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Original Article

Screening for potential fungi from polluted soil for reactive dye remediation

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ABSTRACT

Industrial dye is one of the main sources of pollution to the aquatic and terrestrial environment leading to extensive effects on the ecosystem. Common methods of dye removal from the industrial waste are not efficient and not economical. Mycoremediation on the other hand, is a more preferred method due to its environment-friendly nature. Therefore, the aim of this study is to identify potential fungi that are able to withstand as well as degrade Remazol Brilliant Blue R (RBBR). Soil samples were collected from a textile manufacturing factory in Nilai, Malaysia (Récron). The soil fungi were identified by using Rose Bengal Agar and were later subcultured onto Potato Dextrose Agar. Screening for potential fungi was based on their tolerance towards RBBR at a range of 200 mg/L - 1000 mg/L in Potato Dextrose Broth. This was carried out by 14 days of specimen incubation followed by the study of the tolerance towards RBBR. The dry weight of the fungi obtained from centrifugation was utilized for RBBR tolerance screening while the supernatant was used for decolourization of RBBR. Potential fungal species for RBBR bioremediation were determined based on the highest decolourization activity and the highest biomass (g) obtained from the screening. The current study showed high tolerance of RBBR (up to 1000 mg/L) for *Aspergillus nidulans*, *Aspergillus fumigatus* and *Trichoderma longibrachiatum* and their decolourization activities were as high as 98.47%, 87.80%, and 84.53%, respectively. The current research supports the potential of these fungi species for bioremediation purposes.

KEYWORDS: mycoremediation, Remazol Brilliant Blue R, *Aspergillus nidulans, Aspergillus fumigatus, Trichoderma longibrachiatum.*

INTRODUCTION

Despite being an essential source of income in developing countries in Southeast Asia, the textile industry is also a major contributor of water pollution due to its chemical-intensive nature [1, 2]. The key chemical used in the industry is the reactive dye, to achieve permanent colour on fabrics through strong bonding of the dye to fibers and cellulose [3]. Besides, 30% of total colorants in the printing industry use these dyes which further contributes to dye pollution in the water [4], due to their stability and recalcitrance towards environmental degradations [5].

During the treatment of textile industry effluents, flocculants are used as a neutralizing agent of the particulates and for clumping them down as sediments [6]. The flocculants consist of metal-based substances such as aluminium sulphate (alum), ferric chloride, ferric chlorosulphate ferrous sulphate, and ferrous sulphide [6, 7] that are toxic and cause detrimental effects to the environment [8]. Mycoremediation is often used as the ideal method of removing toxic wastes from the environment as it is more sustainable

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as compared to chemical treatments [9]. However, most bioremediating agents are unable to absorb the reactive dye due to the hydrophobic nature of the dye. Therefore, the most ideal bioremediating agent for this pollutant would be fungal species. Fungi are known for its ability to generate and secrete extracellular enzymes such as hydrolyzing and oxidizing enzymes that could biodegrade pollutants extracellularly, without allowing the pollutants to enter fungi cells [10, 11, 12]. The poor bioavailability of the dye could also be countered by the biosurfactant produced by the fungi and the dye can be uptaken by fungi for degradation [13, 14].

As reported by the Department of Statistics Malaysia in 2016, Malaysia's textile industry has garnered a gross output of RM14 billions from exports in 2014. This has caused a large amount of textile waste including Remazol Brilliant Blue R (RBBR) (Reactive Blue 19) to be released into the waters of Malaysia. RBBR is one of the primary compounds used to produce polymeric dyes [15] which are known for their toxicity towards aquatic ecosystems [16]. Thus, the aim of this study is to determine the potential fungi that have the ability to decolourize RBBR from the soil samples.

METHODS

Sample collection

Collection of fungi samples was carried out at three sites located within the textile manufacturing factory, Récron Sdn Bhd, located at Nilai, Negeri Sembilan, Malaysia, as they used industrial dyes in their manufacturing processes. The fungi were obtained from the surface of the soil, which is about 5 cm deep. 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. Young and pure cultures were acquired from sub-culturing the colonies on potato dextrose agar (PDA).

