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Biodegradation of Agricultural Plant Residues by Some Fungi Isolated From Yemen

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ABSTRACT

Forty eight samples of different plant residues (250g each) are collected from 9 different provinces in Yemen during 2008, which are corn, wheat, barley and cabbage residues. The samples are mycological analyzed on Czapek's agar medium at 28 ± 1° C.

There are 29 species and one variety belonging to 12 fungal genera are obtained from the samples, of which *Alternaria* (3 species), *Aspergillus* (5 species), *Penicillium* (6 species), *Mucor* (2 species) and one variety, *Fusarium* (5 species) contributed the broadest spectra of fungal species.

The ability of 58 fungal isolates representing 29 species and one variety related to 12 genera to produce extracellular plant cell wall degrading enzymes (cellulase, pectin lyase and polygalacturonase) on solid media is tested. Results reviled that most of the tested fungal isolates are able to produce cellulase, whereas a relatively few number of them are able to produce pectinases with variable capabilities.

A. fumigatus followed by *A. terreus* var. *aureus*, then *P. griseofulvum* are the active cellulase producers. Whereas *P. glabrum* is active producer of pectinlyase (PL), while *Acremonium strictum* and *P. chrysogenum* appeared to be good polygalacturonase (PG) producers.

Key words: Cellulase, pectin lyase (PL), polygalacturonase (PG) and biodegradation.

INTRODUCTION

Plant residues include the crop residues such as plant stalks, hulls, leaves and tree wastes processed through a wood chipper. The non-crop residues are also included, such as leaves, grass clippings, tree parts, shrubbery and garden wastes. These residues with their physical and chemical properties contribute to a considerable extent to the carbon cycling of the site, humus formation, soil structure and fertility, as well as, the nutrients and organic matter in soil (Kabli, 2007).

The ability of some microorganisms to metabolize lignin and hemicelluloses (Silva, *et al.,* 2005), beside the increasing energy demand has focused worldwide attention on the utilization of renewable resources, particularly vegetable residues, agricultural and agro-industrial wastes, such as sugarcane bagasse (Acuna-Arguelles, *et al.,* 1994), wheat bran (Singh, *et al.,* 1999), rice husk, rice peel, corn straw, corn cob, fruit peels, orange bagasse and other agricultural residues which have high organic matter content and their disposal arise both economical and environmental problems (de Freitas, *et al.,* 2006).

On the other hand, their major components as cellulose, starch, lignin, xylan, and pectin can be used by several microorganisms both as a source of energy for growth and as carbon source for synthesis of cell biomass and producing enzymes and other products with high commercial value (de Freitas, *et al.,* 2006 and Costa, *et al.,* 2007).

Little literatures are available on mycoflora of Yemeni plant residues. Hence, the present study is designed to determine the biodegradation and fungal content of different agricultural residues in this country. Cell wall degrading enzymes producing potential of the isolated fungi are also assessed.

MATERIALS AND METHODS

Samples collection:

Forty eight samples of plant residues are used in this study (250 g each). They are collected from different agricultural areas in Yemen during 2008. The studied plant residues are corn, wheat, barley and cabbage residues. Samples are separately kept inside clean plastic bags, transferred to the laboratory, and stored at room temperature until mycological analysis (Mahmoud, *et al*., 2011).

Isolation of fungi:

The dilution-plating technique of Johnson and Curl, (1972), is employed. 1 ml of the desired dilution is transferred aseptically into sterile Petri dishes with 15–20 ml of melted Czapek's agar medium supplemented with rose bengal 65 part per million (ppm) and chloramphenicol (250 mg/L).

The dishes are rotated by hand in a broad and slow swirling motion to disperse the plant residues suspension. Plates are incubated at 28 ± 1°C for 5 to 7 days. Three replicates are prepared for each sample. The resulting colonies are isolated, purified and identified according to their macro and microscopic characters depending on different scientific references in this field. Pure cultures of the identified fungi are transferred to potato-dextrose agar (PDA) slants which contained per liter: potato, 200g; dextrose, 20g; agar, 20g and 1000 ml of distilled water. These slants are kept for physiological studies.

