

Bioremoval capacity of phenol by some selected endophytic fungi isolated from *Hibiscus sabdariffa* and batch biodegradation of phenol in paper and pulp effluents

Doaa Montaser Ahmed Khalil^{1,2*}, Mohamed Salah Massoud^{1,3}, Soad A El-Zayat^{1,2}, Magdi A El-Sayed^{1,2,4}

¹Department of Botany, Faculty of Sciences, Aswan University, Aswan, Egypt

²Department of Biological Science, Unit of Environmental Studies and Development, Aswan University, Aswan, Egypt

³Department of Chemistry, Science & Arts College, Alqurayyat, Jouf University, Al-Jawf, Kingdom of Saudi Arabia

⁴Department of Biological Science, Molecular Biotechnology Program, Field of Advanced Basic Sciences, Galala University, New Galala City, Egypt

Received: January 2021, Accepted: April 2021

ABSTRACT

Background and Objectives: The use of endophytic fungi for management of phenol residue in paper and pulp industries has been shown as cost-effective and eco-friendly approach. In this study, isolation of endophytic fungi from roots, stems, and leaves of *Hibiscus sabdariffa* was conducted. Additionally, the isolated fungi were examined for their ability to degrade phenol and its derivatives in paper and pulp industrial samples, using different growth conditions.

Materials and Methods: Out of 35 isolated endophytic fungi, 31 were examined for their phenol biodegradation capacity using Czapek Dox broth medium containing Catechol and Resorcinol as a sole carbon source at final concentrations of 0.4, 0.6 and 0.8%.

Results: A total of 35 fungal species belonging to 18 fungal genera were isolated and identified from different parts of *H. sabdariffa* plants. All strains have the capability for degrading phenol and their derivatives with variable extents. The optimum condition of degrading phenol in paper and pulp effluent samples by *Fusarium poae* 11r7 were at pH 3-5, temperature at 28-35°C, good agitation speed at no agitation and 100 rpm.

Conclusion: All endophytic fungal species can utilize phenol and its derivatives as a carbon source and be the potential to degrade phenol in industrial contaminants.

Keywords: Endophytes; Phenol; Biodegradation; Biodiversity; *Aspergillus*; *Hibiscus*

INTRODUCTION

Fungal endophytes represent a diverse group of unrelated filamentous fungi. They occur in most plants and often stay without causing any immediate, negative effects for the plant (1). Endophytes play a major role in the phytoremediation process and degradation of environmental toxins by direct or indirect process, indirectly by accelerate the phytoremedia-

*Corresponding author: Doaa Montaser Ahmed Khalil, Ph.D, Department of Botany, Faculty of Sciences, Aswan University, Aswan, Egypt; Department of Biological Science, Unit of Environmental Studies and Development, Aswan University, Aswan, Egypt.
Tel: +2001100824842
Fax: +20973480450
Email: doaa.khalil30@sci.aswu.edu.eg

Copyright © 2021 The Authors. Published by Tehran University of Medical Sciences.



This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International license

(<https://creativecommons.org/licenses/by-nc/4.0/>). Noncommercial uses of the work are permitted, provided the original work is properly cited.

tion process through promoting plant growth that has the ability of phytoremediation, or directly through degradation and/or accumulating pollutants by itself. Endophytes have the main role in the ecosystem are decomposers because they are the primary colonizers of dead plant tissues (2).

Hibiscus sabdariffa (Hs, roselle; Malvaceae) is an annual or perennial herb or woody subshrub and locally known as “karkade”, *Hibiscus sabdariffa* used as a diuretic, hypertensive, treating the viscosity of the blood and used traditionally as a portion of food, a herbal drink and as herbal medicine (3). In addition, previous study demonstrated that *H. sabdariffa* is potential source of endophytic fungi with good plant growth promoting properties and antimicrobial activity (4).

Phenol is an aromatic molecule and it is widely distributed naturally and present in the decay of organic materials or coal (5). Phenol is considered hazardous pollutants in many industrial methods such as petroleum strainers, pharmaceuticals, paper industries, textiles, plastics, dyes, and phenolic resin manufacturers (6). For example, the high antibacterial and inhibitory activities of phenols may generate problems in the operation of biological process. Additionally, phenols exhibited negative impacts on the quality of food processing and paper industry due to their odour and coloring properties (7).

Due to the cyclic molecular structure of phenol, the degradation rate is slow and difficult in the environment because of the difficulty of cleaving the benzene ring that causing their accumulation in the effluent environment (8). Even at low concentrations, phenolic compounds are highly toxic to living organisms and deadly to the aquatic system (9). Various treatments technique of wastewater containing high concentrations of phenol represents the most economical and environmental challenges to industries. Biotechnology is considered very effective in dealing with major environmental challenges through utilizing different chemical and physical methods but these methods are very expensive and may produce hazardous by-products. The biological process has been reported to be the most preferring and economical methods for the biodegradation of phenol (10). Biodegradation of phenol by the biological process is preferable to the physiochemical process because it is environmentally friendly and cost-effective (6). Several microorganisms can utilize phenol as carbon and energy sources for their

growth (11). Most of the phenol-degrading microorganisms such as fungi including, *Aspergillus fumigatus*, *Fusarium flocciferum*, *Aspergillus awamori*, *Penicillium chrysogenum*, *Trichosporium cutaneum*, and *Candida tropicalis* have been proved as phenol degrader (12). Fungi can mineralize a wide variety of carbon sources to produce various active enzymes that providing possibilities for the removal of phenol and their derivatives (13). Many reports explained the mechanism of degradation of phenol and the metabolism of aromatic compounds and the mechanism of phenol biodegradation mainly takes place through Meta and Ortho pathways were illustrated (14). Several factors influence the rate of degradation of phenol including, temperature, pH, agitation, and physical properties of contaminants (15). The present study aimed to determine the biodiversity of endophytic fungi associated with *Hibiscus sabdariffa* and investigate the ability of some isolated endophytic fungal species to degrade different concentrations of phenol derivatives, Catechol (1,2 dihydroxy-benzene) and Resorcinol (1,3 dihydroxy benzene) and use of high biodegradation fungi in the degradation of phenolic compounds in the effluent from pulp and paper factory.

MATERIALS AND METHODS

Plant materials. Healthy plant materials of *Hibiscus sabdariffa* were collected from a natural habitat in the desert of Aswan University Campus, Aswan governorate, Egypt which latitude: 24° 5' 15" N32° and 53' 56" E. Fifteen healthy medicinal plants were selected from five different sites in Aswan University area. The climatic environments in this region ranged between moderately to cold dry winter (16).