Degradation study

The mycelium of each fungal species were grown in a series of different RBBR concentrations (0 mg/L (control), 200 mg/L, 400 mg/L, 600 mg/L, 800 mg/L, and 1000 mg/L) in 20 mL PDB media. The experiment was carried out in triplicate. Fast-growing species were measured for decolourization of media while slow-growing species were eliminated.

Incubation was carried out for 14 days before the fungi mycelium were removed from the samples through filtration; then the filter paper was kept for dry weight determination. The filtrate was centrifuged (10,000 rpm) for 20 minutes at room temperature [17]. The absorbance reading for RBBR of the supernatant was determined by using a spectrophotometer at 592 nm wavelength. The control group for each species was used as the blank control for absorbance reading. The determination of RBBR decolourization in the samples was based upon the absorbance reading. The following formula was used to calculate the percentage of RBBR decolourization.

$$\frac{A_i - A_f}{A_i} \times 100\%$$

A_i – The initial absorbance of RBBR.
A_f – The final absorbance of RBBR.

The resulting absorbance was multiplied by the dilution factor to obtain the estimated absorbance reading.

Statistical analysis

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 and RBBR degradation.

RESULTS AND DISCUSSION

Based on the results, 8 species of fungi were isolated in the collected soil samples in which 3 species were identified as potential species while the rest were identified as non-potential species. The 3 species that were identified with tolerance and high decolourization activities towards RBBR are *Aspergillus nidulans*, *Aspergillus fumigatus*, and *Trichoderma longibrachiatum*. In this study, two main criteria were observed in the identification of a good bioremedirating agent, i) High biomass in target pollutant indicating rapid growth in the pollutant [18]; ii) High growth rate to ensure the species will not be outcompeted by native species

during *in situ* treatment and shorter remediation period are needed.

Aspergillus nidulans

In Figure 1, *A. nidulans* shows the highest dried biomass (0.183g) at 600 mg/L of RBBR but shows no significant differences with control, 200, 800 and 1000 mg/L of RBBR. The high tolerance towards RBBR of this species was reflected by the high amount of biomass obtained even at 1000 mg/L of RBBR. *A. nidulans* has the second highest biomass among all 8 species of fungi, indicating high tolerance level towards RBBR. The tolerance towards the RBBR could be attributed to the biofilm formation of *A. nidulans* that forms a protective layer against the dye when the cells attach to each other during the formation [19]. This was also observed during the culture in PDB whereby the fungi formed a layer of paper-like colony that floats on the surface. Hence, this provides protection for *A. nidulans* from the reactive dye RBBR, and thus provides tolerance towards RBBR.

Based on Figure 2, the highest decolourization activity was observed in *A. nidulans* cultured in 200 mg/L of RBBR, with 96.42% decolourization, but showed no significant difference with 400 mg/L. This could be attributed to the presence of laccases which are known to decolourize RBBR [20]. The production of laccases, which are extracellular enzymes, could be induced by the presence of pollutants such as copper, reactive dyes and recalcitrant compounds [21]. *A. nidulans* was reported to carry the laccase gene with a strong promoter that effectively produces the enzyme that counters the dye [22].

Aspergillus fumigatus

Based on Figure 3, the highest biomass (0.21 g) was recorded at 0 mg/L RBBR but showed no significant difference compared to other concentrations, for *A. fumigatus*. The result suggested that *A. fumigatus* has high tolerance towards high concentrations of RBBR. The ability of *A. fumigatus* to produce high biomass at high concentrations of RBBR indicated the ability of *A. fumigatus* to grow rapidly and to compete with the indigenous microbial species for in situ bioremediation. Kaur and Singh [23] has reported biofilm formation in *A. fumigatus* in their study, which could be the reason of the high tolerance of this species towards RBBR. The biofilm creates a barrier between the dye and the fungus and prevents it from reaching the center of the cell mass and reacting with the chitin cell wall of the species.

