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Gas Chromatography-Mass Spectrometry Analysis of Agricultural Residues using Indigenous Laccase producing Fungi (*Albifimbria viridis*) as Herbicides

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Abstract

Background and Objective: Discarded as wastes, parts of the agricultural products can be used for feed productivity as well as management of animal feed production. Production of various products is possible using appropriate processing. The objective of the present study was to use laccase of *Albifimbria viridis* in degradation of agricultural residues and to produce compounds with herbicide properties.

Material and Methods: The fungi were isolated from agricultural soils. The isolates were identified using morphological detection and PCR amplification of the internal transcribed spacer. Supernatants were collected from semi-solid cultures and laccase activity was assessed using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) substrate. This was carried out using n-hexane and degradation of the agricultural residues was investigated using gas chromatography-mass spectrometry.

Results and Conclusion: Growth of the fungal isolate in culture media with tannic acid was studied using scanning electron microscopy. In total, the isolate produced 50 U ml^{-1} laccase. Gas chromatography-mass spectrometry analyses revealed production of oxime, methoxy-phenyl and 2-cyclopenten-1-one for tannic acids, o-guaiacol, tetradecane, hexadecane, octadecane, octadecanoic acid, hexadecanoic acid and benzene, 1,3-bis(1,1-dimethylethyl) for sorghum seeds and 2-acetyl-5-methylfuran, phenol, 2-methoxy and benzene, 1,2-dimethoxy for wheat straw during fungal growth (0.73 mg ml^{-1}). Results have shown that the laccase enzyme produced from *Albifimbria viridis* native strain is capable of hydrolytic cleavage of chemical pollutants from agricultural wastes for herbicide bioremediation.

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1. Introduction

The annual production of more than 1.3 billion tons of residues indicates increases in food and agricultural waste production as one of the world significant challenges [1]. Agricultural and food residues are abundantly found in world. The components of these residues mostly include cellulose, hemicellulose, lignin and other compounds [2-3]. They are widely used from economic, environmental and technological viewpoints [4-5]. These lignocellulosic residues consist of 25-40% hemicellulose, 15-25% lignin and 35-55% cellulose [6]. Such increases result in energy waste and severe environmental, social and economic issues [7]. One of the most promising solutions includes recycle and recover of wastes as well as production of high-value materials. Used mostly as fertilizers and composts, industries can be suggested as the most important users of such wastes. Nowadays, the global energy demand is rapidly increasing as the population grows. Fossil fuels are currently the primary energy source and responsible for various environmental pollutions such as greenhouse gas emissions. Bioethanol has been suggested as a promising

alternative for these fuels and is produced majorly by sugars and starch from food plants such as sugarcanes. Lignocellulosic materials are other options to be used in bioethanol production. Food wastes converted to single-cell proteins can eliminate protein efficiencies and decrease contaminations. The single-cell proteins, as a group of proteins, have been produced from cell biomasses, including higher nutritional values than that plant and animal sources do. These proteins can be derived from bacteria, algae, fungi and yeasts. Fungi are used mostly and include more valuable biomasses than that bacteria and algae do. Oshoma and Eguakun-Owie studied effects of food wastes on *Aspergillus niger* biomass production [8]. Furthermore, fungi can produce laccases (EC 1.10.3.2), such as benzenediol, oxygen oxidoreductase, which can degrade lignocellulosic materials. The Whit Philips Xl30e rot fungi have proven a better ability in laccase production than that algae and yeasts have. Lignocelluloses are the most abundant cellulose and hemicellulose sources in environment, which are further converted to fermentable sugars used in bioethanol production. Lignocellulosic materials can be detected in stems, leaves and barks of plants such as wheat straws, beet molasses and sugarcanes [9]. Presence of guaiacol and syringol subunits in lignocellulose makes its degradation difficult; therefore, further physical, biological and chemical preparations (pretreatment) are needed before the extraction of valuable materials and biomass fermentation. Semi-solid fermentation of such materials by fungi produces biological herbicides. Wheat straw is a low-price agricultural residue, which is produced in large quantities and is used in bioethanol production [10]. Weeds are reported as the most important agricultural materials that affect quality and quantity of the products. Biocontrol methods such as antimicrobials, fungal spore suspensions and bioherbicides have critically been used to address these issues. Several fungi act as herbicides by the production of phytotoxic metabolites [11]. Therefore, the overall aim of the current study was to carry out gas chromatography-mass spectrometry (GC-MS) analysis of agricultural residues from indigenous laccase producing fungi, *Albifimbria viridis*. Furthermore, this study included primary screening of laccase producer fungi and their growth in agriculture residues as well as GC-MS analyses of laccase agricultural residues.

2. Materials and Methods

2.1. Materials

Potato dextrose broth medium was purchased from Quelab, Canada. Sigma Aldrich, Darmstadt, Germany, provided 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The n-hexane was purchased from Sigma-Aldrich, Darmstadt, Germany. The primer pair of ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) was synthesized by Eurofins, Germany. The 5× PCR buffer, dNTP mixture, $MgCl_2$ and RB Taq DNA polymerase were purchased from RNA Biotechnology, Isfahan, Iran.

