for atrazine as 3 µg/L [2]. Excessive exposure to atrazine might cause health hazards to the locals, e.g. damages to the central nervous system and endocrine system, deterioration of immune system, and increase in occurrence of cancer [3, 4, 5].

There are a few possible ways to clean up or reduce the herbicide in the soil, such as volatilization, incineration and chemical treatment. However, the overall cleaning methods aforementioned are expensive and inefficient and may also cause additional pollution to the environment. Thus, the alternative approach of removing atrazine using fungi (mycoremediation) is being proposed.

Fungi are highly versatile, capable of surviving in stressed environment and able to secrete extracellular enzymes to break down into non-toxic substances [6, 7, 8]. Several fungi are known to metabolize various chlorinated aromatic compounds, such as 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine, and polychlorinated biphenyls [9]. Hence, the main objective of this study is to evaluate the tolerance level of different fungi species towards atrazine toxicity. The results obtained could be used to determine the potential of fungi for mycoremediation of atrazine.

MATERIALS AND METHODS

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 through 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 polymerase chain reaction (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 the soil samples.

Atrazine tolerance study

Fungi were screened using 10 mg/L of atrazine in potato dextrose broth (PDA), to find out which species were able to tolerate atrazine, based on the growth diameter of colonies and the distribution pattern. Fungal species with good growth (more than 3 cm) and those well distributed were used in the tolerance study. For tolerance study, young colonies from PDA were transferred to the respective PDB with a range of atrazine (5 mg/L, 10 mg/L, 15 mg/L, and 20 mg/L) and incubated for 7 days. A control set without any atrazine was run simultaneously. After incubation, the culture was filtered using a 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 software SPSS version 22.0. The analysis of variance (ANOVA) test was carried out to determine the significant (95% level of confidence) growth of fungal biomass for tolerance study.

RESULTS AND DISCUSSION

Initially, a total of 11 fungi species screened using 10 mg/L of atrazine in PDA showed that five species, namely *P. chrysogenum*, *P. simplicissimum*, *T. erinaceum*, *A. nidulans* and *G. butleri* exhibited good growth rate on the PDA with diameter > 3 cm of the colonies. Figure 1 shows the biomass obtained for each of the five fungi species cultured in 5 mg/L, 10 mg/L, 15 mg/L and 20 mg/L of atrazine respectively.

Figure 1 also demonstrates the effect of atrazine on the dry weight of *P. chrysogenum*. The highest dry weight (0.216 g) was observed with the control (0 mg/L), which was significantly ($P < 0.05$) higher than the fungi exposed to 10 mg/L, 15 mg/L and 20 mg/L of atrazine. The trend indicates *P. chrysogenum* has reduced tolerance against the toxicity of atrazine at high concentrations.

Atrazine is an organic compound consisting of an s-triazine-ring structure. *P. chrysogenum* has been reported to have the ability to break down monocyclic aromatic hydrocarbons (benzene, toluene, ethyl benzene and xylene) and phenol compounds [10]. Moreover, it has also been reported that *P. chrysogenum* has the ability to produce carboxyl esterase enzyme, which catalyzes the cleavage and formation of ester bonds that are widely distributed in animals, plants and microorganisms [11]. According to Klimek *et al*. [12], *P. chrysogenum* has the ability to break down herbicide glyphosate. The amino acids (possible products of glyphosate breakdown) and ammonia

produced were found to replace the herbicide inside the fungi and help in restoring the mycelial growth. However, *P. chrysogenum* cells were devoid of detectable nitrate reductase activity; thus the isolate seems to be impaired in its ability to convert nitrate to ammonium suggesting that *P. chrysogenum* can only tolerate up to a certain limit of atrazine.

Figure 2 shows *P. simplicissimum* has the highest dry weight for the control which is 0.156 g, while the lowest dry weight of 0.030 g was obtained at 20 mg/L atrazine. Figure 2 shows a decreasing trend which indicates that *P. simplicissimum* did not grow well in high concentration of atrazine. However, it is still a potential fungi species to remediate atrazine as it can tolerate high atrazine toxicity compared to other fungi species.

