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Original Paper

Impact of Some Hafr Al-Batin Ligno-cellulosic Natural Resources: Characterization, Evaluation and Enzymes Production

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The main idea of this paper is the evaluation of two new flora of wild plant species and Date palm fronds collected from the Hafr Al-Batin region in Eastern Saudi Arabia. The chemical properties, including macro-element and micro-element contents, were analyzed for each natural material: Date palm fronds (*Phoenix dactelifera* L.) (DPFs), desert grasses of species (*Stipa capensis*) (DGs), small desert trees of species (*Haloxylon persicum*) (DTs). These Ligno-cellulosic natural resources were evaluated for their suitability as substrates (organic materials) for mushroom mycelium grows and the production of the industrially valuable enzymes. Oyster mushroom was able to degrade three tested substrates producing, the highest levels of lignin-degrading enzyme laccase 182.05 U·ml^{-1}, 140.38 U·ml^{-1}, and 41.75 U·ml^{-1} using DPFs, DGs and DTs, respectively, followed by medium activity of xylanase and cellulase enzymes. Meanwhile, the Chestnut mushroom exhibited notable laccase activity, with 59.23 U·ml^{-1}, 21.54 U·ml^{-1}, and 43.51 U·ml^{-1} using DPFs, DGs and DTs, respectively, though it showed lower xylanase and cellulase activity. Obtained results indicate that the Lignocellulosic materials DPFs, DGs, and DTs have potential for use in lignocellulolytic enzyme production by efficient degrader mushrooms. Further studies are needed to demine the suitable conditions for effective mushroom cultivation and lignocellulolytic enzyme production on an industrial scale.

Keywords: ligno-cellulosic materials, mushrooms, characterization, enzymes production, Hafr Al-Batin

1 Introduction

Saudi Arabia generally has large areas of arid deserts full of normal flora of wild plants that has a wide range of ecological habitats and characterized by diversity of plant species. The wild flora of Hafr Al-Batin region, northeastern Saudi Arabia includes different species, like *Stipa capensis*, *Schimpera arabica*, *Launea nudiculis*, *Rumex vesicarius*, *Citrullus colyocynthis*, *Malva parviflora*, *Artemisia* sp., and *Haloxylon salicornicum* (Mohammed-Ibtisam and Doka, 2018).

In Saudi Arabia there are large amounts of Ligno-cellulosic residues annually from date palm trees, shrubs, wild desert plants and woody products mills as well as field crop residues (El-Juhany., 2001). Most of these Ligno-cellulosic wastes are eliminated by burning or thrown away as refuse which causes an environmental damage.

Many microorganisms able to degrade Ligno-cellulosic biomass, including fungi. Lignocellulolytic enzymes secreted by fungi play agood role in decaying biomass for mineralization of the recalcitrant compounds (Janusz et al., 2017). These fungi usually initiated degradation processes enzymatically to decompose lignin, cellulose, and hemicellulose. Mushrooms considered one of the most valuable degraders in nature, they play a carbon-recycling role in ecosystems (Kim, 2021).

Mushrooms are considered as one of the most important food product in Asia and Central-Eastern Europe due to their good taste, nutritional value and biological activities (Muszynska et al., 2017; Rzymski and Klimaszyk, 2018). Ligno-cellulosic substances, including agricultural, horticultural, forestry, and wood industry wastes, can be used for mushroom cultivation (Agarwal et al., 2016; Jeznabadi et al., 2017). Various studies have shown the cultivation of different mushroom species on Ligno -cellulosic materials including, sawdust of different tree species (Liang et al., 2016), paddy straw (Rajak et al., 2011), wheat-straw (Rühl et al., 2008), indigenous

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grasses (Jongman et al., 2010) and other substrates (Belewu and Belewu, 2005).The chemical composition of substrates used for mushroom cultivation have been vary in the content of protein, carbon, nitrogen and other trace elements. Therefore, it can be expected that the quality of substrate may significantly affect the growth and chemical composition of cultivated mushrooms (Rzymski et al., 2016).

This highlights the aim of the present study, which is to identify new unconventional lignocellulosic natural materials that can be used as substrates for lignocellulolytic enzyme production through the cultivation of efficient degrader mushrooms.

2 Material and Methods

2.1 Tissue Culture Isolation for Mother Culture production

Two different edible mushrooms, Oyster mushroom (*Pleurotus ostreatus*) and Chestnut mushroom (*Agaricus bisporus* var. *avellaneus*) were purchased from a local market to ensure they were edible strains, suitable for cultivation. A piece of the inner cap tissue (cross section) of both mushrooms basidiocarp is placed on Potato Dextrose Agar medium (PDA) for fungal isolation and creating a pure culture mycelium (Petersen and Krisai-Greilhuber, 1996).The plates were incubated at a temperature of 26–28 °C for 10 days. Multiple plates for each fungus were applied to ensure success in isolation. The mycelia from the pure fungal cultures were then maintained at 4 °C on PDA slants.

2.2 Microscopic Examination

The agar plates of *P. ostreatus* and *A. bisporus* var were examined under the Olympus CX40 RF100 light microscope with (40 × Magnification power), coupled to a Canon A620 digital camera to confirm the purity of isolated mushroom mycelium. Purity of isolated mycelium was confirmed by observing uniform hyphae with one shape.

2.3 Nutrient Element Analysis of the Ligno-Cellulosic Natural Resources

Three ligno-cellulosic natural resources including, Date palm fronds (*Phoenix dactelifera* L.) (DPFs), desert grasses of species (*Stipa capensis*) (DGs), small desert trees of species (*Haloxylon persicum*) (DTs) were collected from Hafr Al-Batin City, in the Eastern Province, Saudi Arabia. All plant materials were analyzed for macro-elements (Nitrogen, Phosphorus, Potassium, Calcium and Magnesium), micro-elements (Zinc, Copper, Manganese) using digestion method as recommented by Cottenie et al. (1982). The total protein, organic matter, organic carbon and ash were also evaluated in the Unit of Land Resources Evaluation and Mapping, National Research Centre, Cairo, Egypt according to Cottenie et al. (1982).

