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Study for the enzymatic activity of some fungi isolated from agricultural soil

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Abstract:

Thirty-three fungal species were isolated from sixteen soil samples collected from several agricultural areas in Dhi-Qar governorate and tested for their enzymatic activity to five enzymes (cellulase, laccase, lipase, lignase and amylase). This study showed a good activity for the isolated fungi and all studied fungi showed enzymatic activity, 27 fungi were showed a good activity to produce lignase enzyme, 25 fungi were able to produced lipase, 18 fungi produced cellulase, 17 fungi produced amylase and five fungi were able to produce laccase enzyme.

Key words: Fungi, Agriculture soil, Enzymatic activity

1-Introduction

The enzymes secreted by fungi play an important role in the decomposition process of organic substances. There are different types of fungal enzymes involved in this process such as cellulase, laccase and lignease, etc. The degradation of all organic and agricultural substances depends on the presence of these enzymes (Rabinovich *et al*., 2004). Different microbes participate in the decomposition process of cellulose in the environment in which fungi play an important role in this process. Cellulase enzymes are a group of aqueous enzymes capable of decomposing cellulose into simple sugar constituents such as glucose (Chellapandi and Himanshu, 2008; Khalid *et al*., 2006).

The lignin is a large molecule and has difficult decomposition properties. Lignin content in wood ranges from 18 to 30% and it is highly complex due to its high molecular weight (Abd-Elsalam and El-Hanafy, 2009; Saritha and Arora, 2012).

Although this compound is difficult to decompose, some fungi especially white rot fungi, where able to secrete a group of

enzymes that have the ability to analyze this compound using ligninase and manganese peroxidase and laccases (Lopez *et al*., 2007; Liers *et al*., 2011). In addition, amylase enzyme plays an important role in the digestion of starch and glycogen, which are very important components found in microorganisms, plants and higher organisms (Sales *et al*., 2012). This enzyme which secreted by some microorganisms is very important for use in many industrial processes such as food processing, fermentation, textiles, paper and pharmaceutical industries and others (Rajagopalan and Krishnan, 2008; Souza and Magalhães, 2010).

Materials and methods:

Fungal isolation

Sixteen soil samples were taken from different agricultural areas in Dhi-Qar governorate. Fungi were isolated from soil samples by using dilution method (Wicklow and Wittingham, 1974), using two different types of media Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). The preparation of the isolation media where done according to the direction of the manufacturing company (Hi media).The cultures where incubated at 25 °c for one month. The isolated fungi identified according to the following sources

Raper and Fennell (1973), de Hoog and Guarro (1995), Watanabe (2002), Guarro *et al*. (2012).

Study the enzymatic activity by isolated fungi:-

Cellulase enzyme

The media used contained 7.0g KH_2PO_4, 2.0g K_2HPO_4, 0.1g $MgSO_4.7H_20$, 1.0g $(NH_4)_2SO_4$, 0.6g yeast extract, 10g carboxy methyl cellulose and 15g agar per liter (Ireri *et al*., 2015). After 3-5days of fungal colony growth, the plates were flooded with 0.2% aqueous Congo red solution and distained with 1M NaCl for 15minutes. Appearance of yellow areas around the fungal colony in an otherwise red medium indicated cellulase activity

Lipase enzyme

For lipase activity, the fungi were grown on Peptone Agar medium (10g peptone, 5g NaCl, 0.1g $CaCl.2H_2O$, 16g agar, 1L distilled water; pH6.0) supplemented with 1% Tween 20 separately sterilized and added to the medium. At the end of the incubation period, a visible precipitate around the colony due to the formation of calcium salts of the lauric acid liberated by the enzyme indicated positive lipase activity (Sunitha *et al*., 2013).

Laccase enzyme

Glucose Yeast Extract Peptone Agar medium with 0.05g 1-napthol L 1, pH 6.0 was used. As the fungus grows, the colorless medium turns blue due to oxidation of 1-napthol by laccase enzyme (Sunitha*et al*., 2013).

