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Biochemical study for three keratine baiting and using it to isolation and identification some fungi from soil in Basrah, Iraq

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Abstract — ***fifty soil samples were collected from different areas of Basrah city, Iraq and screened for the presence of keratinophilic and keratinolytic fungi by using three baiting materials. horns , hedgehog quills and hairs in isolation technique during study isolated and identificated (112) species of fungi belonging to 58 genera the deutromycota group appeared highest percentage(62.5)% compared with other phylum the results showed higher number of fungal genera on horns.(58)while less number on hairs (20) genera.tested enzymatic activity for 15 fungal isolates the results appeared all test fungi gave positive activity to produced protease and lipase excepted Aspergillus tamari and Chrysosporium demonbrorium appeared negative activity for production of protease. Also X-Ray technique used to determined crystal structure and solidified of three materials appeared results horn more solidified and has crystal structure at angle (2 θ) while hedgehog quills and hairs have non crystal structure. So determined percentage of sulfur in three baiting materials the results showed higher proportion in horns which reached to (587mg/gm) while less proportion in hairs (85.1 mg/gm)***

Keyword: **keratinophilic and keratinolytic fungi, three baiting materials.**

I. INTRODUCTION

Keratin is a major component of hair, feathers horns and wool .The durability of keratins is a direct consequence of their complex architecture. In addition to keratin, keratinaceous materials such as skin, hair, nails, hoofs contain a large proportion of non-keratin proteins.[1].

It is a fibrous and insoluble structural protein extensively cross-linked with disulphide, hydrogen and hydrophobic bonds, resulting in mechanical stability and resistance to common proteolytic enzyme such as pepsin, trypsin and papain. [2]

Keratin are divided into two types. α-Keratin: It present in wool, hair, and horn. It is in the form of folder chain. β-Keratin: It present in feather in the form of polypeptide chain. A fungi grown on keratinous substances which is important natural material occurring in nature mainly in the form of hairs, wools, feathers, horns, hooves, nails, skin and other cornified appendages, constituting natural baits for these fungi[3].

Soil provides a heterogeneous and complex environment for all soil inhabitants. Soil is also known to harbor different microorganisms including diverse group of fungi Due to their diversity, fungi have been recognized as a source of new enzymes with useful and/or novel cha. Filamentous fungi are particularly interesting due to their easy cultivation, and high production of extracellular enzymes of large industrial potential ex : lipase and protease[4].

Soil Enzymes are usually offered as "cocktails" of several activities rather than a single enzymatic activity However, in many cases the enzyme activities can still act on the same composition, as the composition can have a complex chemical structure having various types of chemical bonds, requiring different enzyme activities for breakdown. Example keratinase, lipase and protease [5].

Keratinophilic fungi are small, well defined and important group of fungi that colonize various keratinous substrates and degrade them to components of low molecular weight. These fungi are present in the environment with variable distribution patterns which depend on factors, such as human and or animal presence. The species of keratinophilic fungi have been divided into three categories depending on their natural habitats: anthrophilic, when human beings are natural host, zoophilic, when animals act as natural host and geophilic [6].

[7] Used Two keratinous fragments, human hair and nails were used for the growth of fungi by hair-baiting technique. During this study isolated number of 25 species belonging to 16 genera

During study [8] Feathers used as keratin baits were sterilized and spread over the moist soil samples .Out of total 76 soil samples, 65 soil samples were found to be positive for the keratinophilic fungi. Fourteen species belonging to a single genus Chrysosporium were isolated throughout the cropping season.

Aims of study: Iraqi soils contains many more keratinophilic and keratinolytic fungi . Therefore an attempt was made in the present study to isolate more onygenalean fungi from different soil habitats by using horns and hedgehog quills as well as hair as baits .and enzymatic activity for 15 fungal isolates beside of biochemical study for three baiting materials. protein (keratin), then one can imagine the quantity of keratin that would have accumulated on earth, since a vast quantity of keratin is shed by the vertebrates. Basrah soils

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contain many more keratinophilic fungi than those presently recorded, and there is need for further taxonomic and ecological studies of this interesting group of organisms the present work is thus carried

II. MATERIALS AND METHODS

A. Samples collection

Collected 50 soil samples during the period 1-1-2012 /1-1-2013 from three different area in Basra city , Iraq.