2.4 Qualitative Assay for Ligninolytic Enzymes Production (Guaiacol Oxidation)

The pure mycelium of two strains were screened for lignocellulolytic enzymes production by growing them on plates of B&K medium containing 4 mM guaiacol (D'Souza et al., 2006). Plates incubated for 7 days at 28 °C in dark, the production of an intense brown color under and around the fungal colony resulting from guaiacol oxidation was considered as a positive reaction for the extracellular enzymes activity (Okino et al., 2000; Abd El Aty et al., 2015; Abd El Aty and Mostafa, 2013). The fungal diameter and the color zone diameter were recorded in millimeters (mm) at three different points and the average values are reported as mean ± SD using MS Excel.

2.5 Ligno-Cellulosic Wastes Preparation and Solid State Fermentation (SSF)

The plant materials were cut randomly into pieces (~1.0–3.0 cm) long. The solid state fermentation was carried out in Erlenmwyer flasks (250 ml) with 3 g of the dried materials: DPFs, DGs and DTs.the solid substrates were moistened by addition of 15 ml of distilled water to each flask. The flasks were covered with hydrophobic cotton and autoclaved at 121 °C for 20 min (Abd El Aty and Ashour, 2022). After cooling, each flask was incoculated with two 15 mm discs of 10 days old cultures separately. The inoculated flasks were incubated for 7, 10 and 14 days at 28 °C under static conditions.

2.5.1 Extraction of the Fungal Degrading Enzymes

At the end of incubation period the crude extracellular enzymes extracted by adding 30 ml of distilled water to each flask and left for 60 min in a rotary shaker at 180 rpm (Mostafa et al., 2016). Then the mixture was filtered through a cloth and the culture filtrate centrifuged for 15 min at 5,000 rpm and 4 °C (Alharbi et al., 2023).

2.6 Enzymes Determination

2.6.1 Laccase Activity

Laccase (EC 1.10.3.2) activity was measured by using the method described by Bournnais et al. (1995) based on the oxidation of the substrate 2,2'-azino–bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS). The reaction mixture contained 600 µL sodium acetate buffer (0.1 M, pH 5.0 at 27 °C), 300 µL ABTS (5 mM), 300 µL

culture filtrate and 1,400 µL distilled water. The mixture was then incubated for 2 min at 30 °C. The absorbance was measured immediately at 420 nm. One unit of laccase activity was defined as activity of an enzyme that catalyzes the conversion of 1 mole of ABTS (ε 420 = 36,000 $M \cdot cm^{-1}$) per min (Abd El-Aty et al., 2022).

2.6.2 Xylanase Activity

The enzyme activity measured according to the method of Warzywoda et al. (1983). Half ml of the filtrate was added to 0.5 ml of 1% xylan in 0.05 M acetate buffer pH 5.0 and incubated for 30 min at 50 °C. The amount of reducing sugar liberated was quantified by the method of Neish (1952) using xylose as standard. One unit of xylanase is defined as the amount of enzyme releasing 1 µmol of xylose equivalents per min under assay conditions.

2.6.3 Cellulase Activity

In accordance with Mandels and Weber (1969) method cellulose activity was determined. 0.5 ml of enzyme solution was added to 0.5 ml of 1% carboxymethylcellulose (CMC) in 0.05 M acetate buffer (pH 5.0). Incubation of the reaction mixture was performed for 30 min at 50 °C. The released reducing sugars were determined by the method of Neish (1952). One unit of cellulase (IU) was defined as the amount of enzyme releasing 1 µmol of glucose per min under the assay conditions.

2.7 Small Scale Fermentation Using Plastic Bags

Heat resistant plastic bags are mainly applicable to both commercial and small scale mushroom cultivation (Siwulski et al., 2019).

2.7.1 Spawn Preparation

wheat grains obtained from local market used as a carrier for oyster and chestnut mushrooms spawn production (Das et al., 2015).

Eighty grams of seeds added to each 500 ml Erlenmeyer conical flask. The flasks were moistened with 160 ml distilled water and sterilized by autoclaving at 121 °C for 20 min. After cooling the seeds inoculated with mycelium of 10-days old cultures under aseptic conditions to prevent contamination. Inoculated flasks were plugged with cotton for gas exchange. Flasks were incubated at 26–28 °C with shaking every while to prevent agglutination of the seeds into one big mass.

2.7.2 Cultivation Design

The substrates were first autoclaved at a temperature of 121 °C for 20 min to eliminate potential competitors (e.g. *Trichoderma* spp.) of mushrooms. The sterilized substrates were cooled down to a temperature of 25 °C and each plastic bag was filled with ~100 g of the substrates with 60–70% moisture content without any free water. The substrates in the bags were afterwards inoculated with 10 g of spawn (on wheat grain), then the bags loosely closed and incubated under the same conditions of spawn preparation in a controlled room Sözbir et al. (2015). Three experimental replicates (3 bags for each substrate) were conducted for each cultivation.

After 30 days' incubation the substrates covered with fungal mycelium were evaluated for Lignocellulolytic enzymes production. From each bag about 10 g was taken and 100 ml distilled water were added to 500 ml conical flask. The flasks were shacked for 60 min at 180 rpm. The supernatant obtained after filtration was evaluated for enzymes availability.

2.8 Spectral Characteristics of Degraded Agricultural Wastes

The spectral characteristics of the plant materials before and after fungal degradation were observed by using field emission scanning electron microscopy (FE-SEM; JEOL 6400 F, USA). The 30 days old bags of different plant materials (DPFs, DGs, DTs), inoculated with both mushrooms and control bags (without inoculation) were examined by transferring pieces of plant materials mixed with growing mycelium, in to glass coverslips, fixed with 2.5% glutaraldehyde. The samples dehydrated in a series of ascending ethanol concentrations (50–100%) (v/v), dried in desiccator, and coated with gold in a sputtercoater (SCD 005; BAL-TEC, Switzerland); then, the examination was done by scanning electron microscope at (100 and 200 µm × magnification) (Barak et al., 1986).