Lignase enzyme

Culture was inoculated onto tannic acid agar plates containing 0.2% tannic acid and incubated at 25C°. Growth was followed for a period of 2 weeks. Positive reaction is indicated by the formation of a yellow to light brown zone around the colony (Sharma *et al*., 2017).

Amylase enzyme

The ability to degrade starch was used as the criterion for determination of ability to produce amylase enzymes. The medium used contained malt extract plus 0.2% soluble starch, pH9. After 3-5 days of incubation, the plates were flooded with an iodine solution and a yellow zone around a colony in an otherwise blue medium indicated amylase activity (Ireri *et al*., 2015).

Statistical analysis

The ANOVA analysis was used by applying Minitab ver.16 to analyze the results statistically. The mean was tested using the least significant difference RLSD test under the probability level 0.05.

Results and discussion

Fungal isolation

Thirty three fungal isolates where isolated from 16 agricultural soil sample. 76.19 percentage of the isolated fungi where belonging to the anamorphic fungi with 26 species, followed by the ascomycetes fungi with 19.04 percentage of appearance and finally the zygomycetes fungi with only 4.76 percentage of appearance.

The appearance of the anamorphic fungi in high percentage may be due to the ability of these fungi to produce large amount of reproductive units and the secretion of different enzymes also the possess of a great ability to tolerate the stress in the environment, all of these features and others made them one of the large groups of fungi in the environment (Serna-Chavez *et al*.,2013).

Enzymatic activity

The enzymatic activity of 33 fungal isolates where done to study their ability to produce (cellulase, lignase, laccase, amylase and lipase) as clear variation between the different fungal species was observed in their enzymatic abilities.

It was observed that all fungi were able to show a variety of enzymatic capabilities, but in varying degrees and also varying number of enzymes that each fungus was able to secrete, and this may be due to the inherent enzymatic activity of each fungus, it is well known that each microorganism possesses enzymatic capacity which differentiate it from the other microorganisms.(Sunitha *et al*., 2013; Patil *et al*., 2015).

A. flavus, *E. nidulans* and *Botryotrichum* sp. where showed the ability to secrete all the studied enzymes, the ability of the rest fungi

ranged from their ability to secrete one to four enzymes.

The results showed that most of the studied fungi were able to produce the lignaes enzyme in which 27 fungal species where able to produce this enzyme with different capabilities of secretion rates from low as in *Alternaria* sp. to high secretion as in *Penicllium* sp. Table1, Fig.1, The statistical analysis for the results showed significant differences ($P < 0.05$) between the tested fungi in their ability to secrete the ligninase enzyme. Lignin is an essential component in the formation of vascular plant cells and fungi play an important role in the degradation of this compound in the environment, and the secretion of this enzyme from the fungi in this study indicate that these fungi play and important role in the degradation of these material and possess great ability to explore it as a source of carbon and energy (Saini *et al*., 2015).

Lipase enzyme come in the second place with 25 fungal species that able to produce it Table 2, Fig. 2. In general, 11 fungal species showed ability to secrete enzyme in very high rates while remaining species ranged from low to high yield. The results of the statistical analysis showed significant differences ($P < 0.05$) between the tested fungi in their ability to produce lipase enzyme. The high susceptibility of fungi to secrete this enzyme may be due to the fact that lipase is a fatty substance found in grains and agricultural materials and can be used by fungi easily as a source of food for growth, which contributed to the increase in the number of fungi that were able to produce this enzyme, soils may also contain fatty substances in the organic content of the soil, and microorganisms including fungi, have an important role in the degradation of fatty substances through the secretion of extracellular lipase enzyme, and many previous studies have indicated the fungal ability to produce lipase (Fadıloğlu and Erkmen,1999;Kow*et al*., 2005).