2.2. Primary screening of laccase producer fungi

Samples from agricultural soil were collected and enriched in liquid tannin broth (1.0 g l^{-1} yeast extract as nitrogen source and 0.04 g l^{-1} $MgSO_4$, 0.0004 g l^{-1} $FeCl_3$ and 2.0 g l^{-1} sodium phosphate, pH 8.0) or tannin extracted from plant seeds (as sole carbon source) was used for primary screening. Then, fungi were isolated on potato dextrose agar (PDA) and supplemented with 1% of tannin or tannic acid. Pure colonies with light brown hollows were collected and tested for laccase activity. Fungi were molecularly identified based on nuclear ribosomal DNA internal transcribed spacer (ITS) [12].

2.3. Scanning electron microscopy

Samples were collected from fungal hyphae and spores on potato dextrose agar media for scanning electron microscopy (SEM) (Philips Xl30 Instrument, the Netherlands). These samples were fixed using chemicals, dehydrated and gold coated in vacuum and then studied using SEM.

2.4. Growth of fungi in agricultural residues

Fungi (1 cm) cultured on PDA were selected and inoculated into agricultural residue semisolid media. After 16 days, the supernatant was filtered and the biomass was assessed by weight [13].

2.5. Gas chromatography-mass spectrometry analysis of laccase producing agricultural residues

The isolated fungi were cultured on potato dextrose broth (PDB) for four days and harvested by centrifugation at 6000 rpm. Then, biomass (22.12 mg ml^{-1}) was added to the basic media (0.04 g l^{-1} magnesium sulfate, 0.0004 g l^{-1} ferric chloride, 2.0 g sodium phosphate and 1.0 g l^{-1} yeast extract per liter, pH 8.0), including 170 g l^{-1} agricultural residues in semi-solid media. After three days, the supernatant was assessed for laccase production at 30 ºC and analyzed using GC-MS method. The supernatant was extracted using n-hexane and degradation of the agricultural residues was assessed using GC-MS (Agilent 7890A Gas Chromatography, USA) with Agilent Mass Spectrometric Detector, equipped with a fused silica capillary column DP-5MS and a direct capillary interface (film dimensions of 30 m × 0.25 mm × 0.25 µm). Injection of the samples was carried out as follows: helium was used as carrier gas at 1.0 ml/min in pulsed splitless state. Initiated at 90 °C for 3 min, GC temperature increased to 300 °C at 10 °C/min. Moreover, 250 °C was used as the injector and detector temperature. To identify the separated peaks, Wiley and Wiley Nist05 Mass Spectral Dataset was used. The enzyme was detected

from a cell-free extract of fungi cultured in liquid or semi-solid media. In semi-solid media, cells were harvested and the cell-free supernatant with concentrated enzyme was used. The enzyme was induced using tannin. Laccase activity was assessed using oxidation of ABTS. The reaction mixture, including 0.5 mM of the substrate (ABTS), 2.8 mL of 0.1 M sodium acetate buffer (pH 4.5) and 100 µl of the culture supernatant, was incubated for 10 min. Absorbance was read against a blank at 420 nm using spectrophotometer. A unit was defined as the quantity of laccase that oxidized 1 µmol of ABTS substrate in 1 min [14].

2.6. Statistical analysis

Analysis of variance was used to estimate the experimental errors as well as significance of the results. Significant differences were reported at $p \leq 0.05$. All experiments were carried out with three replications from separate cultures and values were reported as mean ±SD (standard deviation).

3. Results and Discussion

3.1. Primary screening of laccase producing fungi

In the current study, laccase enzyme was produced by microscopic fungi using solid-bed fermentation technique. The *Albifimbria* (*A.*) *viridis* was cultured on wheat straw, tannic acid and sorghum seed extrudes as substrates. Various hydrolysis byproducts were assessed using GC-MS. The isolate included green-to-black spores and white mycelia in SEM. As an herbicide, this isolate included sporodochial conidiomata, conidiogenous cells and white mycelia (Fig. 1A), which produced oval-shaped black-green conidia (Fig. 1B). The fungi also demonstrated a high laccase activity and eliminated tannin. Identification of the isolate was carried out using molecular method and ITS primers. The BLAST analysis showed a high similarity (99.48%) to *A. viridis* (Fig. 2). In this study, laccase of *A. viridis* in an optimal condition of 30 °C and pH 5.0 in semi-solid media with 1% of tannic acid and ABTS as substrates produced 50 U ml^{-1} laccase. In presence of agricultural residues including tannic acids, sorghum seeds and wheat straws, 23, 34 and 27 U ml^{-1} laccase were produced, respectively. It is noteworthy that laccase production has not been reported in fungi.

3.2. Growth of fungi in agricultural residues

The maximum level of laccase (50.0 U ml^{-1}) was produced by the fungi after 16 days at 30 °C and pH 5.0. However, laccase production in agricultural wastes included 23, 27 and 34 U ml^{-1} from tannic acid, wheat straw and sorghum seed extrude semi-solid media, respectively (Table 1). Fungi can decompose lignocellulosic matters using their enzymes, including laccase, peroxidase and cellobiose dehydrogenase. Decomposition of lignocellulosic compounds by these enzymes is beneficial as it prevents environmental pollutants produced during residual burning [15]. The fungi belonged to ascomycete families while the maximum laccase production has been reported in basidiomycete families. For example, activity of the produced laccase by *Pleurotus eryngii* was 43,761 U l^{-1} after 20 days in presence of various inducers (Tween 80, Cu^{2+} and Fe^{2+}) [16]. In contrast, activity of the produced laccase by *Trametes pubescens* was 333,000 U l^{-1} in presence of Cu^{2+} [17]. In 2017, Myasoedova et al. [18] Investigated laccase production with various substrates in ascomycetes and reported that activity of the enzyme produced from *Myrothecium roridum* VKM F-3565 was 37.2 U ml^{-1} in presence of ABTS at pH 5.0, which was the maximum activity of all [18].