3 Results and Discussion

3.1 Preparation of Mushroom Mycelium

The mycelium of mushroom species *P. ostreatus* and *A. bisporus* var were isolated and maintained in laboratory using PDA medium. The morphological examination of agar plates indicated their purity, without any contamination, as shown in (Figure 1, B & E). In addition to morphological characteristics on solid medium, the microscopic examination using Olympus CX40 RF100 light microscope was applied. Microscpic results in (Figure 1, C & F) showed a uniform hypha, without any different forms in all sides of the slide in both mushrooms, which indicated the purity of isolated mycelium.

Khare et al. (2006) emphasized that, mushroom species were considered as primary decomposers, and can be easly cultivated without high production technology. Mushrooms also characterized by their abilities to grow well under natural conditions on dead woody branches of trees and tree stumps.

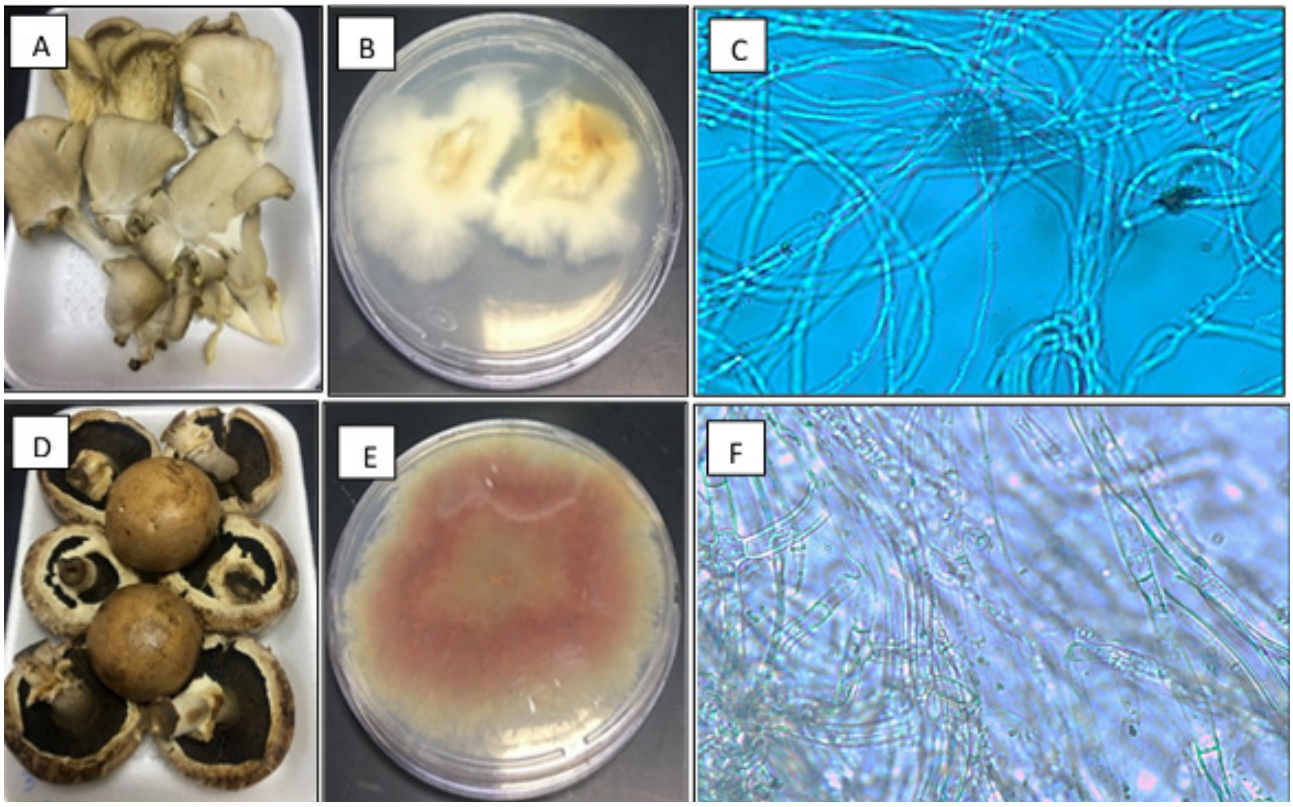

Figure 1 Tissue culture isolation and microscopic examination (40 × magnification) of mushroom mycelium, *P. ostreatus* A, B and C.and mushroom *A. bisporus* var D, E and F

3.2 Chemical Properties of Ligno-Cellulosic Natural Resources (Substrates)

Some chemical properties of tested ligno-cellulosic materials, including protein, organic matter, organic carbon and ash contents are given in (Table 1). Analysis of the macro-elements (Nitrogen, Phosphorus, Potassium, Calcium and Magnesium), micro-elements (Zinc, Copper, Manganese) of 3 replications were calculated for each of the substrates (Table 2). Results indicated that, the highest organic and carbon contents were found in DTs, followed by DGs, and DPFs, in that order. In addition, the ash and protein contents of the substrate DTs was lower than

Table 1 Analysis of different Ligno-cellulosic natural materials

Ligno-cellulosic materials*	Organic carbon (OC) (%)	Organic matter (OM) (%)	Ash (%)	Total protein (%)
DPFs	51.90	89.30	20.80	3.06
DGs	52.70	90.60	90.40	3.69
DTs	87.80	99.50	0.50	1.31

* Date palm fronds (*Phoenix dactelifera* L.) (DPFs), desert grasses of species (*Stipa capensis*) (DGs), small desert trees of species (*Haloxylon persicum*) (DTs); ** the values for all analysis are an average of three replicates

Table 2 Mineral analysis for different Ligno-cellulosic natural materials

Lignocellulosic materials*	Macroelements (%)					Microelements (ppm)		
	Phosphorus (P)	Nitrogen (N)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Copper (Cu)	Manganese (Mn)	Zinc (Zn)
DPFs	ND	0.49	0.052	1.07	0.42	7.30	96.50	12.10
DGs	0.068	0.59	1.88	2.03	0.31	49.30	194.80	0.90
DTs	0.018	0.21	0.023	0.41	0.09	51.35	246.80	5.80

* Date palm fronds (*Phoenix dactelifera* L.) (DPFs), desert grasses of species (*Stipa capensis*) (DGs), small desert trees of species (*Haloxylon persicum*) (DTs); ** the values for all analysis are an average of three replicates

those of the others. In earlier studies, Oak sawdust and Walnut shell applied as substrates showed 4.99% and 6.44% protein content, C (%) 41.86 and 43.17 respectively and N (%) 1.14, 1.47 respectively (Sözbir et al., 2015).