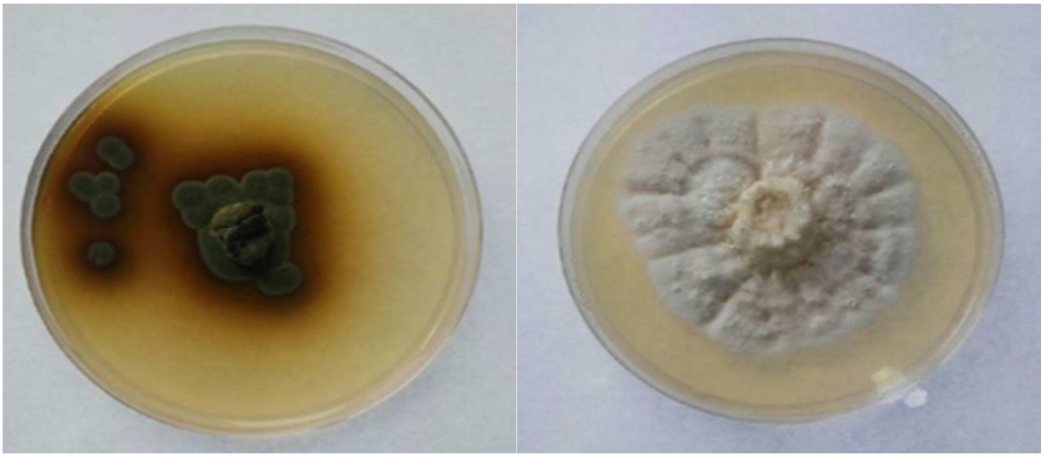

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Fig. 1:Enzymatic activity of lignase enzyme

Table 1: Fungal activity for lignase enzyme

No.	Fungal species	Lignase enzyme activity
1	*Penicillium* sp.2[a]	+++
2	*A. niger*[ab]	+++
3	*Cladosporium herbarum*[ab]	+++
4	*Exserohilum* sp.[abc]	+++
5	*Penicillium* sp.1[bce]	+++
6	*E. nidulans*[cef]	+++
7	*Emericella dentata*[efg]	++
8	*A. flavus*[efgh]	++
9	*Thielavia* sp.[efgh]	++
10	*Botryotrichum* sp.[efghi]	++
11	*Cladosporium* sp.[efghi]	++
12	*Humicola grisea*[efghi]	++
13	*Acremonium* sp.[efghij]	+
14	*Drechslera* sp.[fghijk]	+
15	*Trichoderma* sp.[fghijk]	+
16	*Myrothecium* sp.[fghijk]	+
17	*Gilmaniella* sp. [fghijk]	+
18	*Drechslera tritici*[fghijk]	+
19	*Chaetomium* sp.[fghijk]	+
20	*Fusarium* sp.[fghijk]	+
21	*Stachybotrys* sp.[ghijk]	±
22	*A. terreus*[hijk]	±
23	*A. fumigatus*[ijk]	±
24	*E.undulata*[ijk]	±
25	*Aspergillus candidus*[ijk]	±
26	*Stachybotrys atra*[ijk]	±
27	*Alternaria* sp.[jk]	±
28	*A. versicolor*[k]	–
29	*A. wentii*[k]	–
30	*Eurotium* sp.[k]	–
31	*Mucor* sp.[k]	–
32	*Phialophora* sp. [k]	–
33	*Scopulariopsis* sp. [k]	–
RLSD= 5.01		

- no secretion ♦ ± weakly secretion (1-3)mm.
+ Medium secretion (3-5)mm. ♦ ++ good secretion (5-8) mm.
+++ Strong secretion (8-11) mm.

Table 2:Fungal activity for lipase enzyme

No.	Fungal species	Lipase enzyme Activity
1	*Cladosporium herbarum*[a]	+++
2	*Cladosporium* sp.[b]	+++
3	*Emericella dentata*[bc]	+++
4	*Drechslera tritici*[bcd]	+++
5	*Acremonium* sp.[bcde]	+++
6	*A. niger*[bcde]	+++
7	*Thielavia* sp.[bcdef]	+++
8	*A. versicolor*[bcdefg]	+++
9	*Eurotium* sp.[cdefgh]	+++
10	*Myrothecium* sp.[defgh]	+++
11	*Stachybotrys atra*[defgh]	+++
12	*E. nidulans*[defgh]	++
13	*Botryotrichum* sp.[efgh]	++
14	*A. wentii*[efghi]	++
15	*Drechslera* sp.[efghi]	++
16	*Stachybotrys* sp.[efghi]	++
17	*Exserohilum* sp.[efghij]	++
18	*A. terreus*[efghij]	++
19	*A. flavus*[fghijk]	+
20	*Penicillium* sp.1[ghijk]	+
21	*Scopulariopsis* sp.[ghijk]	+
22	*Aspergillus candidus*[hijk]	+
23	*A. fumigatus*[hijk]	+
24	*Mucor* sp.[ijk]	±
25	*Alternaria* sp.[jk]	±
26	*Chaetomium*sp.[k]	–
27	*E. undulata*[k]	–
28	*Fusarium* sp.[k]	–
29	*Gilmaniella* sp.[k]	–
30	*Humicola grisea*[k]	–
31	*Penicillium* sp.2[k]	–
32	*Phialophora* sp.[k]	–
33	*Trichoderma* sp.[k]	–
RLSD= 5.28		