Biodegradation by enzyme activities of the isolated fungi:

Organisms:

A total of 58 fungal isolates representing 29 species and one variety related to 12 genera recovered during this investigation are screened for their abilities to biodegrade by producing extracellular cell wall degrading enzymes on solid media. These isolates comprised 6 species of *Penicillium,* 5 species and one variety of *Aspergillus*, 5 species of *Fusarium*, 3 species of *Alternaria,* 2 species of each of *Mucor* and *Ulocladium,* and one species of all of the following fungi: *Acremonium, Cochliobolus, Curvularia, Drechslera, Eurotium,* and *Stachybotrys.*

Screening for cellulase production:

Cellulase production is tested as described by Eggins and Pugh, (1962). The medium contained (g/L): $(NH_4)_2SO_4$, 0.5g; L- asparagine, 0.5g; KH_2PO_4, 1.0g; KCl, 0.5g; $MgSO_4.7H_2O$, 0.2g; $CaCl_2$, 0.2g; Yeast extract, 0.5g; cellulose, 10g and agar, 20g. The pH was adjusted to 7 using acetate buffer.

After 7 days incubation at 28°C, plates are flooded with a solution of chloroiodide of zinc (1% of each of $ZnCl_2$ and iodine solution in equal portions). The uncolored zones around colonies indicated hydrolysis of cellulose by the releasing cellulase enzyme (exo-1, 4-β–glucanase). Percent of clear zone is calculated according to Bokhary and Parvez, (1994).

$$\text{Percent of clear zone} = \frac{\text{Diameter of clear zone} - \text{Diameter of colony}}{\text{Diameter of colony}} \times 100$$

Screening for pectinases production:

Isolates are screened on MP-7 and MP-5 media of Hankin, *et al.*, (1971), for pectin lyase (PL) and polygalacturonase (PG), respectively. After growth of organisms for 7 days at 28°C.

The plates are incubated in an inverted position, at 28 ± 1°C for 7 days after which the cultures are flooded with (1% w/v) of hexadecyltrimethyl ammonium bromide (citramide) for 10 minutes. This reagent precipitates intact pectin in the medium and, thus, clear zone around a colony in an otherwise opaque medium indicated degradation of the pectin.

RESULTS AND DISCUSSION

It is possible to isolate 29 species and one variety belonging to 12 fungal genera from the 48 different plant residues samples as shown in Fig. (1) and Table (1).

Penicillium, Aspergillus, Fusarium, Alternaria, and *Ulocladium* are the most common genera
isolated from different plant residues. *Penicillium* is represented by 6 species from which *P. funiculosum* is the most dominant species. *Aspergillus* is represented by 5 species, from which *A. flavipes* and *A. flavus* are the most common. *Fusarium* is represented by the same number of species among which *F. dimerum* and *F. longipes* are dominant. *Alternaria* is in turn represented by 3 species of which *Alternaria longipes* is the only species found in all types of plant residues. On the other hand, *Ulocladium alternariae* is the common species of *Ulocladium*.

Some fungal species are found in some plant residues samples while they are absent in the others. Examples: *Cochliobolus spicifer, Drechslera holmii* and *F. oxysporum* are recovered from corn residues samples. *Curvularia lunata* colonized wheat residues, whereas *A. niger, Eurotium amstelodami, M. fuscus, P. chrysogenum* and *P. griseofulvum* are found in barley residues. *Alternaria tenuissima* and *U. tuberculata* are isolated from cabbage residues only as shown in Table (1).

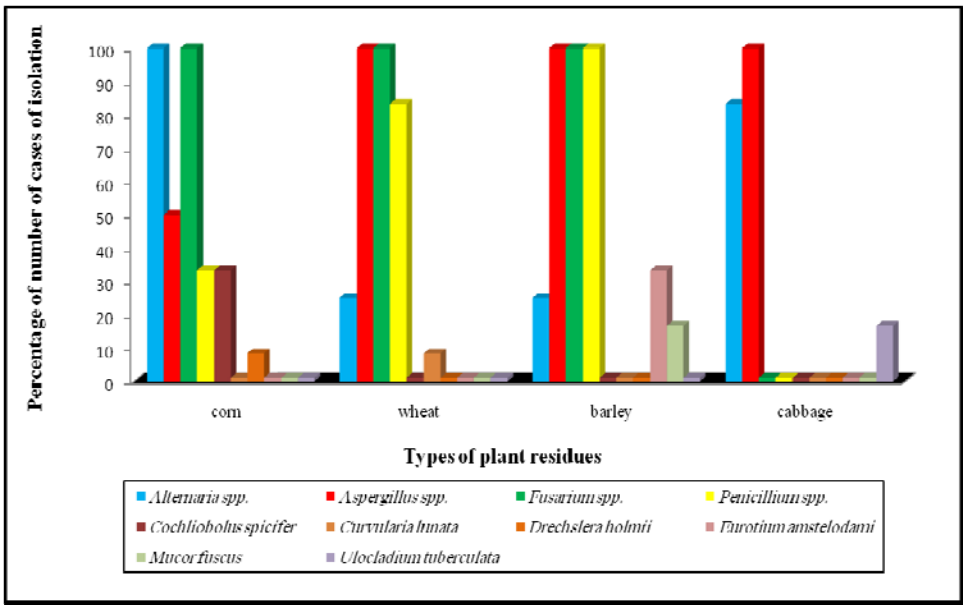

Fig. 1: Mycological analysis of different plant residues types used.