Mineral analysis of all materials showed the availability of macro and micro-elements with different values. The N, P, K, Ca contents of the DGs was more than those of the other substrates. Where the N, K, Ca, Mg of DTs was lower than DPFs and DGs. On the other hand, DPFs contained the highest Zn value of (12.10 ppm) and DTs showed the best content of Mn (246.80 ppm) and Cu (51.35 ppm). Ash content of DPFs (20.80%) is greater than other tested materials, the results were in agreement with Sherif et al. (2010).

3.3 Qualitative assay for Lignocellulolytic enzymes production

The pure isolated mycelium of Oyster mushroom (*Pleurotus ostreatus*) and Chestnut mushroom (*Agaricus bisporus* var. *avellaneus*) were tested for the production of ligninolytic enzymes using B&K medium containing 4 mM guaiacol (Abd El Aty et al., 2016; Alharbi et al., 2023).

Results in (Figure 2) revealed that, after incubating plates for 7 days at 28 °C in dark, Oyster mushroom has a good ability to grow and oxidize guaiacol with (50 mm) and (55 mm) diameter, respectively. Where Chestnut mushroom showed a reddish-brown zone of (40 mm) formed under and around a 17 mm colony diameter after 7 days' incubation. The showed results indicating the good activity of isolated mycelium able to produce lignin-degrading enzymes i.e.. laccase, manganese peroxidase, and lignin peroxidase.

3.4 Evaluation of Mushroom Lignocellulolytic Enzymes Production

Two isolated mushrooms were tested for their ability to produce the lignocellulolytic enzymes by degrading different substrates (DPFs), (DGs), (DTs) without any

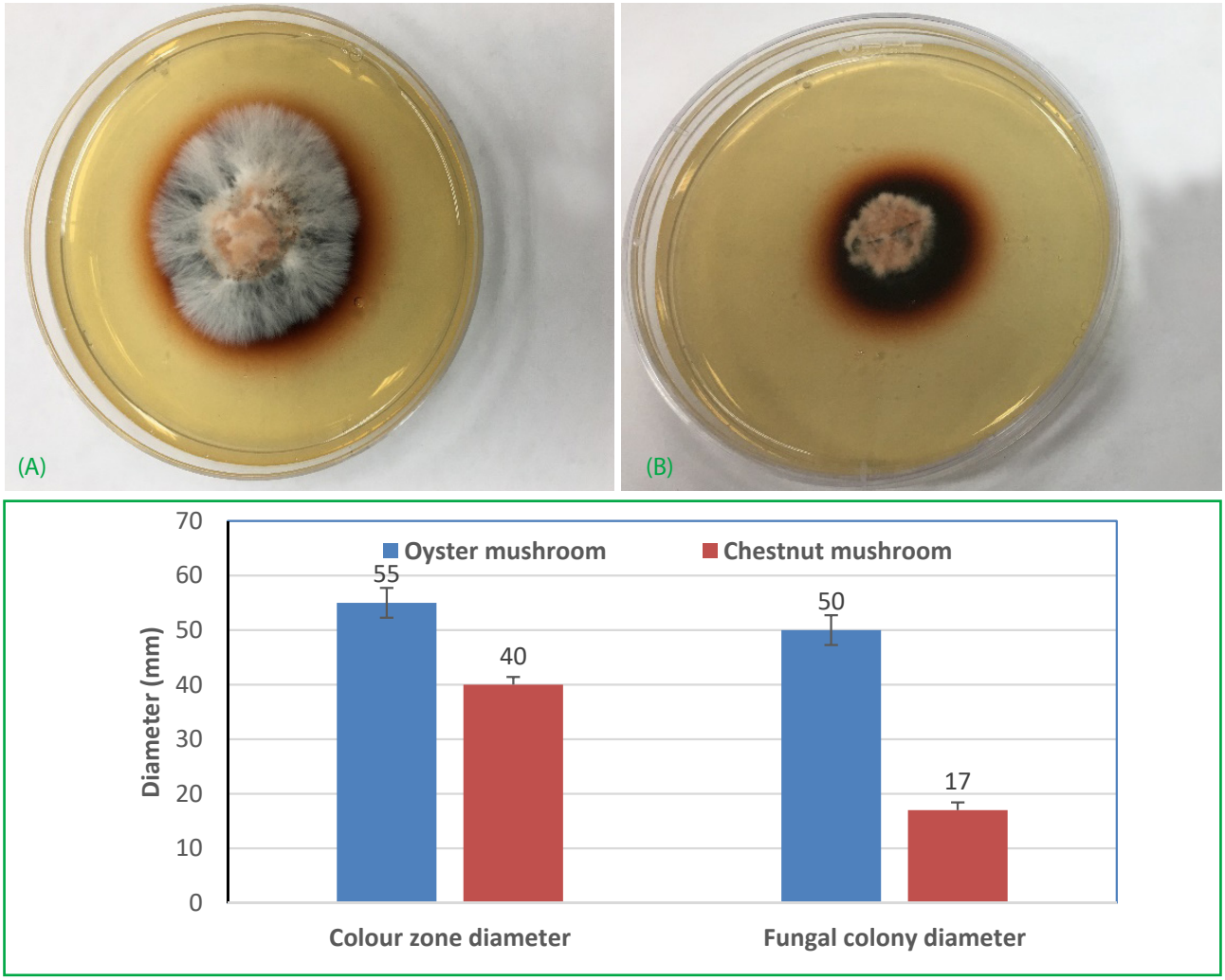

Figure 2 Mycelial growth and oxidation characteristics of Oyster mushroom (A) and Chestnut mushroom (B) on the 7th day of cultivation (the initial disc 10 mm diameter)