There is limited information on the ability of *P. simplicissimum* in remediating atrazine. However, *Penicillium* spp. has demonstrated its ability to degrade different xenobiotic compounds with

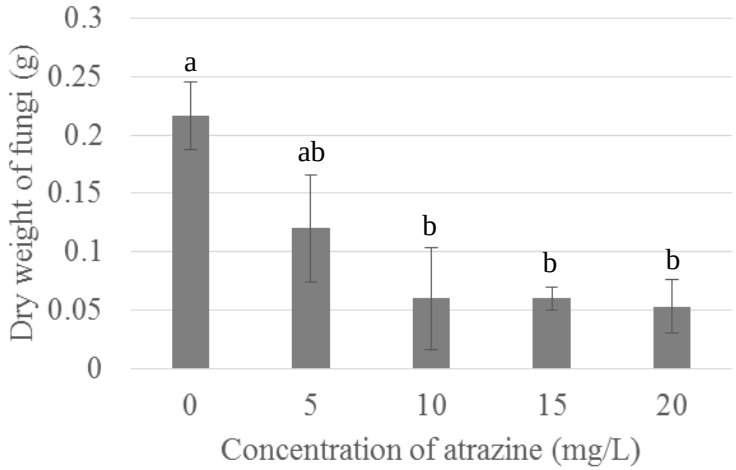

Figure 1. Dry weight (mean ± standard deviation) (g) obtained from *Penicillium chrysogenum* after 7 days of incubation. Alphabets (a, b) in each column indicate different significant mean values (LSD test, $p < 0.05$).

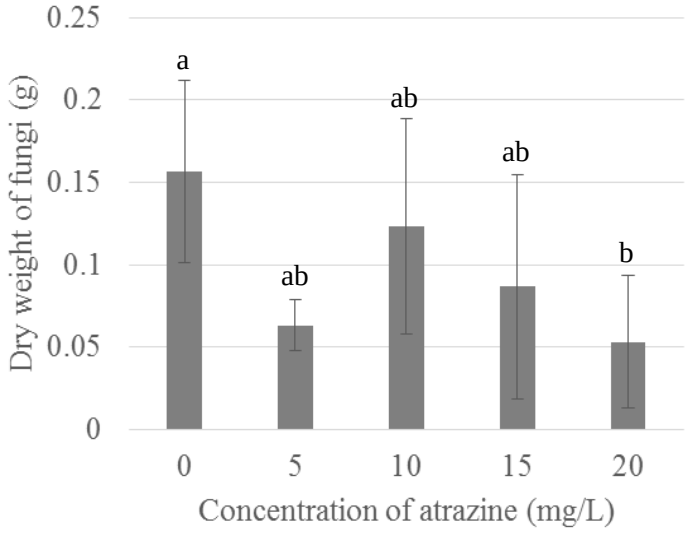

Figure 2. Dry weight (mean ± standard deviation) (g) obtained from *Penicillium simplicissimum* after 7 days of incubation. Alphabets (a, b) in each column indicate different significant mean values (LSD test, $p < 0.05$).

low co-substrate requirements [13]. Based on the study reported by Saraswathy and Hallberg [14], five pyrene-degrading strains of *Penicillium* were isolated from soil of a former gaswork site, and were identified as *P. simplicissimum, P. janthinellum, P. funiculosum, P. harzianum* and *P. terrestre*. Degradation of pyrene was directly correlated with biomass development with *P. simplicissimum* being the second efficient species after *P. terrestre* in pyrene degradation. Another study reported that *P. simplicissimum* SK9117 effectively degraded 8.5 mM of phenol used as the sole source of carbon and energy after 22 days of incubation. The catabolism of phenol produced catechol, hydroquinone, and muconic acid [15].