For *A. fumigatus*, the highest decolourization activity (76.44%) of RBBR was shown at 200 mg/L (Figure 4) but showed no significant difference with 400 and 1000 mg/L. This species produces

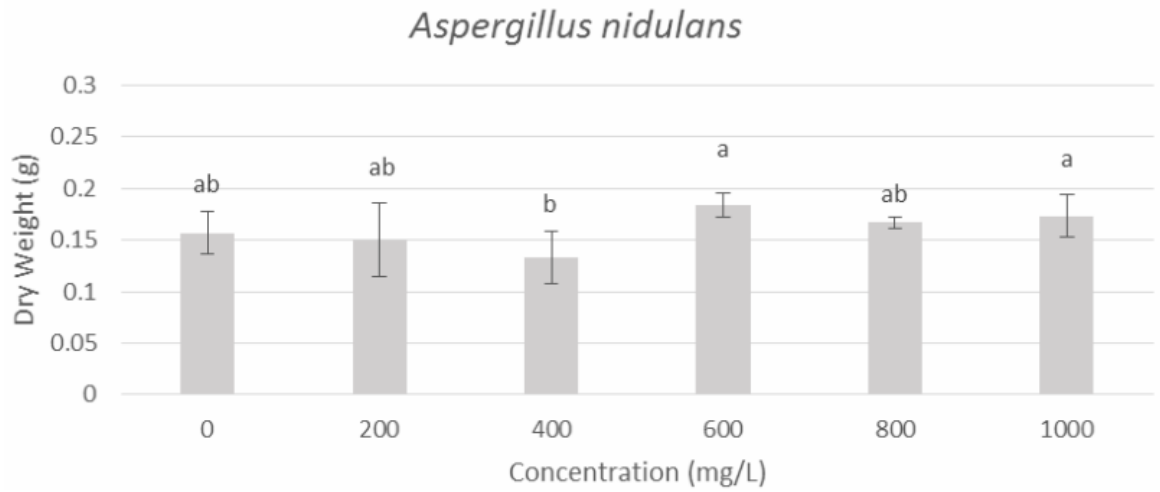

Figure 1. Dry weight (mean ± standard deviation) for *Aspergillus nidulans* in the presence of RBBR after 14 days. Alphabet(s) (a, b) in each column shows different significant mean values of LSD test ($p < 0.05$).