It is worthy to mention that literatures dealing with isolation and identification of fungi from Yemeni plant residues are rare. In this respect, In Al-Taif province, Saudi Arabia, Kabli, (2007), screened the presence of mycoflora in the samples of the litters of some plants which contained deciduous leaves, twigs, flowers, seeds, fruits and plant bark, beside other dead plant materials. He identified twenty one fungal species belonging to ten different genera of which *F. oxysporum* was dominant followed by *A. niger, F. solani, M. racemosus, P. glabrum* and *P. janczewskii.*

P. viridicatum, P. glabrum, P. italicum, P. citrinum, Curvularia inaequalis, A.niger, Aureobasidium sp., *Phanerochaetes* sp. and *Cladosporium* sp., were isolated from decaying vegetables (Martin, *et al.*, 2004).

A. niger was isolated from wastes of sunflower processing industries in Gulbarga, (India), whereas *R. stolonifer, A. niger* and *A. terreus,* were dominated on cassava wastes in the same country (Patil and Dayanand, 2006 and Pothiraj, *et al.*, 2006).

According to data obtained in this investigation, it is clear that there is an apparent difference in both fungal content and species in regard to plant residues types. For example, the wide spectrum of fungal genera and species is recovered from barley residues (9 genera and 18 species), followed by wheat residues (8 genera and 16 species) then corn residues (6 genera and 13 species) and finally, cabbage residues (6 genera and 12 species).

Biodegradation by extracellular enzymes produced by the tested fungi:

Fifty eight fungal isolates representing 29 species and one variety related to 12 genera are screened for their ability to release extracellular cell wall biodegrading enzymes; cellulase, pectinlyase (PL) and polygalacturonase (PG); on solid media. These isolates are belonged to one species for each of *Acremonium, Cochliobolus, Curvularia, Drechslera, Eurotium,* and *Stachybotry,* 2 species of each of *Mucor* and *Ulocladium,* 3 species of *Alternaria,* 5 species and one variety of *Aspergillus*, 5 species of *Fusarium* and 6 species of *Penicillium.* The term production used here is extended to mean both synthesis of enzymes by the fungus as well as the activity of the enzyme in the medium after its production.

Table 1: Existence, collective total count (colonies/g), number of cases of isolation and occurrence remark (out of 12 samples of each type of plant residues) of fungal genera and species isolated from different types of plant residues on Czapek's agar at 28°C.