Isolation and identification of endophytic fungi from *Hibiscus sabdariffa*. The collected samples from the plant material of *Hibiscus sabdariffa* (root, stem, and leaf) were completely rinsed twice in running tap water, then surface-sterilized by successive immersion in 70% ethanol (1 min) and then in 5% (v/v) sodium hypochlorite (5 min). Surface-sterilized roots stem, and leaves were longitudinally cut into approximately ~5 mm segments under sterile conditions and directly placed on a sterilized Petri dishes containing potato dextrose agar supplemented with 100 U/mL chloramphenicol to suppress bacteri-

al growth. Plates were incubated at 28°C for 2-3 wk until formed colonies. The growing tips of mycelia were transferred to new PDA plates for pure culture and maintained by continuous sub-culturing (17). All isolated fungal colonies were identified according to the microscopic observation of mycelia, asexual/sexual spores and colony morphology, cultural characteristics (color, texture, and pigmentation), and spores and spore-bearing structure using standard identification manuals such as Manual of Soil Fungi, The Genera of Hyphomycetes from Soil, Soil fungi in Qatar and other Arab Countries and Raper and Fennell for *Aspergillus* species (18-21). Colonization frequency (CF) was calculated as described by Hata et al. (22) using the following formula:

CF% = Number of segments colonized by endophytes / Total number of segments examined × 100

Biodegradation of phenolic compound by isolated endophytic fungi: Microorganisms. Thirty-one strains of endophytic fungi that were isolated from *H. sabdariffa* were used for screening their ability for biodegradation of phenol and its derivatives at different concentrations.

Medium for degradation studies. Czapek Dox broth medium was used during studies of the biodegradation of phenol by strains of endophytic fungi which had the following composition (g/L) sodium nitrate 3.0, potassium phosphate (dibasic) 1.0, potassium chloride 0.5, and magnesium sulfate heptahydrate 0.5. The pH of the medium was adjusted to 5.5 (23). Biodegradation tests were performed by supplemented the medium with Catechol (1,2dihydroxy-benzene) and Resorcinol (1,3 dihydroxy benzene) in concentrations of 4,6 and 8 (g/L) as sole carbon and energy sources.

Screening for phenol biodegradation potential. Five ml of sterilized CzapekDox broth medium were inoculated with an 8 d culture in discs form (5 mm) from the plate as inoculums and the tubes were incubated under shake culture condition on a rotary shaker for 8 d at 28°C. After an incubation period, the cells were centrifuged at 1200 rpm for 12 min. Residual phenol in the cell-free supernatants was estimated using Folin-Ciocalteu reagents method by taking about 100 µl of each supernatant and adding 100 µl of Folin-Ciocalteu reagents. The mixture was then allowed to stand for 5 min, then 100 µl sodium carbonate (2%)

was added to the mixture and incubated for 60 min at 20°C (24). The absorbance was measured at 725 nm. The residual phenol content was detected. The phenol removal efficiency of the tests strains was calculated according to the following formula:

% Phenol Removal Efficiency (PRE) = $(C_i - C_f / C_i) \times 100$

Where c_i is the initial concentration of phenol (mg/L) and c_f is the final concentration of phenol.

All experiments of phenol biodegradation were done triplicate; the results were expressed as average ± standard deviation (SD) (25).

Batch biodegradation of industrial effluent samples. Six isolates of endophytic fungi that showed the highest degradative potential used for batch biodegradation of industrial effluent samples containing phenol, were selected, *Aspergillus niger* 13r7, *Aspergillus japonicus* 4r2, *Alternaria chlamydospora* 6l4, *Cochliobolus australiensis* 5l7, *Emericella quadrilenata* 1f7 and *Fusarium poae* 11r7.

Medium for batch biodegradation culture. Industrial effluent samples were collected from the Paper and Pulp factory in the Edfu area in the Aswan government, Egypt. The industrial effluent samples were supplemented by adding necessary macro elements, KH_2PO_4 – 1.0 g/L, $MgSO_4$ – 0.50 g/L, KCL – 0.5 g/L, $NaNO_3$ – 3.00 g/L (26).

Screening of isolates with highest phenol batch biodegradation. Each of six endophytic fungi were inoculated in 250 mL flasks containing 50 mL of industrial effluent medium samples and incubated at 28°C at 150 rpm on a rotary shaker for 5 d. Control assay (without inoculums) was performed under the same experimental conditions. Residual Phenol content was measured at various intervals over 5 d by using Folin-Ciocalteu reagents at 725 nm against a blank (24). All experiments of batch phenol biodegradation were done triplicate; the results were expressed as average ± standard deviation (SD). The total phenol contents were determined spectrophotometrically as described above using Folin-Ciocalteu reagent (24).

Factors influence on batch biodegradation rate of phenol. *Fusarium poae* 11r7 were the most effective fungal species and have the highest and quickly batch biodegradation of phenol in industrial effluent samples, so it was selected for further optimal condi-

tions experiments such as temperature, pH, different agitation speed, and incubation time during 5 d to detect the best optimization conditions for highly degradation rate. Experiments were performed in 250 mL Erlenmeyer flask containing 100 mL of Czapek Dox broth medium and autoclaved at 121°C for 15 min then supplemented with industrial effluent samples after medium sterilization. Disc of *Fusarium poae* 11r7 of 5 d old culture on Czapek Dox agar medium was used as inoculums on the tested medium. The above growth medium supplemented with industrial effluent samples without *F. poae* 11r7 inoculum has been used as control. Conditions parameters were varied: for temperature (28, 35, and 45°C), for pH (3.0, 5.0, 7.0, and 9.0 using 1.0 M HCl or 1.0 M NaOH, for incubation at without agitation and agitation at different speeds (100, 150, and 200 rpm) on different 3 shakers and for different incubation time during 5 d (1, 2, 3, 4, and 5 d) (25). Folin-ciocalteu reagent method was used for the estimation of residual phenol content (24). All experiments of phenol biodegradation were done triplicate; the results were expresses as average \pm standard deviation (SD).

Statistical analysis. Data obtained were subjected to a one-way analysis of variance (ANOVA). Means were compared using Tukey's test analysis at the 5% level using Minitab software. The values shown in the figures were carried out by using the R program (R-3.4.3, <https://www.r-project.org/>).

RESULTS

Endophytic fungi associated with *H. sabdariffa*.

From 15 individual plants corresponding to *Hibiscus sabdariffa* (roots, stems, and leaves) plants were collected from different places for the study of endophytic fungi. A total of 34 fungal species belonging to 18 fungal genera in addition to one variety were isolated and identified from *Hibiscus sabdariffa* plants. The results showed that the root tissue of *Hibiscus sabdariffa* harbors the largest number of endophytic fungi (26 fungal isolates) as compared to the stem (10 fungal isolates), whereas only 16 fungal isolated from the leaves. The *Aspergillus* genus was the most dominant fungal genus in all plant parts of *H. sabdariffa* (38.5%) followed by the *Chaetomium* genus (10.88%) and *Macrophomina phaseolina* (Fig. 1B) In this study, the relative abundance of the *Aspergillus*

genus in the different parts of the plant was 22.6%, 10.46%, and 5.44% of the roots, stem, and leaves, respectively, and represented by seven species. The distribution of endophytic fungal species in different parts of the plant was variable, whereas *Aspergillus japonicus* and *Macrophomina phaseolina* were the most common fungal species in the root (24 colonies and 23 colonies respectively). *Aspergillus niger* and *Cochliobolus australiensis* in the stem (14 colonies and 8 colonies respectively). *Cladosporium sphaerospermum* and *chaetomium elatum* were common in the leaf (10 colonies and 7 colonies respectively) (Fig 1A, 1C, 1D).