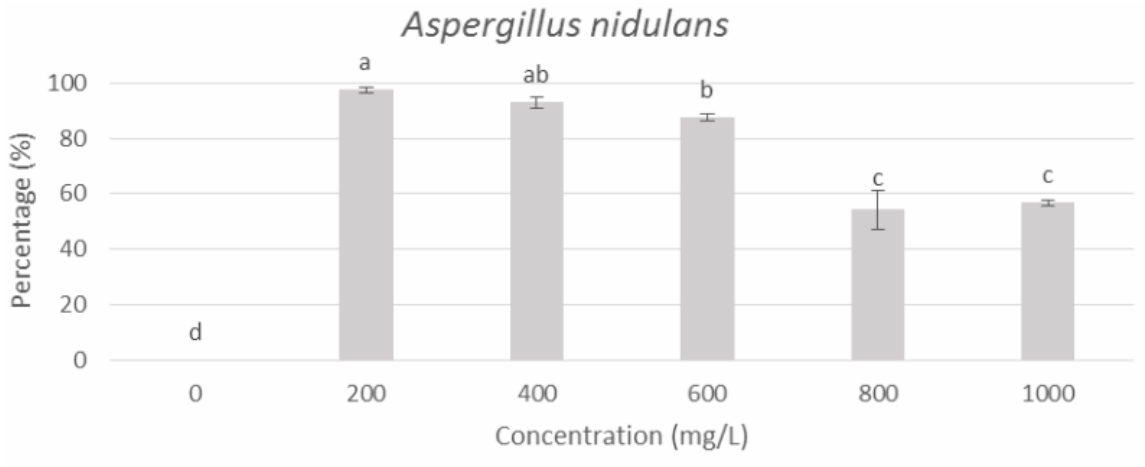

Figure 2. Percentage of RBBR decolourization (mean ± standard deviation) by *Aspergillus nidulans* after 14 days of culture. Alphabet(s) (a, b c, d) in each column shows different significant mean values of LSD test ($p < 0.05$).

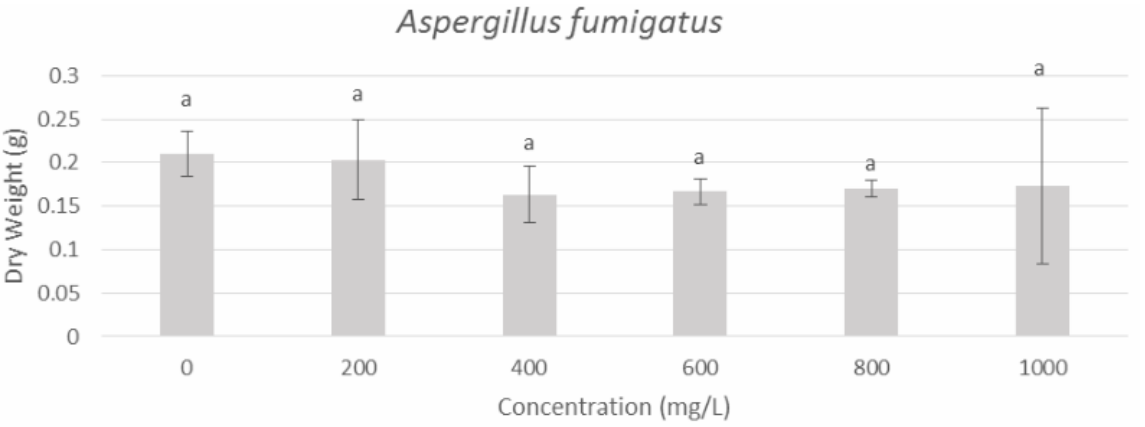

Figure 3. Dry weight (mean ± standard deviation) for *Aspergillus fumigatus* in the presence of RBBR after 14 days. Alphabet(s) (a) in each column shows different significant mean values of LSD test ($p < 0.05$).

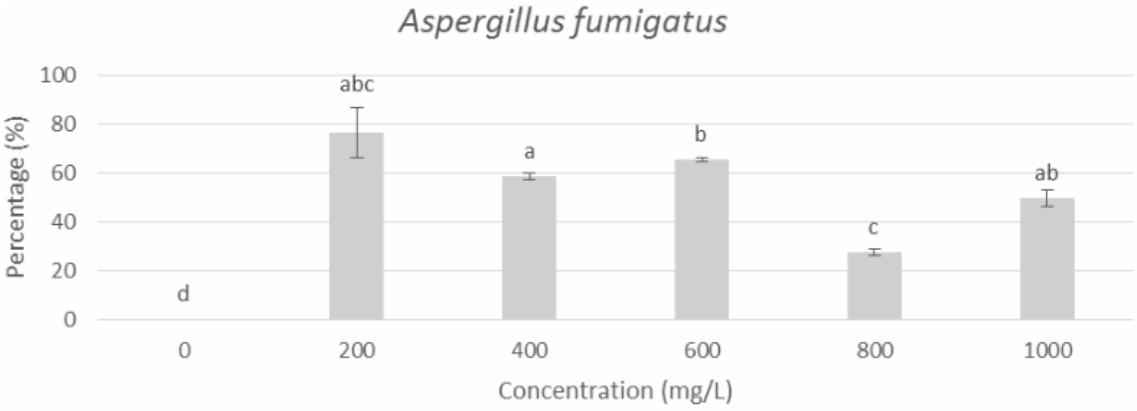

Figure 4. Percentage of RBBR decolourization (mean ± standard deviation) by *Aspergillus fumigatus* after 14 days of culture. Alphabet(s) (a, b, c, d) in each column shows different significant mean values of LSD test ($p < 0.05$).