Plant residues / Genera & species	Corn				Wheat				Barley				Cabbage			
	Ex.	**T.C**	**N.C.I**	**O.R**	**Ex.**	**T.C**	**N.C.I**	**O.R**	**Ex.**	**T.C**	**N.C.I**	**O.R**	**Ex.**	**T.C**	**N.C.I**	**O.R**
Acremonium strictum	-	-	-	-	+	168	6	M	+	788	9	H	+	43	3	L
Alternaria chlamydospora	+	28	3	L	-	-	-	-	-	-	-	-	+	39	3	L
A. longipes	+	1481	12	H	+	88	3	L	+	24	3	L	+	578	6	M
	-	-	-	-	-	-	-	-	-	-	-	-	+	24	1	R
Aspergillus flavipes	-	-	-	-	+	28	1	R	+	20	1	R	+	62	2	R
A. flavus	-	-	-	-	+	26	4	L	+	186	6	M	+	21	1	R
A. fumigatus	+	400	6	M	+	33	6	M	-	-	-	-	+	58	9	H
A. niger	-	-	-	-	-	-	-	-	+	44	8	H	-	-	-	-
A. terreus var. *aureus*	-	-	-	-	+	125	7	H	-	-	-	-	+	32	6	M
Cochliobolus spicifer	+	69	4	L	-	-	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	-	-	-	-	+	18	1	R	-	-	-	-	-	-	-	-
Drechslera holmii	+	21	1	R	-	-	-	-	-	-	-	-	-	-	-	-
Eurotium amstelodami	-	-	-	-	-	-	-	-	+	23	4	L	-	-	-	-
Fusarium dimerum	+	67	3	L	+	159	6	M	+	186	6	M	-	-	-	-
F. longipes	+	2441	12	H	+	2587	11	H	+	1065	12	H	-	-	-	-
F. moniliforme	+	583	6	M	+	78	3	L	-	-	-	-	-	-	-	-
F. oxysporum	+	282	7	H	-	-	-	-	-	-	-	-	-	-	-	-
F. poae	+	1329	9	H	+	56	2	R	-	-	-	-	-	-	-	-
Mucor fuscus	-	-	-	-	-	-	-	-	+	2	2	R	-	-	-	-
M. hiemalis	-	-	-	-	+	2	1	R	+	26	6	M	+	1	1	**R**
Penicillium chrysogenum	-	-	-	-	-	-	-	-	+	21	1	R	-	-	-	-
P. citrinum	-	-	-	-	+	624	4	L	+	1329	10	H	-	-	-	-
P. funiculosum	+	21	1	R	+	22	1	R	+	66	3	L	-	-	-	-
P. glabrum	+	145	3	L	-	-	-	-	+	148	4	L	-	-	-	-
P. griseofulvum	-	-	-	-	-	-	-	-	+	141	5	M	-	-	-	-
P. lanosum	-	-	-	-	+	179	5	M	+	1563	7	H	-	-	-	-
Stachybotrys chartarum	-	-	-	-	-	-	-	-	+	77	2	R	+	27	2	**R**
Ulocladium alternariae	+	248	3	L	+	318	7	H	+	101	6	M	+	778	10	H
U. tuberculata	-	-	-	-	-	-	-	-	-	-	-	-	+	18	2	R
Total count	7871				5309				8198				6787			
Total No. of genera	7				8				9				6			
Total No. of species	13				16				18				12			

+: Present, - : Absent, T.C: Total count, N.C.I: Number of cases of isolation, O.R: Occurrence remark.
H: High occurrence (7-12), M: Moderate occurrence (5-6), L: Low occurrence (3-4), R: Rare occurrence (1-2).

From the data, it is apparent that 19 of the isolates investigated could produce; to a varying extent; more than one enzyme. Results also clearly show that the tested fungi are more able to produce cellulase (exo-1, 4-β- glucanase) than pectin lyase and/or polygalacturonase (Table 2).

Table 2: Cellulolytic and pectinolytic activities* of fungi recovered from different types of plant residues on solid media.

Tested fungi	Source of isolation	N.I.T	Cellulase[a]	Pectinases	
				PL	PG
Acremonium strictum	Wheat residues	1	10.47	N.D	N.D
	Barley residues	1	69.2	58.99	88.37
	Cabbage residues	1	20.3	N.D	N.D
Alternaria chlamydospora	Corn residues	1	22.22	N.D	N.D
	Cabbage residues	1	22.01	N.D	N.D
Alternaria longipes	Corn residues	1	22.39	N.D	N.D
	Wheat residues	1	10.11	N.D	N.D
	Barley residues	1	29.89	5.71	N.D
	Cabbage residues	1	27.13	3.03	N.D
A. flavipes	Wheat residues	1	45.31	N.D	N.D
	Barley residues	1	37.33	N.D	N.D
	Cabbage residues	1	N.D	11.11	N.D
A. flavus	Wheat residues	1	17.48	N.D	N.D
	Barley residues	1	15.35	N.D	N.D
	Cabbage residues	1	18.81	N.D	N.D
A. fumigatus	Corn residues	1	N.D	N.D	N.D
	Wheat residues	1	98.39	N.D	N.D
	Cabbage residues	1	85.91	13.17	N.D
A.niger	Barley residues	1	3.35	N.D	N.D
A. terreus var. *aureus*	Wheat residues	1	97.14	N.D	5.06
	Cabbage residues	1	89.93	N.D	7.46
Cochliobolus spicifer	Corn residues	1	7.67	N.D	N.D
Curvularia lunata	Wheat residues	1	3.37	N.D	N.D
Drechslera holmii	Corn residues	1	27.78	N.D	N.D
Eurotium amstelodami	Barley residues	1	N.D	4.16	N.D
F. dimerum	Corn residues	1	8.94	N.D	N.D
	Wheat residues	1	N.D	N.D	N.D
	Barley residues	1	7.46	N.D	N.D
F. longipes	Corn residues	1	N.D	N.D	N.D
	Wheat residues	1	7.33	N.D	N.D
	Barley residues	1	N.D	N.D	N.D

Table 2: Continued.