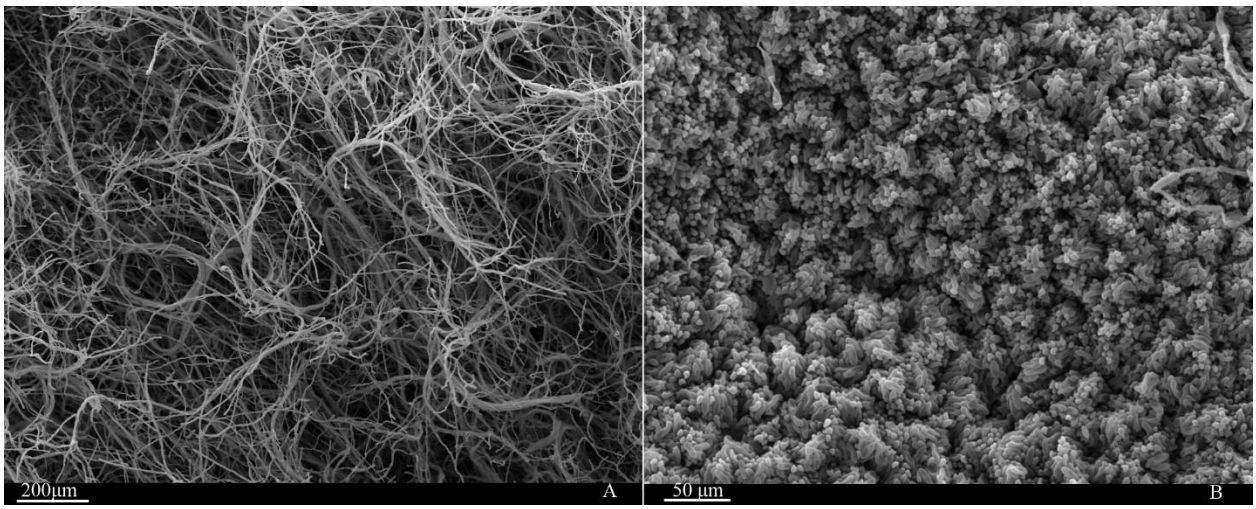


Figure 1. The SEM image of isolated fungi *Albifimbria viridis*. A) Mycelium of fungi (scale bar = 200 µm), B) Spore of fungi (scale bar = 20 µm). Photos were taken by Philips Xl30.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Albifimbria viridis CBS 449.71 ITS region; from TYPE material	1061	1061	99%	0.0	99.48%	NR_153551.1
Albifimbria viridis strain CBS 449.71 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1061	1061	99%	0.0	99.48%	KU845898.1
Albifimbria terrestris CBS 126186 ITS region; from TYPE material	1044	1044	99%	0.0	98.97%	NR_153549.1
Albifimbria lateralis CBS 117712 ITS region; from TYPE material	1044	1044	99%	0.0	98.97%	NR_153548.1
Albifimbria terrestris strain CBS 126186 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1044	1044	99%	0.0	98.97%	KU845883.1
Albifimbria lateralis strain CBS 117712 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1044	1044	99%	0.0	98.97%	KU845881.1

Figure 2. The data blast for detection of herbicide fungi showed 99.48% similarity to Albifimbria viridis a herbicide fungi.

Table 1. The Laccase activities of Albifimbria viridis grown on agricultural waste and tannic acid.

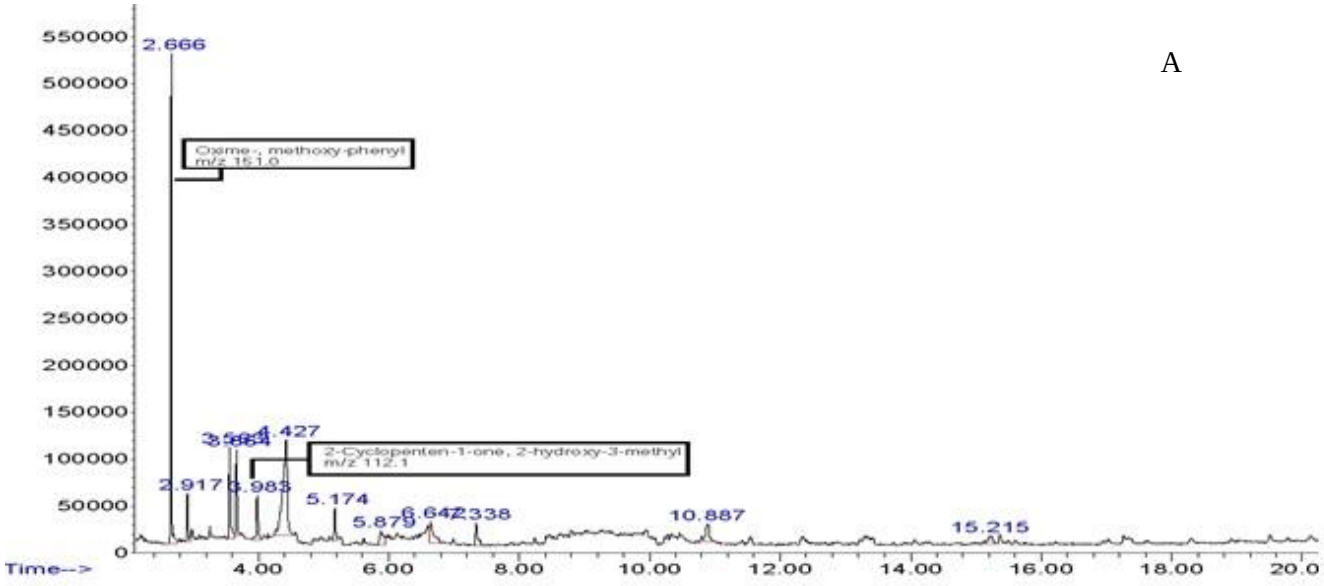
	Semi solid PDA +1% TA	Tannic acid	sorghum	wheat straw
Laccase assay (U ml^{-1})	50 ±1.6	23 ±2.39	34 ±1.97	27 ±2.21
Biomass (mg ml^{-1})	0.73	0.83	0.96	0.87