– no secretion ♦ ± weakly secretion (1-3)mm.
+ Medium secretion (3-5) mm. ♦ ++ good secretion (5-8) mm.
+++ Strong secretion (8-11) mm.

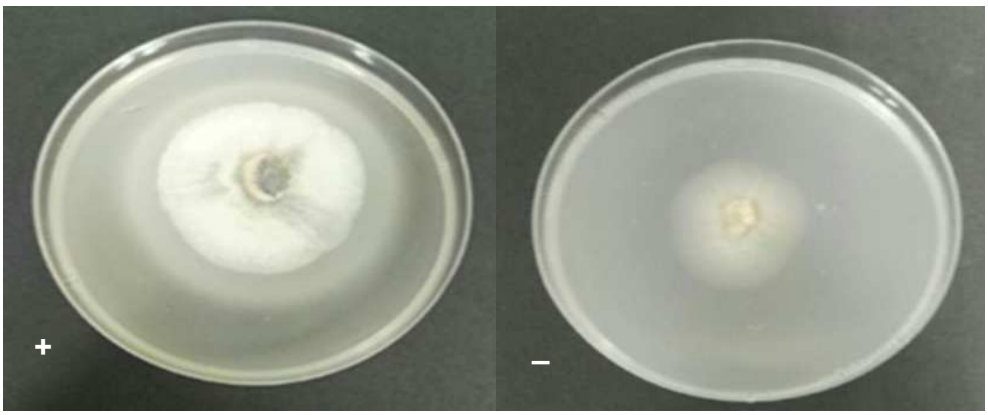

Fig. 2: Enzymatic activity of lipase enzyme

The cellulase enzyme come in the third place with 18 fungal species which able to produce it, Table 3 Fig. 3.The results of statistical analysis showed significant differences ($P<0.05$) between the tested fungi in their ability to produce cellulase enzyme. It is evident that most fungi decomposing plant parts have the ability to secrete cellulase enzymes. This is consistent with Khalid *et al*. (2006) how found that 42 fungal isolate were able to grow on the cellulose-containing medium, and most species have been effective in cellulose analysis. Similar results were obtained in previous studies such as Abdel-Raheem and Shearer (2002) and Bucher *et al*. (2004), which came to the same conclusion as the current study.

17 tested fungi showed the ability to analyze starch and secretion of amylase enzyme,Table 4 Fig. 4, *Botryotrichum* and *Cladosporium* sp. showed the highest efficacy in amylase production, followed by some other fungal species, including *A. flavus* and *A. fumigatus*. The results of statistical analysis showed significant differences ($P <0.05$) between the tested fungi in their ability to secrete amylase enzyme. Many micro-organisms secrete amylase and analyze starch this is may be due to its abundant in plant residues, such as wheat and corn in addition, it is easy to break down and to use as simple source for energy (Omacini *et al*.,2001).