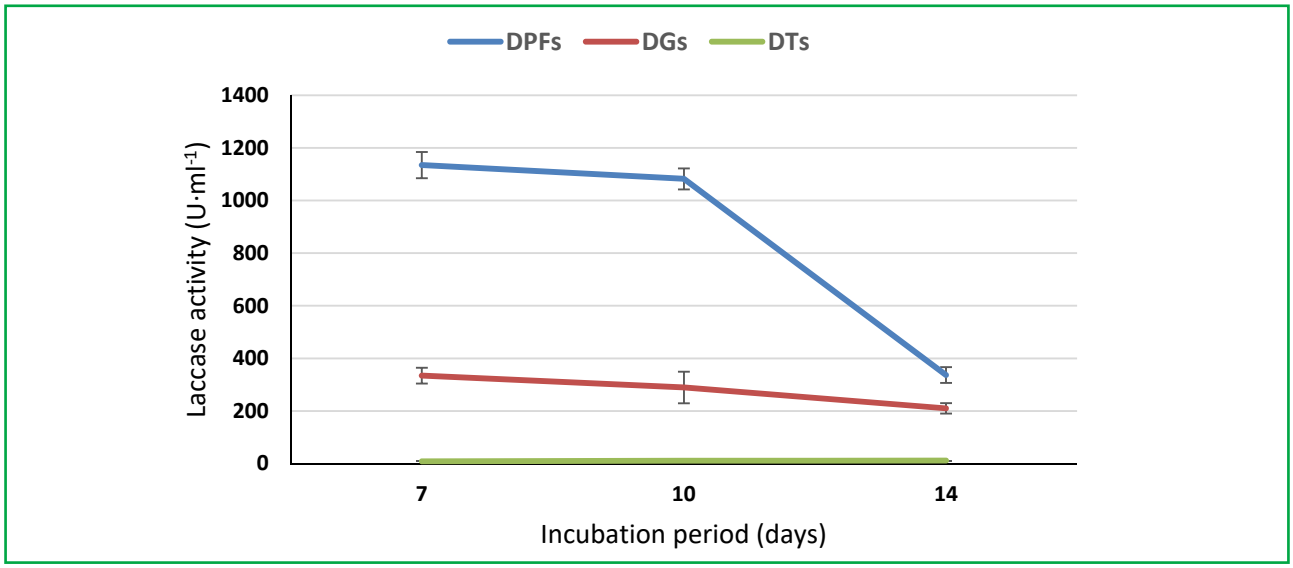

Figure 3 Evaluation of Oster mushroom Lignocellulolytic enzymes production

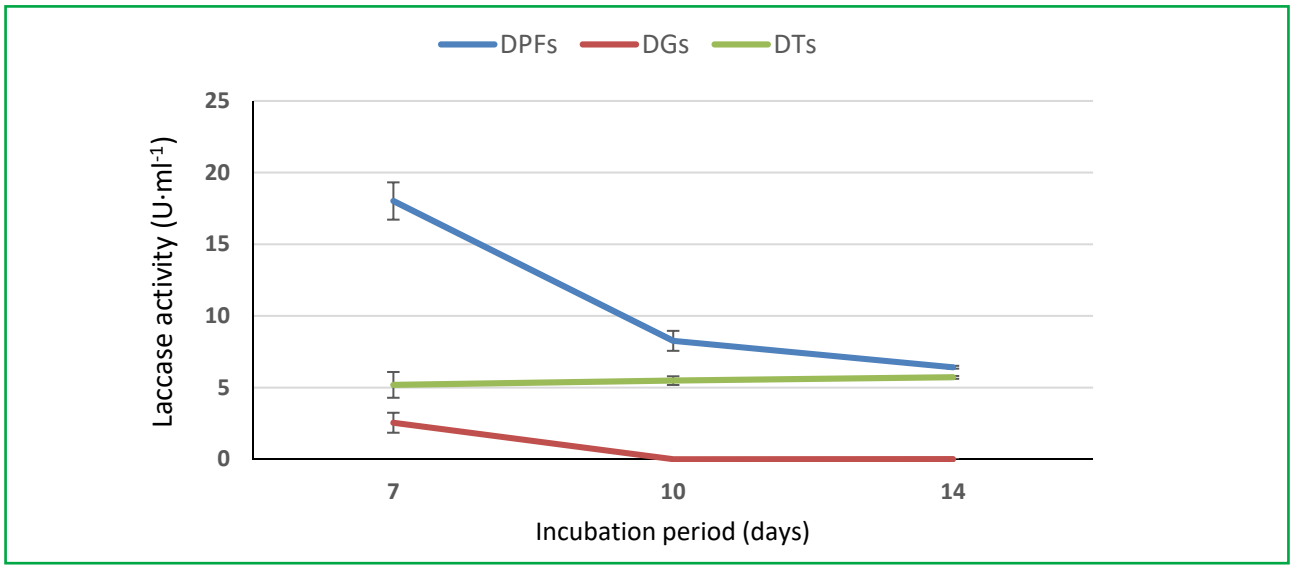

Figure 4 Evaluation of Chestnut mushroom Lignocellulolytic enzymes production

additives. Basidiomycetes fungi initiated degradation processes enzymatically to decompose lignin, cellulose, and hemicellulose (Janusz et al., 2017). Solid state fermentation (SSF) of three tested materials separately using Oyster mushroom indicated the ability to produce good activity of laccase enzyme over the incubation periods 7, 10 and 14 days. Maximum value of laccase enzyme (1,134.388 ±1.31 U·ml^{-1}) obtained using the lignocellulosic material DPFs, followed by DGs (334.4708 ±0.92 U·ml^{-1}) and DTs showed the lowest activity (11.33948 ±0.00 U·ml^{-1}) (Figure 3). Previous studies agree with our obtained results, several species of mushroom were able to decompose lignocellulysic materials producing more than one laccase isoenzyme such as, *Agaricus bisporus* (Bonnen et al., 1994; Othman et al., 2018), *Lentinula edodes* (Nagai et al., 2002), *Pleurotus ostreatus* (Piscitelli et al., 2005), and *Trametes versicolor* (Bertrand et al., 2014), and the produce laccases have different biochemical features.