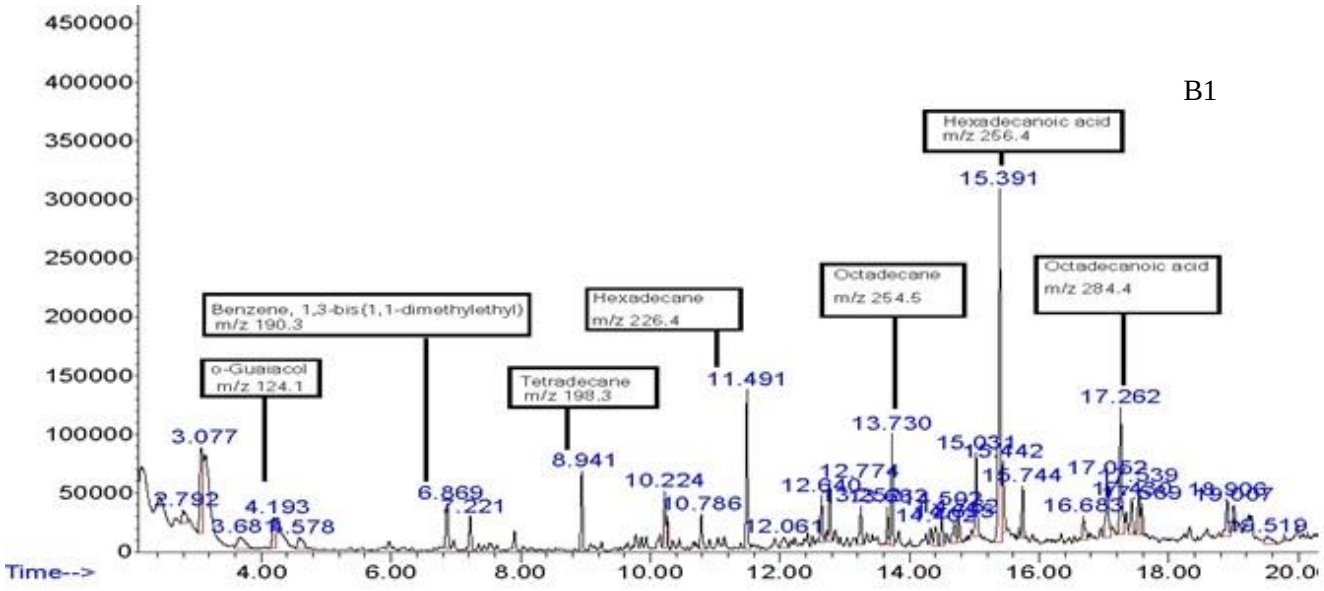
Since use of fungal herbicides is cheaper than use of chemical herbicides and chemical herbicides are harmful for the environment, development of biological herbicides has recently been interested by the researchers. Of various fungi for herbicide production, *Colletotrichum*, *Phoma* and *Sclerotinia* spp. are predominant. For example, *Sclerotinia minor* has been used to control dandelion, white clover and broad-leaf leaves in meadows [19]. Biological herbicides are phytopathogen microorganisms or phytotoxin microbes that are used for the biological control of weeds. Under greenhouse conditions, *Myrothecium verrucaria* killed 100% of Kudzu seeds when treated with silwet L-77. Laccase can produce dicarboxylate, demethylate, phenolic demethylate and methoxy phenolic acids in the initial decomposition phase. In addition to aromatic compounds, laccase is capable of oxidizing iodine and ferrocyanide. Lignin is composed of phenylpropanoid, which is a precursor of lignin. Monolignols include p-hydrophenyl and p-guaiacyl, which are precursors of coumaryl, coniferyl, sinapyl and syringyl alcohols [4-20]. Herman et al. reported that *A. verrucaria* included herbicidal properties, analyzed as a biological herbicide [21].