The dry weights of *T. erinaceum* for the control (0 mg/L), and those exposed to 5 mg/L, 10 mg/L, and 20 mg/L atrazine were significantly higher ($P < 0.05$) than that in 15 mg/L (Figure 3). Mean dry weight of fungi for 20 mg/L was the highest (0.117 g), while for 15 mg/L the lowest mean dry weight (0.030 g) was observed. The trend in Figure 3 is quite consistent which indicates that *T. erinaceum* can tolerate toxicity of atrazine even at high concentration in general, and thus a potential species to remediate atrazine.

There is no report on the ability of *T. erinaceum* in remediating atrazine. However, some other species under the genus *Trichoderma* has been reported to have the ability to degrade organophosphate. According to Fang *et al*. [16], chlorpyrifos, an organophosphate pesticide, could be degraded by *Trichoderma* sp. According to Baarschers and Heitland [17], fungus *T. viride* could hydrolyse fenitrothion and fenitrooxon compounds to 3-methyl-4-nitrophenol which was then further degraded by co-metabolic reactions which indicates the possibility of breaking down the methyl group in the atrazine.

Figure 4 shows dry weight of *A. nidulans* after 7 days of incubation. The dry weight shows the highest growth (0.107 g) with the control and lowest growth (0.026 g) at 10 mg/L of atrazine. The growth of *A. nidulans* is constant at all concentrations except for 10 mg/L which indicates *A. nidulans* is able to tolerate the presence of atrazine and grow well even in high concentrations of atrazine.

A. nidulans is a type of filamentous fungi, which according to Maheswari and Murugesan [18] can absorb 84.35% arsenic in contaminated soil. The ability of *A. nidulans* to tolerate atrazine up to

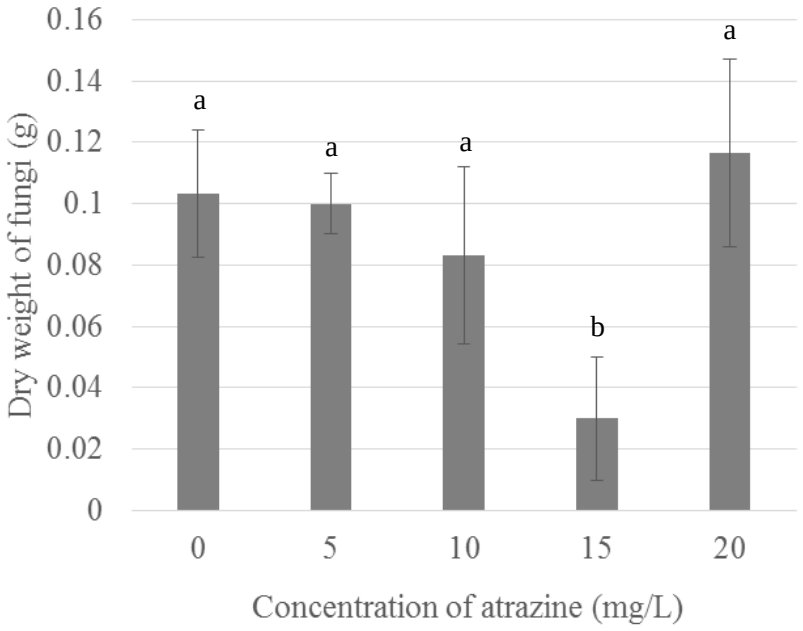

Figure 3. Dry weight (mean ± standard deviation) (g) obtained from *Trichoderma erinaceum* after 7 days of incubation. Alphabets (a, b) in each column indicate different significant mean values (LSD test, $p < 0.05$).

20 mg/L might be due to the bioadsorption mechanism of *A. nidulans*, preventing the pesticide that causes toxicity from entering the cell. Limited studies have been done on this species of fungi, but it belongs to the same genus as *A. niger*, suggesting that they have a similar mechanism of enzymatic degradation of atrazine.

Dry weight of *G. butleri* was affected by different concentrations of atrazine (Figure 5). The dry weight recorded for the fungi exposed to 5 mg/L atrazine was the highest (0.100 g). The lowest dry weight was recorded for the fungi exposed to 15 mg/L, which is 0.030 g. However, there was no significant difference between mean dry weights for all concentrations which indicates that the growth of *G. butleri* is about the same within these range of concentrations of atrazine.