Tested fungi	Source of isolation	N.I.T	Cellulase[a]	Pectinases	
				PL	PG
F. moniliforme	Corn residues	1	N.D	4.27	N.D
	Wheat residues	1	N.D	2.56	13.41
F. oxysporum	Corn residues	1	N.D	N.D	N.D
F. poae	Corn residues	1	3.00	8.00	N.D
	Wheat residues	1	16.82	5.56	N.D
Mucor fuscus	Barley residues	1	N.D	N.D	N.D
M. hiemalis	Wheat residues	1	N.D	N.D	N.D
	Barley residues	1	N.D	N.D	N.D
	Cabbage residues	1	N.D	N.D	N.D
Penicillium chrysogenum	Barley residues	1	44.19	42.86	80.85
P. citrinum	Wheat residues	1	62.22	N.D	N.D
	Barley residues	1	N.D	N.D	N.D
P. funiculosum	Corn residues	1	44.44	N.D	N.D
	Wheat residues	1	N.D	N.D	N.D
	Barley residues	1	15.66	47.83	N.D
P. glabrum	Corn residues	1	9.19	90.95	44.16
	Barley residues	1	N.D	N.D	N.D
P. griseofulvum	Barley residues	1	83.72	19.51	N.D
P. lanosum	Wheat residues	1	75.61	N.D	39.47
	Barley residues	1	50.32	N.D	N.D
Stachybotrys chartarum	Barley residues	1	75.13	N.D	12.12
	Cabbage residues	1	N.D	7.27	N.D
Ulocladium alternariae	Barley residues	1	75.13	N.D	12.12
	Cabbage residues	1	N.D	7.27	N.D
	Corn residues	1	50.61	15.19	45.61
Ulocladium alternariae	Corn residues	1	50.61	15.19	45.61
	Wheat residues	1	8.60	N.D	18.88
	Barley residues	1	50.07	3.74	16.71
	Cabbage residues	1	N.D	N.D	N.D
U. tuberculata	Cabbage residues	1	25.13	9.72	N.D

Activities[*]: The percentage of the ratio of clear zone. N.I.T: Number of isolates tested.
Cellulase[a]: Exo – 1, 4 – β – glucanase. PL: Pectin lyase. PG: Polygalacturonase.
N.D: No enzyme was detected.

In a similar study, Latif, (1990), found that among the fungi isolated from Pakistan, *A. fumigatus* produced great levels of cellulases. On the other hand, enzyme extracts obtained from *R. stolonifer, A. niger* and *A. terreus* were found to be rich in β-glucosidase (Ray, *et al.,* 1993). Three years later, production of cellulases by *A. fumigatus* using wheat straw as a carbon source is studied by Dahot and Noomrio, (1996).

In the same respect, El-shafei, *et al.,* (1990), reported that *A. terreus* showed a higher cellulolytic activity compared with *Trichoderma viride*, which is considered as

standard cellulase and hemicellulase activity. The cellulose degrading potential of *A. terreus* was also confirmed by Ali, *et al.,* (1991).

In addition, most of these species have been reported by many authors as cellulose decomposers but with different cellulolytic activity (De Varies and Visser, 2001; Onsori, *et al.,* 2005; Pothiraj, *et al.,* 2006 and Nwodo-Chinedu, *et al.,* 2007). Fungi produce a large number of cellulases. Because they are the main cellulase-producing microorganisms (McMillan, *et al.,* 2001).

Hamlyn, (1998), reported that *A. niger, A. flavus* and *Penicillium* sp. are considered the main sources of cellulase, amylase, hemicellulase, catalase, pectinase and xylanase. Kabli, (2007), found that *Acremonium strictum, A. flavipes, A. niger, Emericella nidulans, Gliocladium roseum, M. racemosus* and *P. janczewskii,* were capable of producing cellulolytic, pectinolytic and amylolytic enzymes, which indicate their major role in litter decomposition.

The present study revealed that among tested fungi, *P. glabrum* proved to be the most active producer of pectin lyase (PL) enzyme, while *P. chrysogenum* appeared to be good polygalacturonase (PG) producer. Whereas, *Acremonium strictum* is high producer for both enzymes.

A number of 23 isolates could produce at least one of the two pectic enzymes. Pectin lyase (PL) and /or polygalacturonase (PG) are detected in a few fungal cultures (Table 2). *P. glabrum* is active producer of pectin lyase (PL), while *P. chrysogenum* appeared to be good polygalacturonase (PG) producer. Whereas, *Acremonium strictum* is high producer for both enzymes.