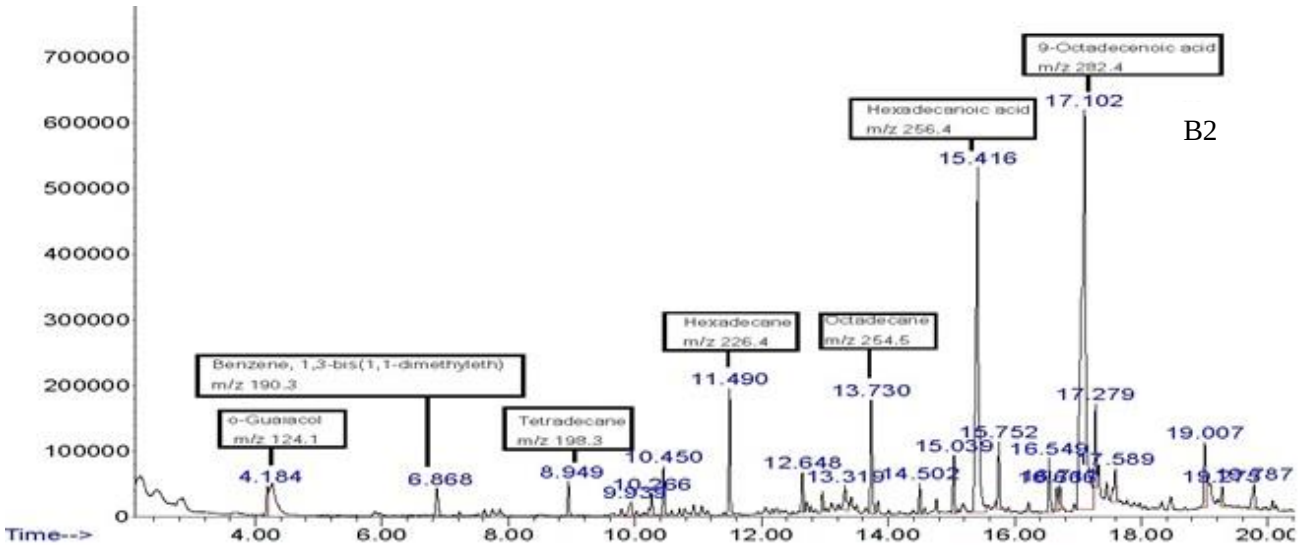
3.3. Gas chromatography-mass spectrometry analysis of laccase producing agricultural residues

Fermented compounds were investigated using mass spectra, retention time, Nist and Wiley Libraries and published data. Results of tannic acid degradation by 0.73 mg ml^{-1} *A. viridis* in semi-solid media are shown in Fig. 3A. Data showed that oxime, methoxy-phenyl, 2-cyclopenten-1-one and 2-hydroxy-3 were produced while triol benzene in control was used 100% by the fungi. Results of sorghum seed extrude degradation by 0.96 mg ml^{-1} *A. viridis* in semi-solid media are shown in Table 2 and Figs. 3B1 and 3B2. Data demonstrated that o-guaiacol, tetradecane, hexadecane, octadecane, octadecanoic acid, hexadecanoic acid and benzene, 1, 3-bis (1, 1-dimethylethyl) were produced by the fungi during fermentation of sorghum seeds. Results of wheat straw degradation by 0.87 mg ml^{-1} biomass of *A. viridis* in semi-solid media are shown in Table 3 and Fig. 3C.

Furthermore, data showed presence of 2-acetyl-5-methylfuran, phenol, 2-methoxy phenol and benzene, 1, 2-dimethoxy. Data from GC-MS analysis revealed that fungi could use agricultural residues as the isolated fungi not only included its herbicidal characteristics but also produced herbicides such as oxime, methoxy-phenyl and 2-cyclopenten-1-one by fermenting lignin and tannin. The GC-MS is an appropriate technique for the assessment of compounds in agricultural wastes.


A
2.666
Oxime-, methoxy-phenyl
m/z 151.0
4.427
2-Cyclopenten-1-one, 2-hydroxy-3-methyl
m/z 112.1
2.917
3.983
5.174
6.642
10.887
15.215
Time-->


B1
Hexadecanoic acid
m/z 256.4
15.391
Octadecane
m/z 254.5
Octadecanoic acid
m/z 284.4
Benzene, 1,3-bis(1,1-dimethylethyl)
m/z 190.3
Hexadecane
m/z 226.4
11.491
o-Guaiacol
m/z 124.1
Tetradecane
m/z 198.3
3.077
8.941
13.730
17.262
6.869
10.224
10.786
4.193
Time-->


B2
9-Octadecenoic acid
m/z 282.4
17.102
Hexadecanoic acid
m/z 256.4
15.416
Hexadecane
m/z 226.4
Octadecane
m/z 254.5
11.490
13.730
Benzene, 1,3-bis(1,1-dimethylethyl)
m/z 190.3
o-Guaiacol
m/z 124.1
Tetradecane
m/z 198.3
4.184
6.868
8.949
10.450
17.279
19.007
Time-->