The results also emphasized that, Chestnut mushroom was able to grow and produce laccase enzyme using three different wastes with varying degrees of efficiency, after 7 days' incubation the highest activity of (18.02011 ±0.75 U·ml^{-1}) obtained using DPFs substrate, followed by DTs (5.713693 ±0.73 U·ml^{-1}) and low activity of (2.549186 ±0.91 U·ml^{-1}) obtained with DGs substrate (Figure 4). On the other hand, both mushrooms possessed very low activities of cellulase and xylanase enzymes after incubation for 14 days using three tested substrates. Studies of Jaturong et al. (2020) indicated

that the variability of waste composition and mushroom species are influential in enzyme production. In this context, our results showed that, the composition of three substrates were more reliable for Oyster mushroom than Chestnut mushroom, that's caused variation in the enzymes production.

3.5 Application of Plastic Bags Fermentation

Mushrooms considered as one of the most important players in lignocellulose degradation depending on the production of both hydrolytic and oxidative enzymes. Hydrolytic enzymes (cellulases and hemicellulases) are responsible for degradation of polysaccharide, while oxidative enzymes (laccases) are responsible for lignin degradation and modification (Jaturong et al., 2020). Small scale fermentation of three ligno-cellulosic substrates using plastic bags was evaluated to confirm the suitability for mushroom cultivation and low cost enzymes production. Morphological results showed that After 30 days' incubation the substrates covered with heavy Oyster mushroom mycelium, and a medium growth was shown in the bags inoculated with Chestnut mushroom.

Results of Lignocellulolytic enzymes assay in (Table 3) indicated the ability of both mushrooms to survive and produce appreciate enzymes activity after long period of incubation. The maximum activity of laccase (182.05 U·ml^{-1}), xylanase (0.54 U·ml^{-1}) and cellulose (0.31 U·ml^{-1}) was obtained with Oyster mushroom using the ligno-cellulosic DPFs substrate. Also good activity of three enzymes (140.38 U·ml^{-1}) (0.34 U·ml^{-1}) (0.24 U·ml^{-1}), respectively was observed using DGs substrate and medium activity with DTs substrate.

The fungal fermentation of three Ligno-cellulosic materials using Chestnut mushroom showed the ability to produce all enzymes laccase, xylanase and cellulose with activity of 59.23, 0.58, 0.19 U·ml^{-1}, respectively using DPFs substrate. Only two enzymes produced by fungal fermentation of DGs, laccase enzyme (21.54 U·ml^{-1}) and Xylanase activity (0.75 U·ml^{-1}). But only laccase activity given by fermentation of DTs with absence of other enzymes.

Finally, from observed results the Oyster mushroom showed better production of lignocellulolytic enzymes than Chestnut mushroom. On the other hand, Elisashvili et al. (2003) showed that solid state fermentation of the agro-industrial waste tree leaves (*Fagus sylvatica*) using *Pleurotus ostreatus* produced 6.3–8.0 U·l^{-1} laccase activity, 160–1,400 U·ml^{-1} total xylanase, 14–15 U·ml^{-1} total cellulose.

3.6 SEM of the Ligno-Cellulosic Natural Substrates

Morphological characterization of ligno-cellulosic materials surfaces (DPFs, DGs and DTs) before and after the mushroom fermentation was analyzed to show the effect of fungal growth and confirm their ability to colonize these materials as nutrient sources individually. (Figs 5, 6 & 7a, b) represented the SEM micrographs for unfermented materials DPFs, DGs and DTs respectively, without fungal cultivation (control). The SEM micrographs revealed that, the unfermented substrates showed clean surfaces without any fungal hyphal mycelium and no changes occurred in their normal surface form.

The surface of the three substrates cultivated with Oster mushroom (Figs 5, 6, & 7c, d) seems rough with many cracks and heavy growth of hyphal mycelium spread over and inside three different materials.

Moreover, the investigation reflecting successful growth of Chestnut mushroom inside and outside tested substrates (Figs 5, 6, & 7e, f) with density and effects lower than Oster mushroom. Low desity of Chestnut mycelium was agreed with obtained results of enzymes assay, which showed low production of lignocellulolytic enzymes.

Table 3 Small scale fermentation using plastic bags

Mushrooms	Ligno-cellulosic substrates	Enzymes activity (U·ml^{-1})		
		Laccase	Xylanase	Cellulase
Oster mushroom	DPFs	182.05	0.54	0.31
	DGs	140.38	0.34	0.24
	DTs	41.75	0.39	0.22
Chestnut mushroom	DPFs	59.23	0.58	0.19
	DGs	21.54	0.75	0
	DTs	43.51	0	0