Biodegradation of phenol by endophytic fungi from *H. sabdariffa*. In this present study, 31 endophytic fungal species representing 91.18% of the total 34 isolates have the capability of biodegradation of two phenol derivatives, Catechol (1,2 dihydroxy-benzene and Resorcinol (1,3 dihydroxy benzene) at three concentrations (0.4, 0.6 and 0.8%, w:v) with variable extents.

As shown in Table 1, the highest degradation of the fungal strains in range between (75.85 to 97.85%, 57.63 to 98.50%, and 10.34 to 83.45%) at 0.4%, 0.6% and 0.8% concentration of 1,2 dihydroxy benzene respectively. The highest degradation rate by 97.85 and 97.82% of 1,2 dihydroxy-benzene at final concentration of 0.4% was observed in *Emericella quadrilinata* and *Cochliobolus australiensis* 9r2, respectively (Table 1). On the other hand, *Emericella nidulans* var. *echinulata* showed the lowest degradation rate by 75.63% of 1,2 dihydroxy benzene (Table 1). With increasing of concentration of 1,2 dihydroxy-benzene (catechol), the capability of endophytic fungal species differed with increasing or decreasing in the ability of biodegradation. There were decreasing in the rate of biodegradation in all species such as *Cochliobolus australiensis* 9r2 (95.30%), *Macrophomina phaseolina* (95.50%) except in *Aspergillus flavus* 13r3, *Aspergillus japonicus* 1r2, *Aspergillus oryzae* 33r5, *Emericella quadrilinata* 1f7, *Emericella nidulans* var. *echinulata*, *Emericella* sp. 3r3, *Fusarium solani* 22r5 and *Phoma leveillei* which had increasing in the biodegradation but with increasing of catechol concentration to 0.8%, there were decreasing in biodegradation rate in all fungal strains.

As shown in Table 2, the highest degradation of the fungal strains occurred when they used Resorcinol (1,3 dihydroxy benzene) as a carbon source in

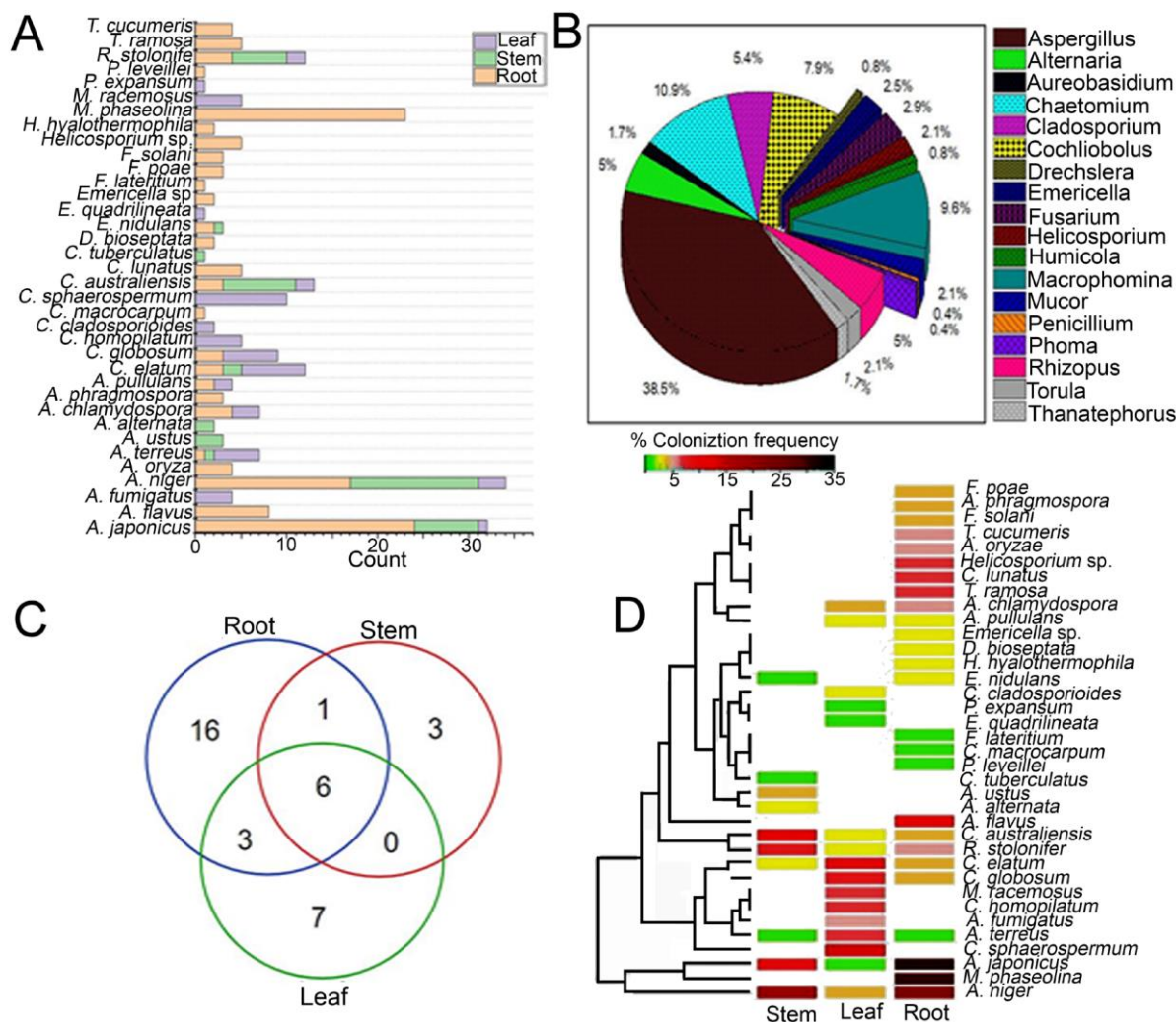


Fig. 1. Diversity profile of endophytic fungi associated with *H. sabdariffa*, A Total count of fungal species, B% Genera Dominance of fungal species. C Venn diagram of the total number and mutual occurrence of fungal species of the root, stem, and leaf, D heat map illustrate % Colonization frequency (CF) of fungal species

the range between (94.54 to 99.85%, 97.93 to 99.67% and 83.30 to 99.04%) at 0.4% 0.6%, and 0.8% concentrations. The highest degradation rate by 99.85% and 99.66% of 1,3 dihydroxy-benzene (Resorcinol) at final concentration of 0.4% was observed in *Aspergillus fumigatus* 2712 and *Aspergillus flavus* 12r5, respectively (Table 2). On the other hand, *Alternaria chlamydospora* 614 showed the lowest degradation rate by 94.54. With increasing the concentration of 1,3 dihydroxy-benzene (Resorcinol) to 0.6%, the ability of endophytic fungal species differed with increasing or decreasing in the ability of biodegradation. There were decreasing in the rate of biodegradation in all species except in *Alternaria chlamydospora* 614, *Aspergillus terreus* 1r7, *Cochliobolus australiensis* 8r2, *Cochliobolus australiensis* 9r2 and *Macropho-*

mina phaseolina which had increasing in the biodegradation. The tested endophytic fungal species can degrade phenol up to a concentration of 0.8%. The limit of phenols in paper and pulp industries was 22 mg L⁻¹.