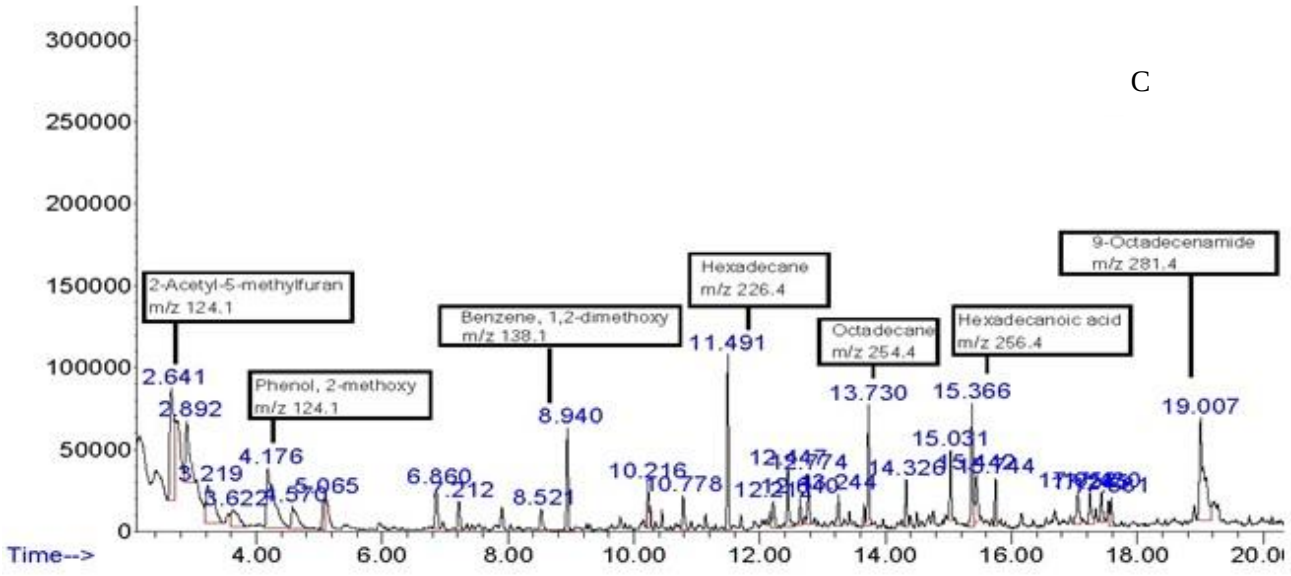


Figure 3. GC-MS analyses for (A) tannic acid hydrolysis by Albifimbria viridis in semi-solid medium (B) Sorghum seed excrude hydrolysis by Albifimbria viridis in semi-solid medium. 1) Sorghum with fungi, 2) blank of sorghum (C) wheat straw hydrolysis by Albifimbria viridis in semi-solid media

Table 2. Production of by- product from sorghum seed excrude by Albifimbria viridis

No	Compound	Qual of Sorghum	m/z	Time
1	Phenol, 2-methoxy	94	124.1	4.19
2	Benzene	96	190.3	6.87
2	Tetradecane	96	198.3	8.94
3	Hexadecane	97	226.4	11.49
4	Heptadecane	97	240.5	12.64
5	Eicosane	91	282.5	12.64
6	Nonadecane	91	266.5	12.64
7	Octacosane	91	394.8	12.64
8	Heneicosane	91	296.6	12.78
9	3-Methyltridecane Tridecane	90	198.3	13.25
13	4-Heptafluorobutyryloxyhexadecane	91	438.5	13.66
14	1-Heptadecanol	91	256.4	13.66
15	Octadecane	91	212.4	13.73
17	Hexamethyl-pyranoindane	93	244.3	14.40
18	Galaxolide	90	258.4	14.40
19	Phthalic acid	90	166.3	14.50
20	Phthalic acid, isobutyl nonyl ester	90	348.5	14.50
21	Docosane	91	310.6	14.69
27	Hexadecanoic acid	99	256.4	15.39
28	Tridecanoic acid	93	214.3	15.39
34	Octadecanoic acid	98	284.5	17.26
35	1-Eicosanol	91	298.5	17.54
36	Trifluoroacetic acid	91	114.02	17.54
38	Dichloroacetic acid	93	367.4	17.54

In the present study, GC-MS analysis identified compounds from the decomposition of tannic acids [oxime, methoxy-phenyl, 2-cyclopenten-1-one and bis(2-ethylhexyl)phthalate], sorg-hum seeds [phenol, 2-methoxy, benzene and 1,3-bis(1,1-dimethylethyl octadecanoic acid] and wheat straws (2-acetyl-5-methylfuran, phenol, 2-methoxy phenol and benzene, 1,2-dimethoxy), which could be used for the biological control of weeds. These compounds were produced during the decomposition of lignocellulosic by laccase. Dursun et al. [22] reported that oxime, methoxy-phenyl (46.07%) was one of the major compounds extracted from volatile compounds of ultra-high-temperature milks. Such compounds are usually associated to the degrees of heat treatments on milks and relationships between the packing materials and milks.

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Table 3. Production of by product by Albifimbria viridis from wheat straw.