It is worthy to mention that, under our experimental conditions, *F. oxysporum, M. fuscus,* and *M. hiemalis* failed to exhibit pectinolytic and cellulolytic activities.

According to several researchers, pectinases were produced by various fungi including *Aspergillus* sp. (Patial and Dayanand, 2006), *Penicillium* sp. (Silva, *et al.,* 2002), and *Thermoascus aurantiacus* (Martins, *et al.,* 2002) when cultivated on agro industrial wastes (Giese, *et al.,* 2008).

In a similar study, Kabli, (2007), found that *Acremonium strictum, A. flavipes, A. niger, Emericella nidulans, Gliocladium roseum, M. racemosus* and *P. janczewskii* , were capable of producing cellulolytic, pectinolytic and amylolytic enzymes, which indicated their major role in litter decomposition.

Said, *et al.,* (1991), showed that *P. frequentans* produced high levels of extracellular pectinases. Also, pectinases synthesis by *P. frequentans* was detected by Kawano, *et al.,* (1999), whereas, extracellular polygalacturonases from *P. frequentans* was partially purified and biochemically characterized by Barense, *et al.,* (2001).

Martin, *et al.,* (2004), tested the pectin lyase and polygalacturonase production by *Moniliella* and *Penicillium* sp. in solid-state fermentation. They found that the two tested fungi produced polygalacturonase and pectin lyase on a mixture of orange bagasse, sugarcane bagasse and wheat bran as substrate.

It was mentioned by Teixeira, *et al.,* (2000), that the main sources for the pectinolytic enzymes are yeast, bacteria and a large variety of filamentous fungi, of which the most relevant ones are aspergilli. But our data didn't support such information, because aspergilli showed moderate to weak ability in pectinases production. These findings are supported by the following researchers who reported that, from the genus *Aspergillus,* a few of the potential fungal cultures explored for the production for pectinases which are *A. niger* (Solis-Pereyra, *et al.,* 1996), *A. awamori* (Blandino, *et al.,* 2002), and *A. foetidus* (Sebastian, *et al.,* 1996). Beside that the production of pectinases by aspergilli affected by the temperature where they

prefer high temperatures than low or moderate temperatures, as assessed by Mahmoud and Omar (2001) who found that the production of PL and PME by *A. flavus, A. fumigatus* and *A. niger* was enhanced with incubation at 35°C.

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ARABIC SUMMARY

التحلل الحيوي لبقايا نباتية زراعية بواسطة بعض الفطريات المعزولة من اليمن

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لقد تم جمع 48 عينة من بقايا نباتية مختلفة (250 جم لكل منها)، من تسع محافظات مختلفة في اليمن خلال العام 2008، والتي كانت عبارة عن بقايا كل من الذرة، القمح، الشعير والكرنب. تم التحليل الفطري لهذه العينات على بيئة الشبك آجار عند درجة حرارة 28 ± 1° م.

أمكن عزل وتعريف 23 نوعاً وصنفاً واحداً تنتمي إلى 12 جنساً فطرياً من العينات، وكان كل من: الألترناريا ممثلاً بـ (3 أنواع)، الأسبرجلس (5 أنواع وصنف واحد)، الفيوزاريوم (5)، البنيسيليوم (6) والميوكر (2) هي أكثر الأجناس الفطرية شيوعاً.

تم اختبار قدرة 58 عزلة فطرية تمثل 23 نوعاً وصنفاً واحداً تنتمي إلى 12 جنساً على انتاج الإنزيمات المحللة للجدار الخلوي (السليوليز– البكتين لييز– البولي جالاكترونيز) على بيئات صلبة. أوضحت النتائج أن معظم العزلات الفطرية المختبرة كانت قادرة على انتاج السليوليز بدرجات متفاوتة، في حين أن عدد قليل منها كان قادراً على انتاج البكتينيز ولكن بقدرات متفاوتة.

أوضحت الدراسة أن فطرة الأسبرجلس فيوميجاتس يليها الأسبرجلس تيريس الصنف أيريس ثم البنيسيليوم جرايسيوفولفم، كانت أكثر الفطريات النشطة في انتاج السليوليز. أما البنيسيلوم جلابرم كان منتجاً نشطاً للبكتين لييز، في حين أن الأكريمونيوم ستريكتم والبنيسيليوم كريسوجينوم أبديا كفاءة عالية في انتاج البولي جالاكترونيز.