Batch biodegradation of industrial effluent sample by endophytic fungi. A batch cultivation experiment was carried out using phenol as limiting substrate for 6 endophytic fungi named *Alternaria chlamydospora* 614, *Aspergillus japonicus* 4r2, *Aspergillus niger* 13r7, *Cochliobolus australiensis* 517, *Fusarium poae* 11r7 and *Emericella quadrileneata* 1f7. Initial phenol concentration in industrial effluent medium was taken as 278 mg/L. The extent of phenol concentration was investigated for several batch residence

Table 1. Potency of different fungal species on percentage degradation of Catechol at 0.4, 0.6, 0.8 % concentrations, incubated at 28°C for 8 d

Species of endophytic fungi	0.4	0.6	0.8
<i>Alternaria chlamydospora</i> 614	97.01 ± 0.08 ^{AB}	94.58 ± 0.89 ^{ABC}	59.68 ± 1.03 ^{EFGHI}
<i>Aspergillus flavus</i> 12r5	85.95 ± 0.76 ^{GHI}	74.46 ± 0.73 ^L	49.68 ± 1.20 ^{IJKL}
<i>Aspergillus flavus</i> 13r3	92.41 ± 0.37 ^D	92.63 ± 0.72 ^{BCD}	55.08 ± 2.16 ^{GHIJK}
<i>Aspergillus fumigates</i> 2712	84.23 ± 0.17 ^J	73.80 ± 0.32 ^L	44.48 ± 2.28 ^{KLM}
<i>Aspergillus japonicus</i> 1r2	86.10 ± 1.13 ^{GHI}	90.17 ± 0.50 ^{CDE}	10.34 ± 1.99 ^S
<i>Aspergillus japonicus</i> 4r2	94.06 ± 0.93 ^{CD}	92.24 ± 0.09 ^{BCD}	23.97 ± 0.23 ^P
<i>Aspergillus niger</i> 13r7	95.38 ± 0.07 ^{BC}	92.48 ± 0.23 ^{BCD}	28.48 ± 5.75 ^{OP}
<i>Aspergillus oryzae</i> 33r5	93.76 ± 0.08 ^{CD}	93.77 ± 0.50 ^{BC}	79.35 ± 2.28 ^{ABC}
<i>Aspergillus oryzae</i> 7r3	81.84 ± 0.51 ^{KL}	81.35 ± 2.23 ^{IJK}	20.67 ± 4.27 ^{QRS}
<i>Aspergillus terreus</i> 1r7	81.84 ± 0.51 ^{KL}	66.02 ± 3.22 ^M	76.38 ± 0.07 ^{ABC}
<i>Aspergillus terreus</i> 2612	79.14 ± 0.83 ^M	77.60 ± 1.31 ^{KL}	59.26 ± 0.68 ^{FGHI}
<i>Aspergillus terreus</i> 1s6	92.02 ± 0.81 ^{DE}	88.86 ± 0.63 ^{DEF}	45.59 ± 2.62 ^{IJKLM}
<i>Chaetomium elatum</i> 6s6	89.72 ± 0.54 ^{EF}	85.32 ± 0.96 ^{FGHI}	61.36 ± 1.25 ^{EFGH}
<i>Chaetomium homophilatum</i> 3315	92.45 ± 0.07 ^D	82.26 ± 0.51	70.05 ± 1.54 ^{CDEF}
<i>Cochliobolus australiensis</i> 517	86.57 ± 0.88 ^{GH}	79.70 ± 0.24 ^{JK}	83.45 ± 0.97 ^A
<i>Cochliobolus australiensis</i> 8r2	93.70 ± 1.37 ^{CD}	88.43 ± 0.03 ^{DEFG}	75.35 ± 1.77 ^{ABCD}
<i>Cochliobolus australiensis</i> 9r2	97.82 ± 0.64 ^A	95.30 ± 0.36 ^{AB}	37.22 ± 2.28 ^{MNO}
<i>Drechslera bioseptata</i> 18r2	95.11 ± 0.29 ^{BC}	92.57 ± 0.40 ^{BCD}	70.89 ± 0.51 ^{BCDE}
<i>Emericella nidulans</i> var. <i>echinulata</i> 30s5	75.85 ± 0.03 ^{LM}	86.07 ± 0.10 ^{EFGH}	81.45 ± 2.62 ^{AB}
<i>Emericella nidulans</i> var. <i>echinulata</i> 31s5	80.40 ± 0.54 ^N	84.39 ± 0.46 ^{FGHI}	69.91 ± 0.86 ^{CDEF}
<i>Emericella quadrilenata</i> 1f7	97.85 ± 0.03 ^A	98.50 ± 0.64	40.89 ± 3.25 ^{LM}
<i>Emericella ruglosa</i> 3s4	89.45 ± 0.30 ^F	88.72 ± 0.86 ^{DEF}	34.15 ± 0.22 ^{NOP}
<i>Emericella</i> sp. 3r3	79.23 ± 0.24 ^M	85.79 ± 0.55 ^{EFGHI}	55.40 ± 2.00 ^{GHIJK}
<i>Fusarium poae</i> 11r7	85.54 ± 0.64 ^{GHIJ}	83.99 ± 0.17 ^{GHIJ}	64.89 ± 0.40 ^{DEFG}
<i>Fusarium solani</i> 22r5	83.38 ± 0.05 ^{JK}	85.58 ± 0.53 ^{FGHI}	46.52 ± 3.06 ^{IJKLM}
<i>Helicosporium</i> sp.	86.03 ± 0.90 ^{GHI}	76.97 ± 0.84 ^{KL}	38.48 ± 0.57 ^{LM}
<i>Humicola hyalothermophila</i>	97.12 ± 0.45 ^{AB}	94.17 ± 0.28 ^{ABC}	52.99 ± 4.78 ^{HJK}
<i>Macrophomina phaseolina</i>	96.52 ± 0.35 ^{AB}	95.50 ± 0.74 ^{AB}	82.05 ± 0.07 ^{AB}
<i>Penicillium expansum</i>	89.58 ± 0.26 ^F	57.63 ± 3.95 ^N	45.03 ± 10.16 ^{IJKLMN}
<i>Phoma leveillei</i>	84.39 ± 0.43 ^{HJ}	96.58 ± 0.13 ^{AB}	14.34 ± 0.91 ^{RS}
<i>Thanatephorus cucumeris</i>	86.70 ± 0.33 ^G	86.60 ± 0.13 ^{EFGH}	55.96 ± 1.42 ^{GHIJ}

times by intermittent sampling, showing the biodegradation potential of six selected endophytic fungi in degrading synthetic phenol waste. There were decrease in the concentration of phenol during 5 days, in 2nd day, *Fusarium poae* 11r7 considered the most effective fungi in decreasing the phenol percentage (by 37.4%) followed by *Aspergillus japonicus* 4r2 (by 42.34%), while the lowest phenol percentage (71.82%) was observed in *Cochliobolus australiensis* (Fig. 2). In 3rd, 4th, 5th the value of phenol concentration decrease gradually until reach to 5th day that there were no any phenol content in all six species. Reduction in phenol level proved the biodegradation.