No	Compound	Qual of wheat straw	m/z	Time
1	2-Acetyl-5-methylfuran	90	124.1	2.64
2	Phenol,2-methoxy-	97	124.1	4.18
3	Mequinol	93	124.1	4.18
4	1-Acetyl-2-methyl-1-cyclopentene	91	124.1	4.18
5	Benzene	96	138.1	5.06
6	Tetradecane	90	198.3	7.21
7	Benzene, 1,2-dimethoxy	91	164.2	8.52
8	Docosane	91	310.6	10.22
9	Hexadecane	98	226.4	11.49
10	3, 5-Dimethyl-2-cyclohexen-1-one	96	150.2	12.45
11	Heneicosane	91	296.6	12.64
12	Octadecane	91	254.5	12.64
13	2, 6, 10, 15-Tetramethylheptadecane	90	296.6	12.64
14	Nonadecane	91	268.5	13.73
15	Pentadecane	91	212.4	13.73
16	n-Hexadecanoic acid	96	256.4	15.37
17	Docosane	91	310.6	15.74
18	Heptadecane	91	240.5	15.74
19	Tricosane	91	324.6	17.05
20	Heptacosane	91	380.7	17.05
21	Octacosane	91	394.8	17.05
22	Pentacosane	90	352.7	17.05
23	Hexacosane	90	366.7	17.05
24	Octadecanoic acid	94	284.5	17.24
25	Dotriacontane	91	450.9	17.58
26	Pentatriacontane	91	492.9	17.58
27	Tetratetracontane	91	619.2	17.58
28	9-Octadecenamide	95	281.5	19.01

Peng et al. [23] studied structural characteristics of the hemicellulose from delignified wheat straws and found that wheat straw hemicellulose included uronic acid and arabinoxylan. The hemicellulose pyrolysis produced various compounds such as 2-cyclopenten-1-one, which is used as essence and spice in chemical and food industries. In the current study, laccase production by fungal isolates as biological herbicides from agricultural residues as the sole carbon sources was investigated for the first time.

4. Conclusion

Lignin includes significant potentials for the sustainable production of fuels, biomasses and chemical intermediates. The effective use of lignin needs its depolymerization to low-molecular-weight phenolics and aromatics that can serve as the building blocks for the chemical synthesis of high-value products. Natural ability of laccase to degrade lignin by laccase mediators is currently suggested with the potential breakthrough use for lignin valorization. In this study, laccase enzyme was used for the biodegradation of agricultural wastes on semisolid media using herbicide fungi during lignin degradation, which resulted in production of valuable chemical materials as well.

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflicts of interest.

References

1. Vilarino M, Franco C, Quarrington C. Food loss and waste reduction as an integral part of a circular economy. Front Environ Sci. 2017; 5: 1-5. doi: 10.3389/fenvs.2017.00021

2. Salihu A, Abbas O, Sallau A, Alam M. Agricultural residues for cellulolytic enzyme production by *Aspergillus niger*: Effects of pretreatment. 3 Biotech. 2015; 5(6):1101-1106. doi: 10.1007/s13205-015-0294-5

3. Jalili Tabaii M, Emtiazi G. Comparison of bacterial cellulose production among different strains and fermented media. Appl Food Biotechnol. 2015; 3(1), 35-41. doi: 10.22037/afb.v3i1.10582

4. Haghdan S, Renneckar S, Smith G. Sources of Lignin. Lignin in Polymer Composites, Canada, 2016:1-11. doi: 10.1016/b978-0-323-35565-0.00001-1

5. Darabzadeh N, Hamidi-Esfahani Z, Hejazi P. Improvement of cellulase production and its characteristics by inducing mutation on *Trichoderma reesei* 2414 under solid state fermentation on rice by-products. Appl Food Biotechnol. 2018; 5(1): 11-18. doi: 10.22037/afb.v5i1.18651

6. Adhikari S, Nam H, Chakraborty J. Conversion of solid Wastes to Fuels and Chemicals Through Pyrolysis. Waste Biorefinery, United States, 2018: 239-263.
doi: 10.1016/b978-0-444-63992-9.00008-2

7. Diacono M, Persiani A, Testani E, Montemurro F, Ciaccia C. Recycling agricultural wastes and by-products in organic farming: Biofertilizer production, yield performance and carbon footprint analysis. Sustain. 2019; 11(14): 3824.
doi: 10.3390/su11143824

8. Oshoma C, Eguakun-Owie S. Conversion of food waste to single cell protein using *Aspergillus Niger*. J Appl Sci Environ Manage. 2018; 22(3):350-355
doi: 10.4314/jasem.v22i3.10

9. Tuong An Tran T, Kim Phung Le T, Phong Mai T, Quan Nguyen D. Bioethanol Production from Lignocellulosic Biomass. Alcohol Fuels - Current Technologies and Future Prospect. 2020. 1-13
doi: 10.5772/intechopen.86437

10. Tarraran L, Mazzoli R. Alternative strategies for lignocellulose fermentation through lactic acid bacteria: The state of the art and perspectives. FEMS Microbiol Lett. 2018; 365(15):1-14
doi: 10.1093/femsle/fny126

11. Parsazad M, Babaeipour V, MalekSabet N, Mohammadian J, Masoumian M. Optimization of 2,6-dimethoxy benzoquinone production through wheat germ fermentation by *Saccharomyces cerevisiae*. Appl Food Biotechnol. 2020; 7(3): 161-169.

12. Ahmadi Khozani M, Emtiazi G, Aghaei SS, Ghasemi SM, Zolfaghari MR. Application of fungal laccase for heavy metals precipitation using tannin as a natural mediator. Int J Environ Sci Technol. 2020; 17(11): 4549-4562.