Gongronella sp. is a rare fungus. Batch cultures with a progressive increase of fungicide concentrations were conducted by Martin *et al*. [19].

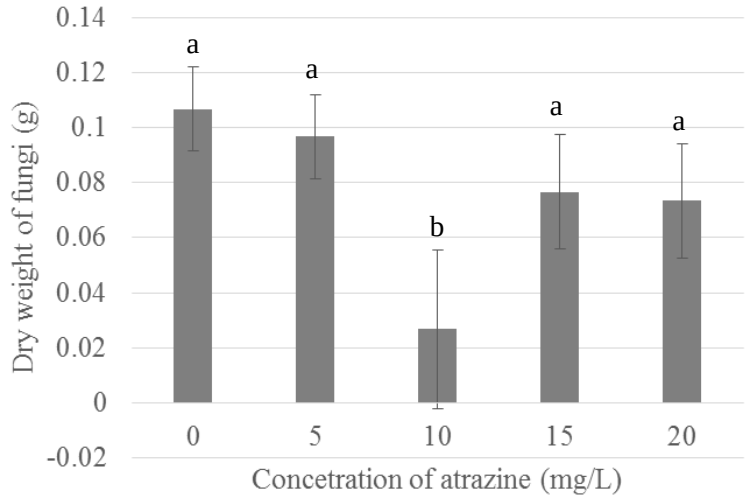

Figure 4. Dry weight (mean ± standard deviation) (g) obtained from *Aspergillus nidulans* after 7 days of incubation. Alphabets (a, b) in each column indicate different significant mean values (LSD test, $p < 0.05$).

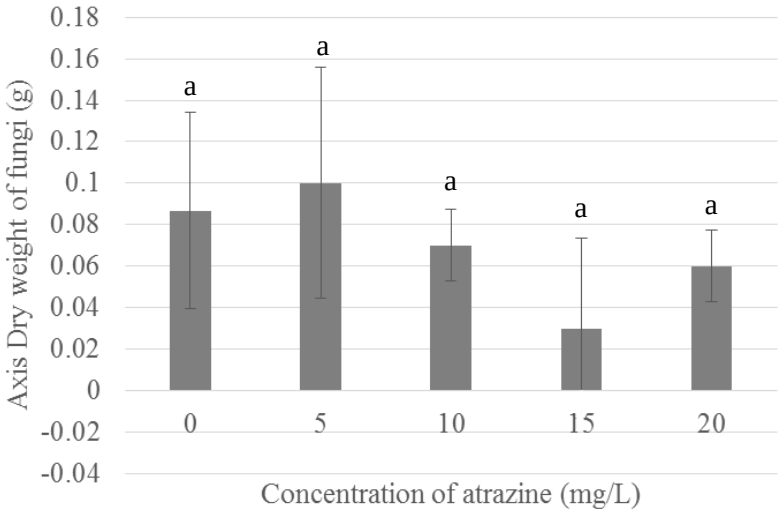

Figure 5. Dry weight (mean ± standard deviation) (g) obtained from *Gongronella butleri* after 7 days of incubation. Alphabets (a, b) in each column indicate different significant mean values (LSD test, $p < 0.05$).

The high tolerance to metalaxyl and folpet shown by *Gongronella sp.* (which carry methyl group) might be correlated with their degradation ability. Another study reported that *G. butleri* that was isolated from the soil samples from Vietnam (Nha Trang and Hanoi) has the ability to degrade poly-aromatic hydrocarbon [20] which also has a ring structure as atrazine.

CONCLUSIONS

In conclusion, *T. erinaceum* and *A. nidulans* were determined to have the potential to remediate atrazine with the highest mean dry mass obtained for the highest tested concentration of atrazine (20 mg/L), which is 0.117 g and 0.073 g respectively. Mycoremediation is still novel to this society, and more studies are required to study the distinctive mechanism and characteristics of each fungi species in atrazine remediating purpose.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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