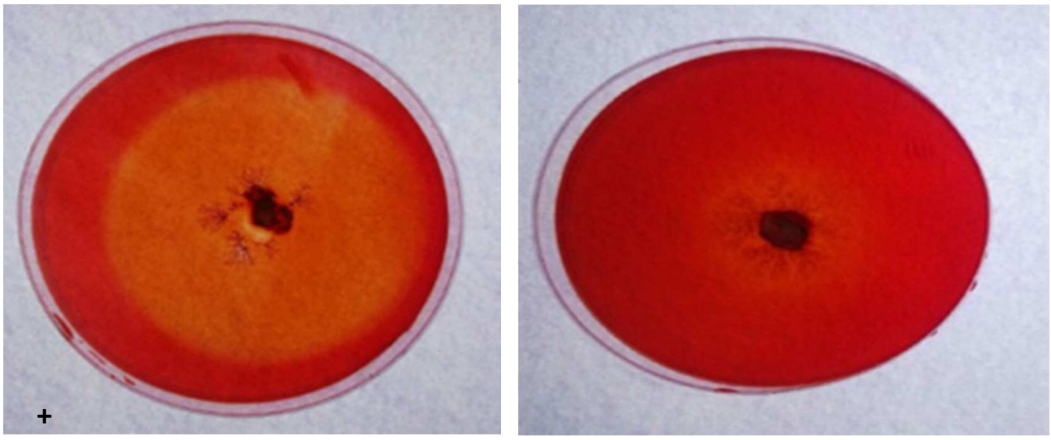

Fig. 3: Enzymatic activity of cellulase enzyme

Table 3: Fungal activity for cellulase enzyme

No	Fungal species	Cellulase enzyme Activity
1	*Cladosporium* sp.[a]	+++
2	*Cladosporium herbarum*[b]	+++
3	*Thielavia* sp.[c]	+++
4	*Penicillium* sp.2[cd]	++
5	*Eurotium* sp.[cde]	++
6	*Penicillium* sp.1[def]	++
7	*Stachybotrys* sp.[defg]	++
8	*Botryotrichum* sp[.defg]	++
9	*Phialophora* sp.[efgh]	++
10	*Alternaria* sp.[fgh]	++
11	*Gilmaniella* sp. [ghi]	+
12	*A. flavus* [hi]	+
13	*E. nidulans*[hij]	+
14	*Aspergillus candidus*[ijk]	±
15	*Scopulariopsis* sp.[jkl]	±
16	*Drechslera tritici*[kl]	±
17	*Stachybotrys atra*[kl]	±
18	*Acremonium* sp.[kl]	±
19	*A. fumigatus*[L]	–
20	*A. niger*[L]	–
21	*A. terreus* [L]	–
22	*A. versicolor* [L]	–
23	*A. wentii*[L]	–
24	*Chaetomium* sp.[L]	–
25	*Drechslera* sp.[L]	–
26	*Emericella dentata*[L]	–
27	*E. undulata*[L]	–
28	*Exserohilum* sp.[L]	–
29	*Fusarium* sp.[L]	–
30	*Humicola grisea*[L]	–
31	*Mucor* sp.[L]	–
32	*Myrothecium* sp.[L]	–
33	*Trichoderma* sp.[L]	–
RLSD= 2.37		

– no secretion ♦ ± weakly secretion (1-3)mm.
+ medium secretion (3-5)mm. ♦ ++ good secretion (5-8) mm.
+++ strong secretion (8-11)mm.