13. Safari Sinegani AA, Emtiazi G, Hajrasuliha S. Comparative studies of extracellular fungal laccases under different conditions. J Agric Sci Technol. 2007; 9(1): 69-76.
doi: 10.1007/s13762-020-02992-7

14. Shrestha P, Joshi B, Joshi J, Malla R, Sreerama L. Isolation and physicochemical characterization of laccase from *Ganoderma lucidum*-CDBT1 isolated from its native habitat in Nepal. BioMed Res Int. 2016; 2016(4): 1-10.
doi: 10.1155/2016/3238909

15. Choudhary M, Sharma P, Jat H, Nehra V, McDonald A, Garg N. Crop residue degradation by fungi isolated from conservation agriculture fields under rice-wheat system of North-West India. IntJ Recy Organ Waste Agric. 2016; 5(4):349-360.
doi: 10.1007/s40093-016-0145-3

16. Akpinar M, Ozturk Urek R. Induction of fungal laccase production under solid state bioprocessing of new agroindustrial waste and its application on dye decolorization. 3 Biotech. 2017; 7(2):2-10
doi: 10.1007/s13205-017-0742-5

17. Brijwani K, Rigdon A, Vadlani P. Fungal laccases: Production, function and applications in food processing. Enzyme Res. 2010; 2010:1-10.
doi: 10.4061/2010/149748

18. Myasoedova N, Renfeld Z, Podieiablonskaia E, Samoilova A, Chernykh A, Classen T, Pietruszka J, Kolomytseva M, Golovleva L. Novel laccase-producing ascomycetes. Microbiol. 2017; 86(4): 503-511.
doi: 10.1134/s0026261717030110

19. Harding D, Raizada M. Controlling weeds with fungi, bacteria and viruses: A review. Front Plant Sci. 2015; 6:1-14
doi:10.3389/fpls.2015.00659

20. Hoagland R, Douglas Boyette C, Abbas H. Myrothecium verrucaria isolates and formulations as bioherbicide agents for kudzu. Biocontrol Sci Technol. 2007; 17(7): 721-731.
doi: 10.1080/09583150701527268

21. Herman T, Pawlowski M, Domier L, Hartman G. First report of *Albifimbria verrucaria* causing leaf spot on glycine latifolia. Plant Dis. 2020; 104(2):576.

22. Dursun A, Guler Z, Emre Şekerli Y. Characterization of volatile compounds and organic acids in ultra-high-temperature milk packaged in tetra brik cartons. Int J Food Prop. 2016; 20(7): 1511-152.
doi: 10.1080/10942912.2016.1213280

23. Peng Y, Wu S. The structural and thermal characteristics of wheat straw hemicellulose. J Anal Appl Pyrolysis. 2010; 88(2): 134-139.
doi:10.1016/j.jaap.2010.03.006

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تجزیه کروماتوگرافی گازی-طیف‌سنجی جرمی ضایعات کشاورزی حاوی قارچ تولیدکننده آنزیم لاکاز (*Albifimbria viridis*) به‌عنوان علف کش

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چکیده

سابقه و هدف: دورریز یا ضایعات، بخشی از فرآورده‌های کشاورزی می‌باشد که می‌تواند برای بهره وری خوراک دام و همچنین مدیریت تولید خوراک دام مورد استفاده قرار گیرد. تولید فرآورده‌های گوناگون با استفاده از فرایند مناسب امکان‌پذیر است. هدف مطالعه حاضر استفاده از آنزیم لاکاز *آلبیفیمبریا ویریدیس* در تجزیه پسماند کشاورزی به‌منظور تولید ترکیبات با خاصیت علف‌کش بود.

مواد و روش ها: قارچ از خاک کشاورزی جداسازی شد. شناسایی جدایه با روش‌های ریخت‌شناسی[۱] و مولکولی با استفاده از پرایمر ITS[۲] انجام گردید.مایع رویی[۳] از کشت‌های نیمه جامد جمع‌آوری و فعالیت آنزیم لاکاز با استفاده سوبسترای[۴] ۲،۲´-آزینو-بیس (۳-اتیل بنزوتیازولین-۶-سولفونیک اسید) و با استفاده از هگزان و تجزیه ضایعات کشاورزی به‌روش کروماتوگرافی گازی-طیف‌سنجی جرمی ارزیابی شد..

یافته‌ها و نتیجه‌گیری: رشد جدایه قارچی در محیط کشت حاوی تانیک اسید با استفاده از میکروسکوپ الکترونی روبشی[۵] مطالعه و آنزیم لاکاز بافعالیت $50\ U\ ml^{-1}$ تولید شد. تجزیه کروماتوگرافی گازی-طیف‌سنجی جرمی تولید اکسیم متوکسی فنیل و ۲-سیکلوپنتن۱-وان برای اسید تانیک، گایاکل، تترادکان، هگزادکان، اکتادکان، اکتادکانوئیک اسید، هگزادکانوئیک اسید و بنزن ۱،۳-بیس(۱،۱ دی متیل) برای بذر سورگوم و ۲-استیل-۵-متیل فوران، فنول ۲-متوکسی، و بنزن ۱،۲-دی متوکسی برای کاه گندم، در مدت رشد قارچ ($0/73 mg\ ml^{-1}$) را نشان داد. نتایج نشان داده است که آنزیم لاکاز تولید شده توسط *آلبیفیمبریا ویریدیس*، سویه بومی قادر به گسستگی آبکافتی[۶] آلاینده‌های شیمیایی از پسماند کشاورزی به‌منظور زیست‌تصفیه علف‌کش می‌باشد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

۱ Morphological
۲ Internal Transcribed Spacer
۳ Supernatant
Substrate ۴
۵ Scanning Electron Microscopy
۶ hydrolytic cleavage