extracellular enzymes such as manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase [24]. In addition to this, *A. fumigatus* increases laccase production during conidation, which is the spore-producing stage of the fungus life-cycle [25]. Albeit having low specificity, laccase is able to degrade phenolic molecules such as industrial dye, which is analogous to RBBR [20, 26]. Enzymes such as LiP and MnP are able to decolourize synthetic dyes which share similar phenolic structures with RBBR [27, 28]. *A. fumigatus* was used as the bioremediating agent in the remediation of the dye effluent and has shown excellent results with up to 100% decolourization in 72 hours of incubation [29]. This shows the potential of *A. fumigatus* as a good bioremediating agent for RBBR.

Trichoderma longibrachiatum

Based on Figure 5, the highest biomass for *T. longibrachiatum* was recorded at 1000 mg/L (0.127 g) but does not show significant differences with 0 mg/L (0.093 g) and 200 mg/L (0.090 g). The current results showed low biomass of *T. longibrachiatum* as compared to *Aspergillus nidulans* and *A. fumigatus* indicating its lower tolerance towards high concentrations of RBBR. The high resistance of reactive dyes towards UV degradation is attributed to the strong covalent bond formed between the fibre polymers and the reactive group of the dye [15]. However, when the bonds are formed with proteins from the cell wall of the fungi, which mostly consists of chitin, the cell wall may rupture due to disruption of the cell wall structure.

Figure 6 shows that *T. longibrachiatum* has the highest ($p < 0.05$) decolourization activity of 79.47% at 200 mg/L RBBR. The lowest ($p < 0.05$) decolourization activitiy of RBBR was found in the control group, which is 0%. It was shown that *T. longibrachiatum* was able to decolourize organic dyes and degrade complex molecules such as anthracene which are phenolic molecules with similar structures as RBBR [30]. As laccase, LiP, and MnP are also produced in *T. longibrachiatum* [31], it is safe to assume that laccase is involved in the dye decolourization mechanism. Based on Gochev & Krastanov [32]'s study *T. longibrachiatum* is able to produce high levels of laccase and this characteristic is essential for a good bioremediating agent. However, there are limited studies on the effect of *Trichoderma* species on dye decolourization; hence studies are required to further understand the dye decolourizing mechanism of *Trichoderma* species. Hence, although *T. longibrachiatum* has high decolourization ability, it has poor tolerance towards high concentrations of RBBR.

CONCLUSION

Three potential fungi species, namely *Aspergillus nidulans*, *Aspergillus fumigatus*, and *Trichoderma longibrachiatum* were isolated from the soil samples