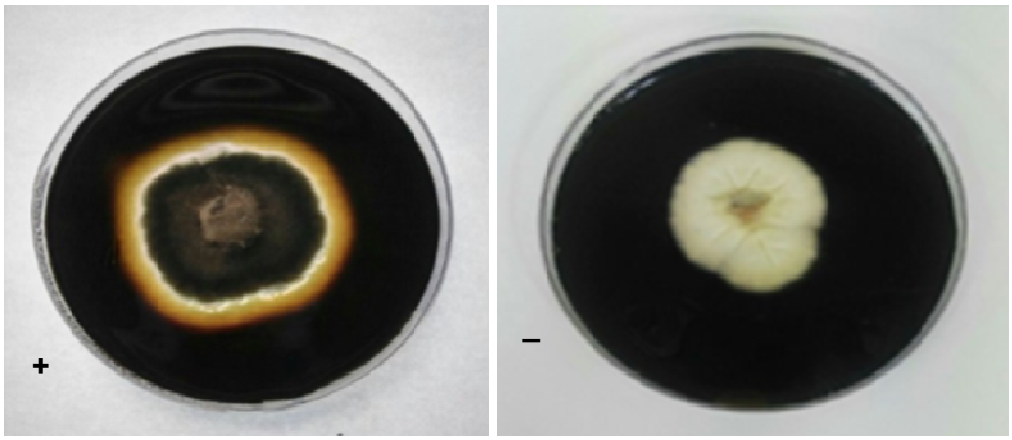

Fig.4: Enzymatic activity of amylase enzyme

Table 4: Fungal activity for amylase enzyme

No.	Fungal species	Amylase enzyme Activity
1	*Botryotrichum* sp.[a]	++
2	*Cladosporium* sp.[a]	++
3	*Drechslera tritici*[a]	+
4	*Exserohilum* sp.[b]	+
5	*A. flavues*[b]	+
6	*Cladosporium herbarum*[b]	+
7	*A. fumigatus*[c]	+
8	*Drechslera* sp.[c]	+
9	*E. nidulans*[cd]	±
10	*Stachybotrys* sp.[cde]	±
11	*Eurotium* sp.[de]	±
12	*Gilmaniella sp.*[de]	±
13	*Penicillium* sp.1 [de]	±
14	*A. niger*[ef]	±
15	*Emericella dentata*[ef]	±
16	*E. undulata*[ef]	±
17	*Humicola grisea*[ef]	±
18	*Acremonium* sp.[f]	–
19	*Alternaria* sp.[f]	–
20	*Aspergillus candidus*[f]	–
21	*A. terreus*[f]	–
22	*A. versicolor*[f]	–
23	*A. wentii*[f]	–
24	*Chaetomium* sp.[f]	–
25	*Fusarium* sp.[f]	–
26	*Mucor* sp.[f]	–
27	*Myrothecium* sp.[f]	–
28	*Penicillium* sp.2 [f]	–
29	*Phialophora* sp.[f]	–
30	*Scopulariopsis* sp. [f]	–
31	*Stachybotrys atra*[f]	–
32	*Trichoderma* sp.[f]	–
33	*Thielavia* sp.[f]	–
RLSD= 1.33		

-- not secretion ♦ ± weakly secretion (1-3)mm.
+ Medium secretion (3-5) mm. ♦ ++ good secretion (5-8) mm.
+++ Strong secretion (8-11) mm.

The laccase enzyme come in the end stage with only five fungi were able to produce it, *E.nidulans* was the best in its secretion for this enzyme, while the other four fungi produced it in very small or medium quantities Table 5, Fig. 5. The results of the statistical analysis showed significant differences ($P < 0.05$) between the tested fungi in their ability to produce laccase enzyme. The laccase enzyme plays a key role in the degradation of pollutants in the environment due to the activity of free radicals during the oxidation of aromatic compounds, phenolic compounds and amines, and this enzyme is used in biotechnology applications as a biocatalyst and must of the fungal secretion of this enzyme was by Basidomycota (Brijwani*et al*., 2010 ; Pozdnyakova *et al.,* 2011).

In the recent years some studies showed that some anamorphic fungi could secret this enzyme and this is may be due to the mutations

as the result from the nature of the environment in which the fungi live (Panuthai *et al*., 2012).This is consistent with the findings of Pragathi *et al*. (2013) how found that very few fungi can secrete this enzyme in his study.