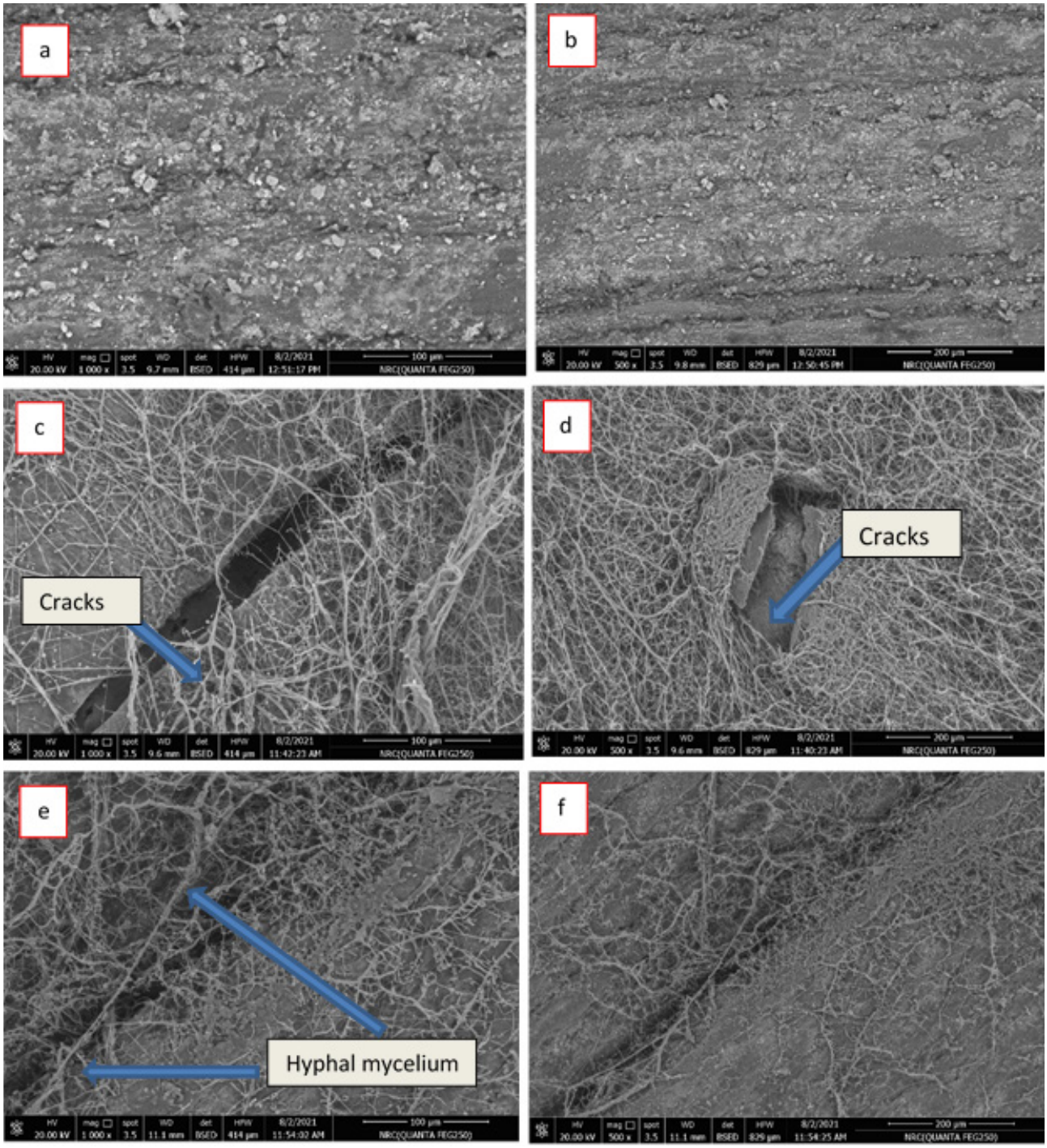

Figure 5 SEM micrographs of DPFs at (100 and 200 µm × magnification), without fungal cultivation (control) a, b, the plant material cultivated with Oyster mushroom c, d and cultivated with Chestnut mushroom e, f for 30 days

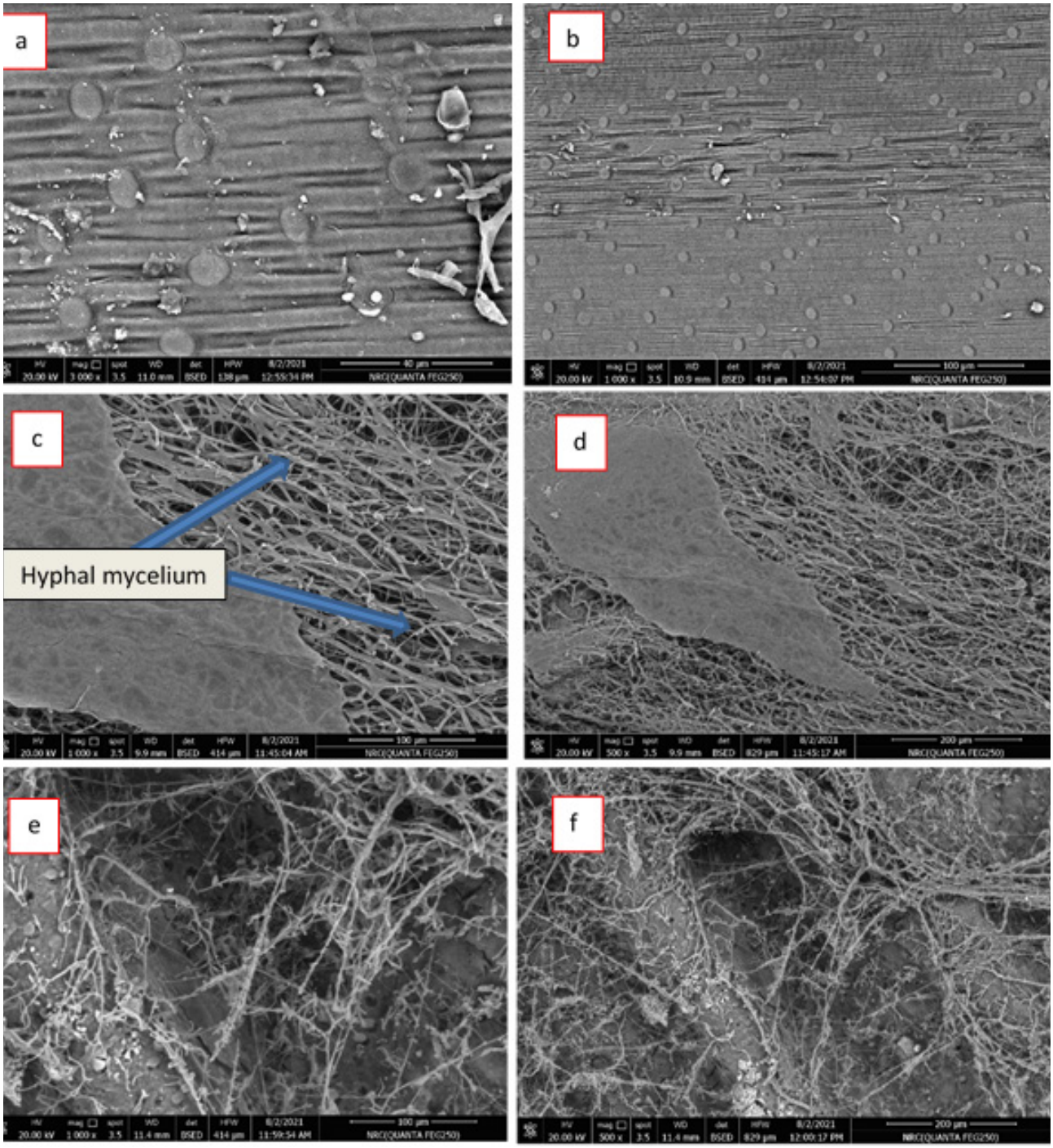

Figure 6 SEM micrographs of DGs at (100 and 200 µm × magnification), without fungal cultivation (control) a, b, the plant material cultivated with Oyster mushroom c, d and cultivated with Chestnut mushroom e, f for 30 days