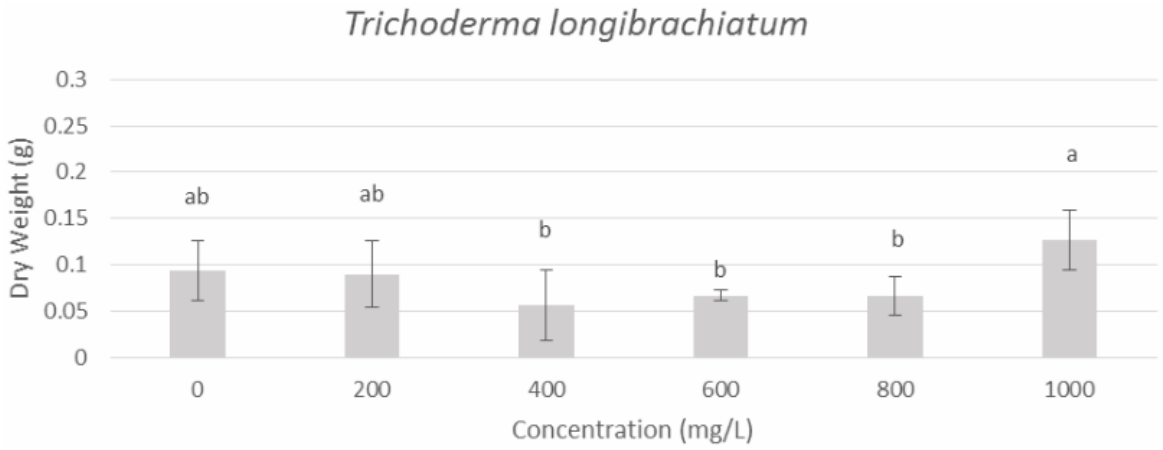

Figure 5. Dry weight (mean ± standard deviation) of *Trichoderma longibrachiatum* in the presence of RBBR after 14 days. Alphabet(s) (a, b) in each column shows different significant mean values of LSD test ($p < 0.05$).

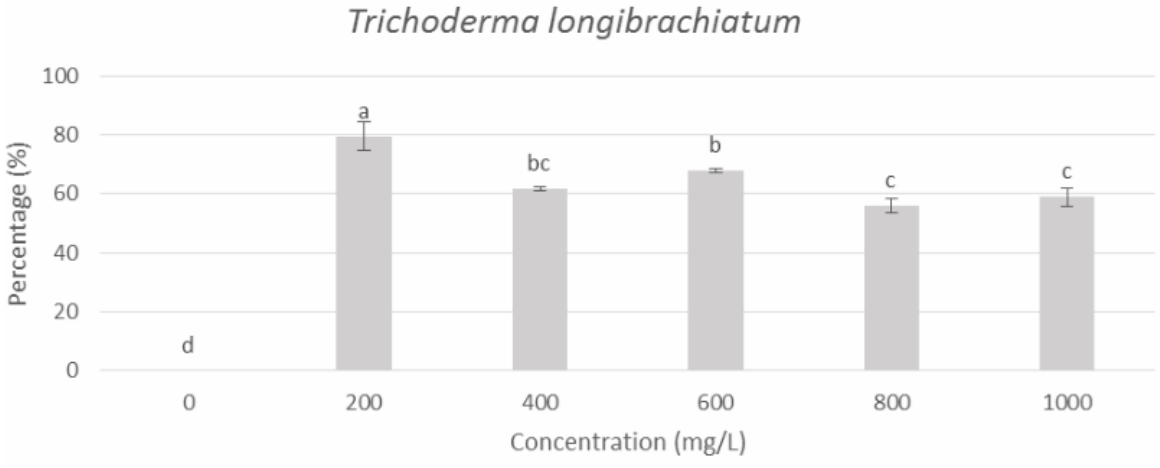

Figure 6. Percentage of RBBR decolourization (mean ± standard deviation) by *Trichoderma longibrachiatum* after 14 days of culture. Alphabet(s) (a, b, c, d) in each column shows different significant mean values of LSD test ($p < 0.05$).