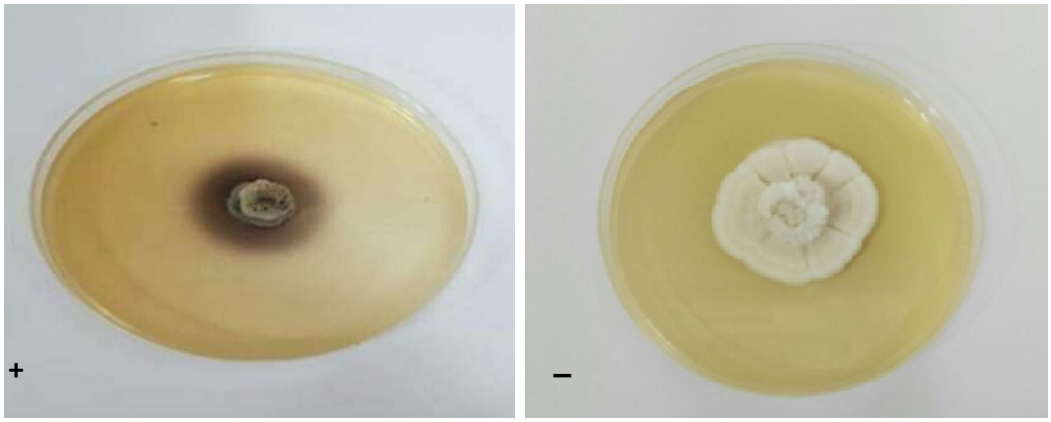

Fig.5: Enzymatic activity of laccase enzyme

Table 5: Fungal activity for laccase enzyme

No.	Fungal species	Lacase enzyme Activity
1	*E. nidulans*[a]	+++
2	*Humicola grisea*[b]	++
3	*A. terreus*[c]	+
4	*Botryotrichum* sp.[d]	±
5	*A. flavus*[d]	±
6	*Acremonium* sp.[d]	–
7	*Alternaria* sp.[d]	–
8	*Aspergillus candidus*[d]	–
9	*A. fumigatus*[d]	–
10	*A. niger*[d]	–
11	*A. versicolor*[d]	–
12	*A. wentii*[d]	–
13	*Chaetomium* sp.[d]	–
14	*Cladosporium herbarum*[d]	–
15	*Cladosporium* sp.[d]	–
16	*Drechslera tritici*[d]	–
17	*Drechslera* sp.[d]	–
18	*Emericella dentata*[d]	–
19	*E. undulata*[d]	–
20	*Eurotium* sp.[d]	–
21	*Exserohilum* sp.[d]	–
22	*Fusarium* sp.[d]	–
23	*Gilmaniella* sp.[d]	–
24	*Mucor* sp.[d]	–
25	*Myrothecium* sp.[d]	–
26	*Penicillium* sp.1[d]	–
27	*Penicillium* sp.2[d]	–
28	*Phialophora* sp.[d]	–
29	*Scopulariopsis* sp.[d]	–
30	*Stachybotrys atra*[d]	–
31	*Stachybotrys* sp.[d]	–
32	*Trichoderma* sp.[d]	–
33	*Thielavia* sp.[d]	–
RLSD= 1.04		

– no secretion ♦ ± weakly secretion (1-3)mm. + medium secretion (3-5)mm. ♦ ++ good secretion (5-8) mm.

+++ strong secretion (8-11)mm.

Conclusion

During this study fungi isolated from all soil samples and all isolated fungi in showed a good enzymatic activity, this reflect the fungi play an important role in the degradation and recycling of different organic materials in the environment, most of the isolated fungi appear a good ability to secrete lignase enzyme, while laccase enzyme came in end with only five species able to secrete this enzyme.

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دراسة الفعالية الانزيمية لبعض الفطريات المعزولة من الترب الزراعية

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الخلاصة:-

تم عزل ثلاثة وثلاثين نوعًا فطريًا من ستة عشر عينة تربة جمعت من عدة مناطق الزراعية في محافظة ذي قار واختبار فعاليتها الإنزيمية لخمسة انواع من الانزيمات هي السيليليز، اللاكيز، اللاييز، اللجنيزوالاماليز. أظهرت هذه الدراسة نشاطا انزيمياً جيدًا للفطريات المعزولة، وأظهرت جميع الفطريات المدروسة نشاطًا إنزيميًا ولكن بنسب متفاوتة، حيث تمكن 27 نوعًا من الفطريات من انتاج إنزيم اللجنيز، 25 نوع من الفطريات تمكن من إنتاج إنزيم اللاييز ، 18 نوعًا من الفطريات تمكن من إنتاج انزيم السيليليز، 17 نوع من الفطريات تمكن من انتاج انزيم الأماليز و5 أنواع من الفطريات كانت قادرة على إنتاج انزيم اللاكيز.