The highest amounts of phenol degradation were observed on 5 day while *Emericella quadrilenata* 1f7 consume all phenol content in the fourth day. Results from this study showed that *Fusarium poae* 11r7 has potential to be used in biodegradation of industrial effluent containing phenol.

Factors influence on batch biodegradation rate of phenol. Numerous factors are affecting the degradation rate of phenolic compounds such as temperature, pH, agitation speed, and incubation period. *Fusarium poae* 11r7 has been selected due to its high batch biodegradation rate of industrial effluent sam-

Table 2. Potency of different fungal species on percentage degradation of Resorcinol at 0.4, 0.6, 0.8% concentrations, incubated at 28°C for 8 d

Species of endophytic fungi	0.4	0.6	0.8
<i>Alternaria chlamydospora</i> 614	94.54 ± 0.70 ^{JK}	97.06 ± 0.06 ^{BCDE}	90.32 ± 1.68 ^{GHIJ}
<i>Aspergillus flavus</i> 12r5	99.66 ± 0.14 ^{AB}	92.31 ± 0.97 ^{KLMN}	90.77 ± 0.08 ^{FGHI}
<i>Aspergillus flavus</i> 13r3	95.37 ± 0.63 ^{IJK}	93.82 ± 0.37 ^{HHIGKLM}	92.92 ± 0.59 ^{DEF}
<i>Aspergillus fumigates</i> 2712	99.85 ± 0.00 ^A	94.73 ± 0.68 ^{EFGHIJ}	91.07 ± 0.10 ^{FGHI}
<i>Aspergillus japonicus</i> 1r2	96.39 ± 0.30 ^{GHI}	89.81 ± 1.00 ^P	89.68 ± 0.45 ^{HJ}
<i>Aspergillus japonicus</i> 4r2	97.32 ± 0.61 ^{EFGH}	79.93 ± 0.41 ^R	85.55 ± 0.20 ^{KL}
<i>Aspergillus niger</i> 13r7	97.52 ± 0.90 ^{CDEFGH}	97.41 ± 0.30 ^{ABCD}	80.55 ± 1.27 ^M
<i>Aspergillus oryzae</i> 33r5	98.45 ± 0.69 ^{ABCDEF}	91.56 ± 0.63 ^{MNO}	96.38 ± 0.14 ^B
<i>Aspergillus oryzae</i> 7r3	98.72 ± 0.22 ^{ABCDEF}	94.19 ± 0.80 ^{GHIJKL}	84.25 ± 0.06 ^L
<i>Aspergillus terreus</i> 1r7	94.35 ± 0.73 ^K	95.20 ± 0.14 ^{DEFGHI}	94.00 ± 1.35 ^{BCDE}
<i>Aspergillus terreus</i> 2612	98.49 ± 0.59 ^{ABCDEF}	92.12 ± 0.94 ^{LMNO}	89.16 ± 0.60 ^{IJ}
<i>Aspergillus terreus</i> 1s6	99.22 ± 0.17 ^{ABCD}	97.46 ± 0.80 ^{ABCD}	84.12 ± 0.66 ^L
<i>Chaetomium elatum</i> 6s6	99.02 ± 0.19 ^{ABCDE}	96.78 ± 0.11 ^{BCDEF}	95.61 ± 0.14 ^{BC}
<i>Chaetomium homophilatum</i> 3315	98.83 ± 0.40 ^{ABCDEF}	96.60 ± 1.00 ^{BCDEF}	92.31 ± 0.14 ^{EFG}
<i>Cochliobolus australiensis</i> 8r2	98.73 ± 0.40 ^{ABCDEF}	98.97 ± 0.91 ^{AB}	95.07 ± 0.24 ^{BCD}
<i>Cochliobolus australiensis</i> 517	97.87 ± 0.03 ^{BCDEFG}	87.50 ± 0.43 ^P	99.20 ± 0.14 ^A
<i>Cochliobolus australiensis</i> 9r2	97.87 ± 0.03 ^{BCDEFG}	98.32 ± 0.40 ^{ABC}	91.55 ± 0.45 ^{EFGHI}
<i>Drechslera bioseptata</i> 18r2	97.89 ± 0.04 ^{BCDEFG}	96.32 ± 0.12 ^{CDEFG}	99.04 ± 0.48 ^A
<i>Emericella nidulans</i> var. <i>echinulata</i> 31s5	98.24 ± 0.88 ^{ABCDEF}	95.69 ± 0.53 ^{DEFGH}	95.02 ± 1.21 ^{BCD}
<i>Emericella nidulans</i> var. <i>echinulata</i> 30s5	97.03 ± 0.03 ^{FGHI}	94.57 ± 0.37 ^{FGHIJK}	93.64 ± 0.90 ^{CDE}
<i>Emericella quadrilenata</i> 1f7	95.79 ± 0.62 ^{HJK}	89.74 ± 0.57 ^{OP}	83.72 ± 0.49 ^L
<i>Emericella ruglosa</i> 3s4	97.36 ± 0.30 ^{EFGH}	94.13 ± 0.86 ^{GHIJKL}	87.82 ± 0.14 ^{JK}
<i>Emericella</i> sp. 3r3	99.50 ± 0.07 ^{AB}	98.48 ± 0.14 ^{ABC}	94.02 ± 0.27 ^{BCDE}
<i>Fusarium poae</i> 11r7	98.95 ± 0.25 ^{ABCDEF}	92.87 ± 0.34 ^{IJKLM}	98.92 ± 0.49 ^A
<i>Fusarium solani</i> 22r5	98.56 ± 0.52 ^{ABCDEF}	89.93 ± 0.97 ^{NO}	85.33 ± 0.02 ^{KL}
<i>Helicosporium</i> sp.	99.31 ± 0.08 ^{ABC}	96.71 ± 0.74 ^{BCDEF}	91.12 ± 0.22 ^{FGHI}
<i>Humicola hyalothermophila</i>	99.25 ± 0.03 ^{ABC}	96.22 ± 0.06 ^{CDEFG}	85.43 ± 0.41 ^{KL}
<i>Macrophomina phaseolina</i>	99.12 ± 0.30 ^{ABCDE}	99.67 ± 0.09 ^A	98.95 ± 0.12 ^A
<i>Penicillium expansum</i>	99.00 ± 0.72 ^{ABCDE}	98.18 ± 0.63 ^{ABC}	92.15 ± 0.16 ^{EFGH}
<i>Phoma leveilei</i>	96.32 ± 0.14 ^{GHIJ}	86.64 ± 0.23 ^Q	83.30 ± 0.20 ^I
<i>Thanatephorus cucumeris</i>	97.43 ± 0.55 ^{DEFGH}	92.75 ± 0.37 ^{IJKLM}	93.65 ± 1.13 ^{CDE}

All values are mean ± SD, Values in the same column with different superscript letters are significantly different, P < 0.05 (ANOVA after Tukey's test analysis).

ples containing phenol. As shown in Fig. 3, temperature has significant impact on degradation efficiency of the selected fungal strains, *Fusarium poae* 11r7 with optimal temperature in the range of 28-35°C. The most suitable pH value for the selected fungal strain *Fusarium poae* 11r7 in increasing the batch biodegradation rate was pH 5, pH 3, and pH7 respectively for 5 d while pH 9 showed the lowest batch degradation rate.