collected from Récron Nilai. These three fungi were able to tolerate and decolourize high concentrations of Remazol Brilliant Blue R. *A. nidulans* gave the best results as it showed higher decolourization and produced a relatively high growth of biomass.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Yesilada, O., Birhanli, E. and Geckil, H. 2018, Mycoremed. Environ. Sustain., 121-153.
2. Kant, R. 2012, Nat. Sci., 4, 22-26.
3. Wilson, S. 2017, Three Types of Dyes for Dyeing Cellulosic Fibers, First Source Worldwide, LLC. Retrieved from http://www.fsw.cc/three-types-of-dyes/
4. Stork. 2002, Developments in the textile printing industry. Boxmear, The Netherlands: Stork Textile Printing Group.
5. McMullan, G., Meehan, C., Conneely, A., Kirby, N., Robinson, T., Nigam, P., Banat, I. M., Marchant, R. and Smyth, W. F. 2001, Appl. Microbiol. Biotechnol., 56, 81-87.
6. Butler, E., Hung, Y. T., Ahmad, M. A. and Fu, Y. P. 2016, Natural Resources and Control Processes. Handbook of Environmental Engineering, 17.
7. Tiroyaone, H. Z. 2012. J. Waste Water Treat. Analy., 3, 4.
8. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K. and Sutton, D. J. 2012, Experientia Suppl., 101, 133-164.
9. Kulshreshtha, S., Mathur, N. and Bhatnagar, P. 2014, AMB Express, 4(1), 29.
10. Raymond, P., Mshandete, A. M. and Kajumulo, K. A. 2015, Biotechnol. Res. Int., 24, 1-9.
11. Purnomo, A. S., Mori, T., Putra, S. R. and Kondo, R. 2013, Int. Biodeterior. Biodegra., 4, 40-44.
12. Ong, G. H, Ho, C. C., Lim, V. B. F., Wong, K. K. and Wong, L. S. 2018, Remed. J., 28(3), 91-95.
13. Bhardwaj, G., Cameotra, S. S. and Chopra, H. K. 2013, Mol. Phylogenetics Evol., 4, 160.
14. Karlapudi, A. P., Venkateswarulu, T. C., Tammineedi, J., Kanumuri, L., Ravuru, B. K., Dirisala, V. and Kodali, V. P. 2018, Petrol., 4(3), 241-249.
15. Singh, J. P. and Verma, S. 2017, Wet Processing. Woven Terry Fabrics, 1, 101-117.
16. Hadibarata, T. and Kristanti, R. A. 2012, J. Chil. Chem. Soc., 57(2), 1095-1098.

17. Garg, N. and Gupta, H. 2010, J. Pharm. Bioallied Sci, 2(1), 38-43.
18. Nierman, W. C. and Nelson, K. E. 2002, Adv. Appl. Microbiol., 201-248e.
19. Kadry, A. A., El-Ganiny, A. M., Mosbah, R. A. and Kaminskyj, S. G. W. 2017, Med. Mycol., 56(5), 621-630.
20. Mechichi, T., Mhiri, N. and Sayadi, S. 2006, Chemosphere, 64(6), 998-1005.
21. Plácido, J. and Capareda, S. 2015, Bioresour. Bioprocess, 2, 23.
22. Li, W., Yu, J., Li, Z. and Yin, W. B. 2019, Sci. China Life Sci., 62, 84-94.
23. Kaur, S. and Singh, S. 2013, Med. Mycol., 52(1), 2-9.
24. Vivekanand, V., Dwivedi, P., Pareek, N. and Singh, R. P. 2011, Appl. Biochem. Biotechnol., 165(1), 204-220.
25. Malcolm, D., Richardson, K. B. and Hope, W. 2009, Clin. Mycol., 271-296.
26. Jin, X. and Ning, Y. 2013, J. Hazard. Mater., 262, 870-877.
27. Verma, P. and Madamwar, D. 2002, Folia Microbiol., 47(3), 283-286.
28. Asses, N., Ayed, L., Hkiri, N. and Hamdi, M. 2018, BioMed. Res. Int., 1-9.
29. Jin, X. C., Liu, G. Q., Xu, Z. H. and Tao, W. 2006, Appl. Microbiol. Biotechnol., 74(1), 239-243.
30. Andreolli, M., Lampis, S., Brignoli, P. and Vallini, G. 2016, Environ. Sci. Pollut. Res., 23(9), 9134-9143.
31. Velázquez-Cedeño, M. A., Farnet, A. M., Ferré, E. and Savoie, J. M. 2004, Mycoae, 96(4), 712-719.
32. Gochev, V. K. and Krastanov, A. I. 2007, Bulg. J. Agric. Sci., 13(2), 171.