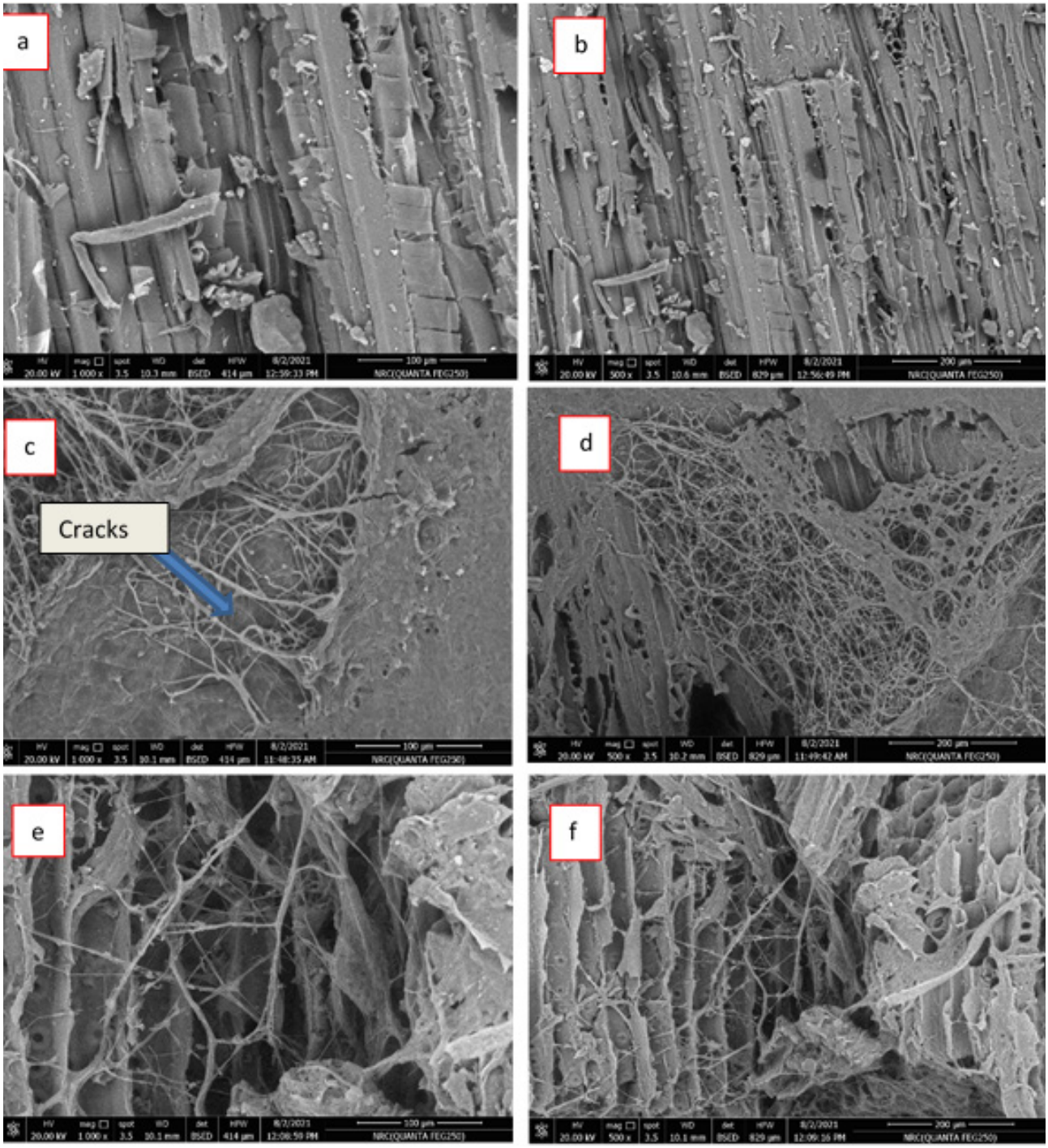

Figure 7 SEM micrographs of DTs at (100 and 200 µm × magnification), without fungal cultivation (control) a, b, the plant material cultivated with Oyster mushroom c, d and cultivated with Chestnut mushroom e, f for 30 days

4 Conclusions

In this work, a new study was conducted on natural flora collected from Hafr Al-Batin Eastern Region, Saudi Arabia. Three lingo-cellulosic natural resources were characterized and evaluated for new approach for cultivation of edible mushrooms and production of industrially important lignocellulolytic enzymes. In the latter context, the chemical properties of Date palm fronds (*Phoenix dactelifera* L.) (DPFs), desert grasses (*Stipa capensis*) (DGs), small desert trees (*Haloxylon persicum*) (DTs) were tested for protein, organic matter, organic carbon and ash contents as well as the macro-elements and micro-elements evaluated. The three substrates inoculated with mycelium of two different mushrooms after isolation and qualitative detection of lignin degrading enzymes production. Fermentation experiments indicated the suitability of different substrates for fungal growth and production of lignocellulolytic enzymes including laccase, cellulase, xylanase with different activities at various incubation periods. Out of tested enzymes laccase showed the maximum production 1,134.388 and 182.05 $U \cdot ml^{-1}$ by fungal fermentation of DPFs substrate using Oster mushroom. Microscopical investigations of fermented substrates including SEM, confirmed the presence of cracks and heavy mycelium growth on the surface and in between materials obey the following order DPFs >DGs >DTs. Finally, our new study on Hafr Al-Batin ligno-cellulosic natural resources, exhibited their good chemical properties that can be used as nutrient substrates for mushrooms growth. In addition, production of valuable lignocellulolytic enzymes which can be used in various applications, including the textile industry, biofuel production, the food and drink industry, pharmaceutical industry, and waste treatment.

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