The incubation of batch biodegradation of *Fusarium poae* 11r7 at static condition and agitation speed

of 100 rpm showed increasing in biodegradation rate of phenol due to decreasing the absorbance of phenol. With increasing the agitation speed to 150 and 200 rpm, there were decreasing in batch biodegradation rate observed. The different incubation periods on the degradation rate of phenol by *Fusarium poae* 11r7 resulted the increment of degradation rate of phenol with increasing the incubation period (decreasing in absorbance). At the 5 d, maximum phenol degradation occurred because of the maximum utilization of all content of phenol.

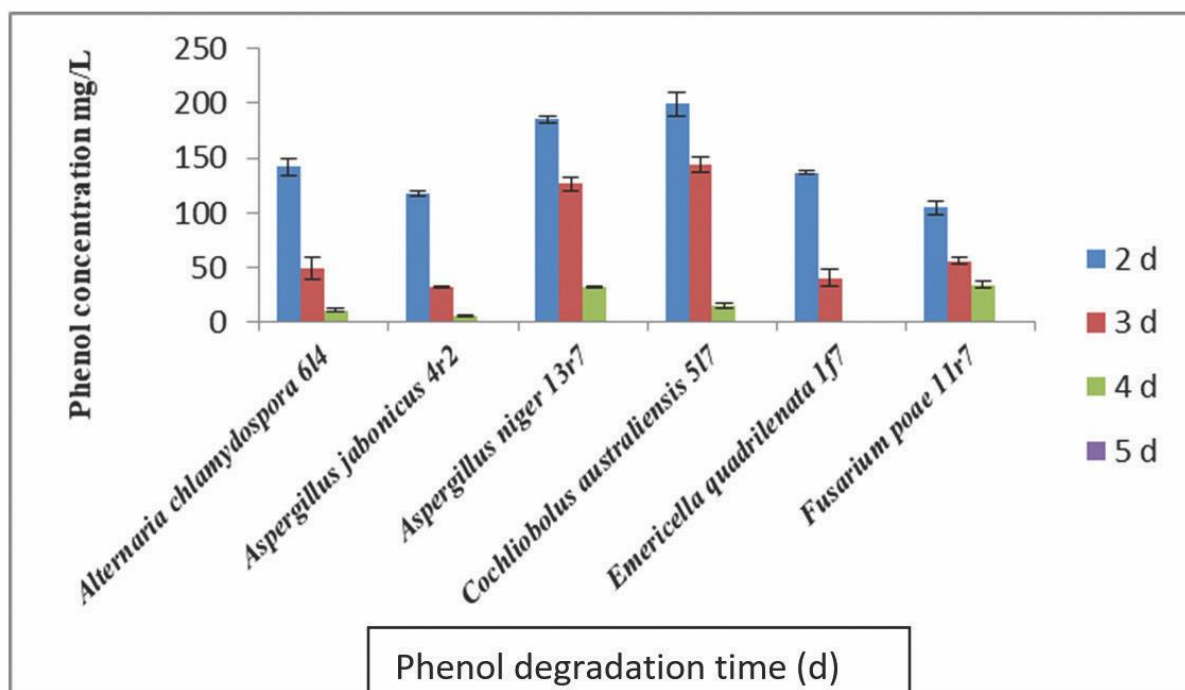


Fig. 2. Batch biodegradation of phenol in Paper and Pulp Effluent samples by some species of endophytic fungi during 5 d.

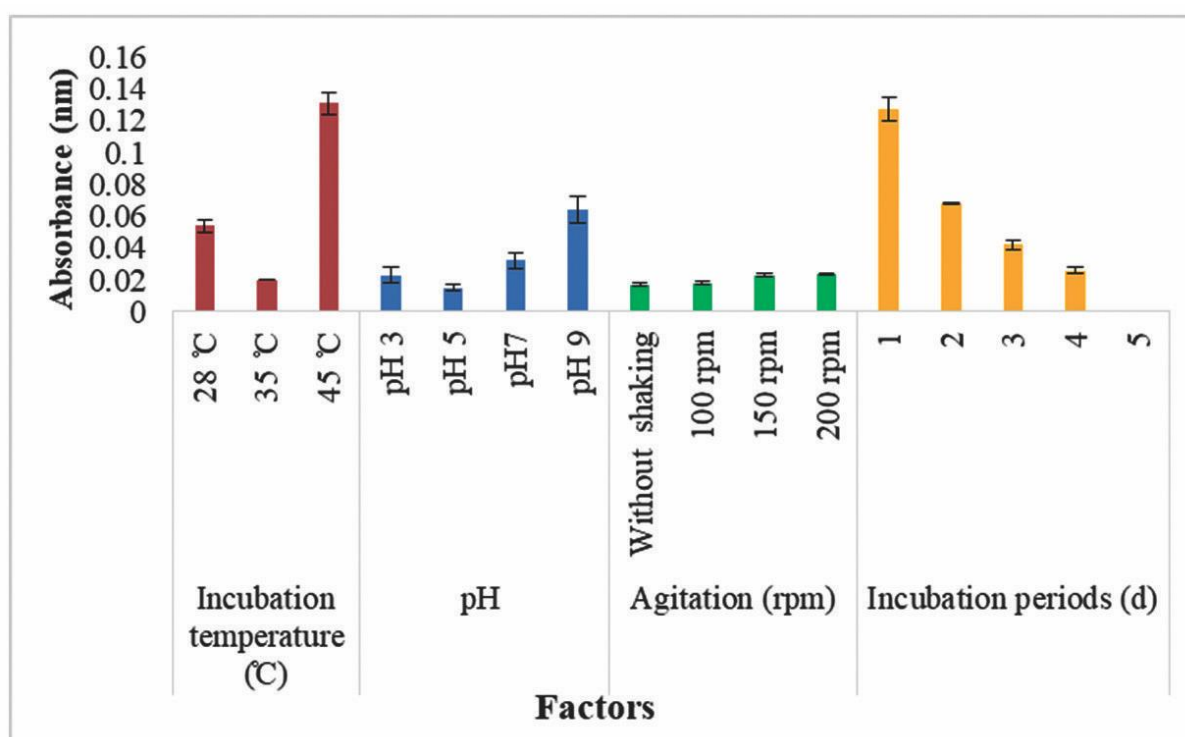


Fig. 3. Effect of different factors on the degradation rate of phenol in paper and pulp effluent samples by using *Fusarium poae* 11r7

DISCUSSION

The relationship between endophytes and host plants involves both antagonism and mutualism. Plants have many mechanisms to limit the growth of endophytes which include producing a variety of toxic metabolites. But by the time of co-evolution, endophytes have gradually formed a variety of tolerant mechanisms towards host metabolites by producing exoenzymes and mycotoxins. These enzymes may also degrade macromolecule compounds into small molecules or convert more toxic substances into less toxic to increase their adaptability (27). A total of 35 fungal species belonging to 18 fungal genera were isolated and identified from different parts of *Hibiscus sabdariffa* plants. *Aspergillus* was the most dominant genus followed by *Chaetomium* genus and *Machrophomina phaseolina*. *Aspergillus* genus was isolated with 7 species that appeared in all parts of plants followed by 3 species of *Chaetomium* genus and *Machrophomina phaseolina* that is in agreement with the study of Kamel et al. (28) who isolated five species of *Aspergillus* and two varieties of *A. terreus* and 2 species of *Chaetomium* genus and *Machrophomina phaseolina* as endophytic fungi from *Euphorbia geniculata* at the same region of our study. Likewise, *Aspergillus* genus exhibited the highest colonization frequency among different endophytic fungi isolated from *H. sabdariffa* (4). *Aspergillus* species are characterized by high secretion of secondary metabolites with various biological activities which may attributed to high dominance of *Aspergillus* genus in *H. sabdariffa* (29).

Nath and Joshi (4) also isolated eight endophytic fungi from different parts of the plant *H. sabdariffa* L., mostly being isolated from the leaf segments. Patil et al. (30) isolated different endophytic fungal species from variable medicinal plants such as *Alternaria* spp., *Rhizopus* spp., *Curvularia* spp., and *Trichoderma* spp. Biodiversity of endophytic fungal species was reported such as *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp. and *Fusarium* sp. that were isolated from *Camellia oleifera* (31). *Aspergillus* sp., *Alternaria* sp., *Chaetomium* sp. and *Cladosporium* sp. were isolated from traditional medicinal plants (32).

Phenol and its derivatives are principle pollutants that are highly toxic even at low concentrations in the industrial effluent and are considered one of the largest groups of environmental pollutants due to their

presence in many industrial effluents (8). In the past few years, there has been several reports that focused on the capability of fungi in degrading phenol and its derivatives and used as a novel for degradation of environmental pollutants including hydrocarbons, polychlorinated biphenyl's (PCBs), polyaromatic hydrocarbons (PAHs), and metals (27). Biodegradation of phenol by fungi is influenced by several factors such as the substrate concentration that considered as an important factor in phenol biodegradation. Also phenol itself is well known to inhibit microbial growth, especially at greater concentrations. This phenomenon is commonly known as substrate inhibition. The high concentration of phenol can affect the growth of microorganisms, and the concentration at which inhibition occurs varies from one compound to another (10). All endophytic fungal strains have the capability for degrading phenol and their derivatives at different concentrations (0.4%, 0.6% and, 0.8%) with variable extents. Stoilova et al. (33) studied biodegradation of phenol and other derivatives by *Aspergillus awamori* at different concentration (0.3, 0.6, 1,2 and, 3 g/L) and revealed that the fungal strain utilized phenol with the varying extent depending on phenol concentration and the rate of degradation decreased gradually with increasing the phenol concentration above 0.6 g/L. Santos et al. (34) and Kennes and Lema, (35) reported the fungal strains, *Graphium* sp. and *Phanerochaete chrysosporium* were significantly degrade phenol at concentration of 0.3 and, 0.05 g/L, respectively. In the present study, the endophytic fungal species could degrade phenol up to a concentration of 0.8%. Stoilova et al. (12) studied the rate of degradation of catechol, 2,4-dichlorophenol and 2,6-dimethoxyphenol with different concentrations (0.1%, 0.2% and 3.0%) by *Aspergillus awamori*. The capability of tested endophytic fungal species on the degradation of phenol and its derivatives decreased or increased with increased the concentration of phenol. The capability of different species of endophytic fungi on the degradation of phenol and its derivatives differed from species to species and from derivatives to derivatives.

In batch biodegradation studies, *Fusarium poae* 11r7 was able to degrade phenol in paper and pulp effluent samples with an initial concentration of 278 mg/L to 104 mg/L within 5 d of incubation period. Sharma and Gupta (36) revealed that *Aspergillus niger* was able to degrade phenol content of paper and pulp effluent samples with an initial concentration

of 268 mg/L to 119 mg/L after five d of incubation. Bernats and Juhna (8) and Lu et al. (37) studied batch biodegradation of phenol in pharmaceutical and coking wastewaters by fungi, respectively. Chhaya and Gupte (38) studied the capability of degrading phenolic compounds olive mill wastewaters by 89% in 5 d.

In batch biodegradation of *Fusarium poae* 11r7, the optimal temperature with highest biodegradation rate was 28-35°C. The phenol biodegradation rate decreased rapidly at temperature up to 35°C because the catalytic activity of the enzymes started to decrease over that temperature. The optimal pH for phenol biodegradation by *Fusarium poae* 11r7 was found to be at pH 3-5 and 7. Sharma and Gupta (36) studied the influence of different pH values on the biodegradation rate of phenol and indicated that the maximum phenol degradation occurs at neutral pH. At acidic or basic pH there was decreasing in phenol degradation since the culture utilizes less carbon source. Bernats and Juhna (8) studied factors affecting the biodegradation of phenol and indicated that the optimal conditions (pH and temperature) of biodegradation were at pH 5-6 and temperature 25°C. Briefly, the optimum conditions for phenol degradation with *Fusarium poae* 11r7 were found at pH 3-5 and temperature 28-35°C. These results indicated that the pH and temperature parameters are important factors for fungi growth and subsequently, increasing the efficacy of phenol biodegrading.

Static condition and agitation speed of 100 rpm showed increasing in the degradation rate of phenol by *Fusarium poae* 11r7 due to decreasing the absorbance of phenol. Maximum phenol degradation has occurred on the 5 d because of the maximum utilization of all content of phenol. Santos and Linardi (5) reported that *Graphium*, *Aspergillus*, *Fusarium* and *Penicillium* can utilize aromatic and aliphatic hydrocarbons and *Graphium* sp. FLB4 degraded 75% of 10 mM of phenol in 168 h.

CONCLUSION

In this study, 34 endophytic fungi were isolated from different parts of the medical plant *H. sabdariffa*. All the isolated endophytic fungi were screened for their ability to degrade phenol residues in the industrial pulp samples. Out of them, 31 fungi species showed considerable ability to degrade phenol

derivatives. The potency of biodegradation of phenol derivatives differed from species to species and from phenol derivatives to another. In general, six endophytic fungal species were found to have significant activity in biodegrading industrial effluent samples containing phenol. *Fusarium poae* 11r7 considered the most effective fungi in decreasing the phenol percentage by 37.4% in batch biodegradation, thus *F. poae* 11r7 considered as the potential candidate for the biodegradation of industrial effluent samples containing phenol. The optimum temperature and pH for *Fusarium poae* 11r7 were found to be 28-35°C, and 3-5, respectively. Static condition and agitation rate 100 rpm were found to be the optimal conditions for biodegradation of phenol.

REFERENCES

1. Gouda S, Das G, Sen SK, Shin HS, Patra JK. Endophytes: a treasure house of bioactive compounds of medicinal importance. *Front Microbiol* 2016;7:1538.
2. Osés R, Valenzuela S, Freer J, Sanfuentes E, Rodriguez J. Fungal endophytes in xylem of healthy Chilean trees and their possible role in early wood decay. *Fungal Divers* 2008;33:77-86.
3. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. – a phytochemical and pharmacological review. *Food Chem* 2014;165:424-443.
4. Nath A, Joshi SR. Ultrastructural effect on mastitis pathogens by extract of endophytic fungi associated with ethnoveterinary plant, *Hibiscus sabdariffa* L. *J Microsc Ultrastruct* 2015;3:38-43.
5. Santos VL, Linardi VR. Biodegradation of phenol by a filamentous fungi isolated from industrial effluents – identification and degradation potential. *Process Biochem* 2004;39:1001-1006.
6. Wang L, Li Y, Yu P, Xie Z, Luo Y, Lin Y. Biodegradation of phenol at high concentration by a novel fungal strain *Paecilomyces variotii* JH6. *J Hazard Mater* 2010;183:366-371.
7. Eaton AD, American Public Health Association, American Water Works Association, Water Environment Federation (2005). Standard methods for the examination of water and wastewater. 21st ed. APHA-AWWA-WEF. Washington DC.
8. Bernats M, Juhna T. Factors governing degradation of phenol in pharmaceutical wastewater by white-rot fungi: a batch study. *Open Biotechnol J* 2015;9:(Suppl 1-M10) 93-99.
9. Przybulewska K, Wiczorek A, Nowak A, Pochrzyszcz

- M. The isolation of microorganism capable of phenol degradation. *Pol J Microbiol* 2006;55:63-67.
10. Al-Khalid T, El-Naas MH. Aerobic biodegradation of phenols: a comprehensive review. *Crit Rev Env Sci Tech* 2012;42:1631-1690.
 11. Zhou W, Guo W, Zhou H, Chen X. Phenol degradation by *Sulfobacillus acidophilus* TPY via the meta-pathway. *Microbiol Res* 2016;190:37-45.
 12. Stoilova I, Krastanov A, Stanchev V, Daniel D, Gerginova M, Alexieva Z. Biodegradation of high amounts of phenol, catechol, 2,4-dichlorophenol and 2,6-dimethoxyphenol by *Aspergillus awamori* cells. *Enzyme Microb Technol* 2006;39:1036-1041.
 13. Lika K, Papadakis IA. Modeling the biodegradation of phenolic compounds by microalgae. *J Sea Res* 2009;62:135-146.
 14. Cerniglia CE (1984). Microbial transformations of aromatic hydrocarbons. In: *Petroleum Microbiology*. Ed, Atlas RM. Macmillan, New York, pp. 99-128.
 15. El-Naas MH, Al-Muhtaseb SA, Makhlof S. Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. *J Hazard Mater* 2009;164:720-725.
 16. Ali AH, Abdelrahman M, Radwan U, El-Zayat S, El-Sayed MA. Effect of *Thermomyces* fungal endophyte isolated from extreme hot desert-adapted plant on heat stress tolerance of cucumber. *Appl Soil Ecol* 2018;124:155-162.
 17. Rossman AY, Tulloss RE, O'Dell TE, Thorn RG (1998). Protocols for an all taxa biodiversity inventory of fungi in Costa Rican conservation area. Parkway publishers. Inc, Boone. North Carolina.
 18. Gilman JC (1957). A manual Of Soil Fungi. 2nd ed. Iowa State Univ Press. Ames. Iowa. USA.
 19. Barron GL (1968). The genera Of Hyphomycetes from Soil. Williams & Wilkins Co. Baltimore.
 20. Moubasher AH (1993). Soil fungi in Qatar and other Arab countries. the center for scientific and applied research, university of Qatar, Doha, Qatar.
 21. Raper KB, Fennell DI (1965). The genus *Aspergillus*. Baltimore: Williams & Wilkins. Edinburgh: E. & S. Livingstone.
 22. Hata K, Futai K. Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge, *thecodiplosis japonensis*. *Can J Bot* 1995;73:384-390.
 23. Stoilova I, Krastanov A, Bui H. Biodegradation of mixed phenolic compounds by a microbial association of *Aspergillus awamori* and *Thermoascus aurantiacus*. *EJEAFChe* 2008;7:2625-2633.
 24. Folin O, Ciocalteu V. On tyrosine and tryptophane determinations in proteins. *J Biol Chem* 1927;73:627-650.
 25. Leonard D, Lindley ND. Growth of *Ralstonia eutropha* on inhibitory concentrations of phenol: diminished growth can be attributed to hydrophobic perturbation of phenol hydroxylase activity. *Enzyme Microb Technol* 1999;25:271-277.
 26. Dos Passos CT, de Medeiros Burkert JF, Kalil SJ, Burkert CAV. Biodegradation of phenol by a newly *Aspergillus* sp. strain isolated from a contaminated soil in southern Brazil. *Quim Nova* 2009;32:950-954.
 27. Krishnamurthy YL, Naik BS (2017). Endophytic fungi bioremediation. In: *Endophytes: crop productivity and protection. Sustainable development and biodiversity*. Eds, Maheshwari DK, Annapurna K. Springer, Cham, Switzerland AG, pp.16:47-60.
 28. Kamel NM, Abdel-Motaal FF, El-Zayat SA. Endophytic fungi from the medicinal herb *Euphorbia geniculata* as a potential source for bioactive metabolites. *Arch Microbiol* 2020;202:247-255.
 29. El-hawary SS, Moawad AS, Bahr HS, Abdelmohsen UR, Mohammed R. Natural product diversity from the endophytic fungi of the genus *Aspergillus*. *RSC Adv* 2020;10:22058-22079.
 30. Patil RH, Patil MP, Maheshwari VL (2016). Chapter 5- Bioactive secondary metabolites from endophytic fungi: a review of biotechnological production and their potential applications. In: *Studies in natural products chemistry*. Ed, Rahman AU. Elsevier, Amsterdam, Netherlands, pp.189-205.
 31. Yu J, Wu Y, He Z, Li M, Zhu K, Gao B. Diversity and antifungal activity of endophytic fungi associated with *Camellia oleifera*. *Mycobiology* 2018;46:85-91.
 32. Devi NN, Singh MS. Endophytic fungi associated with traditional medicinal plants of Manipur. *Int J Pharm Sci Rev Res* 2015;33:127-132.
 33. Stoilova I, Krastanov A, Yanakieva I, Kratchanova M, Yemendjiev H. Biodegradation of mixed phenolic compounds by *Aspergillus awamori* NRRL 3112. *Int Biodeterior Biodegrad* 2007;60:342-346.
 34. Santos VL, Heilbuth NM, Braga DT, Monteiro AS, Linardi VR. Phenol degradation by a *Graphium* spp. FIB4 isolated from industrial effluents. *J Basic Microbiol* 2003;43:238-248.
 35. Kennes C, Lema JM. Simultaneous biodegradation of p-cresol and phenol by the basidiomycete *Phanerochaete chrysosporium*. *J Ind Microbiol* 1994;13:311-314.
 36. Sharma N, Gupta VC. Batch biodegradation of phenol of paper and pulp effluent by *Aspergillus Niger*. *Int J Chem Eng Appl* 2012;3:182-186.
 37. Lu Y, Yan L, Wang Y, Zhou S, Fu J, Zhang J. Biodegradation of phenolic compounds from coking wastewater by immobilized white rot fungus *Phanerochaete chrysosporium*. *J Hazard Mater* 2009;165:1091-1097.
 38. Chhaya U, Gupte A. Possible role of laccase from *Fusarium incarnatum* UC-14 in bioremediation of bisphenol, a using reverse micelles system. *J Hazard Mater* 2013;254-255:149-156.