The samples including rodent holes burrows and sediments

B. Culture Media

During this study prepared Malt extract agar (MEA), Potato Carrot Agar (PCA), Potato dextrose agar(PDA)as information in (Himedia company , India)

C. Examination and identification samples

Put samples in the sterile plastic bag , transferred to the [10], [11], [12], [13], [14], [15], [16], [17] ,[18] ,[19], [20], [21], [22] ,[23] maintained on potato dextrose agar (PDA) The isolates were identified using the relevant publications

D. Occurrence percentage

Calculated the percentage of the occurrence of the genera based on the following equation:-

%Occurrence = Number of positive samples / total number of samples * 100

E. Determine the enzymatic activity of fungi

15fungal isolates tested the ability to production the extracellular enzymes on the solid media a protease enzyme and lipase, as follows:

1-protease enzyme Use the culture media described in [24] containing gelatin as substrate to test the production of the enzyme protease and use theFraziers reagent to detect the production of the enzyme, as to be a clear zone Formed about the colony fungal after the addition of reagent and left for a few minutes then pour it into Evidence on the ability of fungal isolates on the exploitation of protein production enzyme protease.

2–Lipase enzyme Use the culture medium described in the [25] to test the production of an enzyme lipase as to be white deposits around the fungal colony indicate on enzyme lipase secretion

F. Powder X-ray diffraction studies (PXRD)

Powders of horns, hedgehog quills and hair powder were performed by grinding 1 gm of each in a liquid nitrogen environment. Powder X-ray diffraction studies were carried out using a PANalytical X'PERT Pro X-ray

G. Ione Exchange chromatography

Sample preparation for horn, hedgehog quills and hair depended on [26] Diffract meter with Cu Kα radiation as the X-ray source. The diffraction patterns were recorded in the 2θ range of 20 °A to 60 °A.

III. RESULTS

Table (1) :fungal species which isolated and Identification during study

S.	Fungal species
1	*Achaetomium luteum* Rai & Tewari
2	*Achaetomium strumarium* Rai *et al.*
3	*Acremonium stricutum* WiGams
4	*Alternaria alternate* (Fr.) Keissl.
5	*Apanoascus durus* Cano and Cuarro
6	*Aphanoascus clathratus* Cano and Guarro
7	*Aphanoascus mephilialis* (Malloch & Cain) Cano & Guarro
8	*Aphanoascus saturnoideus* Cano &Guarro
9	*Arachniotus ruber* (Van Tieghem)Schroeter
10	*Arthrographis kalrae* (Tewari & Macpherson) Sigler & Carmichael
11	*Ascotricha bosei* D.Hawksw
12	*Aspergillu flavus* Link
13	*Aspergillus fumigates* Fres.
14	*Aspergillus nidulance* (Eidam) Winter
15	*Aspergillus niger* Tieghem
16	*Aspergillus ochraceous* Wilhelm
17	*Aspergillus oryza* (Ahlburg) Cohn
18	*Aspergillus tamari* Kita
19	*Aspergillus terreus* Thom
20	*Aspergillus versicolor* (Vuill.) Tiraboschi
21	*Aspergillus. Candidus* Link
22	*Aspergillus. Wenttii* Wehmer
23	*Aspergillus.penicilliodis* Spegi
24	*Auxarthron californiense* Orr and Kuehn
25	*Beauveria bassiana* (Bals.)Vuill
26	*Bipolaris papendrofi* (Van derAa) Alcorn
27	*Botryotrichum keratinophilum* Kushwaha and Agrawal
28	*Cephaliophora irregularis* Thaxter
29	*Chaetomidium fimeti* (Fuckel) Sacc
30	*Chaetomium atrobrunneum* Ames
31	*Chaetomium fusiforme* Chivers
32	*Chaetomium globosum* Kunze
33	*Chaetomium spirale* Zopf
34	*Chrysosporium demonbreunii* Ajello &Cheng
35	*Chrysosporium indicum* (Randhawa & Sandhu) Garg
36	*Chrysosporium keratinophilum*

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	D.Frey ex Carmichael
37	*Chrysosporium merdarium* (Link ex Greve) Carmichael
38	*Chrysosporium pannicola* (Corda) Van Oorschot & Stalpers
39	*Chrysosporium pseudomerdarium* Van Oorschot
40	*Chrysosporium queenslandicum* Apinis and Rees
41	*Chrysosporium sulfureum* (Field .) Van Oorschot & Samson
42	*Chrysosporium tropicum* Carmichael
43	*Chrysosporium.* anamorph of *Renispora flavissima* Sigler *et al*
44	*Cladosporium cladosporoides* (Fres.) de Vries
45	*Colletotrichum coccodes (Wallr) Hughes*
46	*Curvularia clavata* Jain (dr Mustafa source)
47	*Drechslera papendorfii* (Van der Aa) M.B.Ellis
48	*Emericella nidulance* (Eidam) Vuill
49	Engydontium album (Limber) de Hoog
50	*Eurotium chevalieri* Mangin
51	*Exerohilum holmii* (Luttr.) Leonard and Suggs
52	*Fusarium moniliforme* Sheldon
53	*Fusarium oxysporum* Schlecht : Fr.
54	*Fusarium proliferatum* (Matsushima) Nirenberg
55	*Fusarium solani* (Mart).Sacc
56	*Fusarium verticillioides*(Sacc.) Nirenberg
57	*Geotrichum candidum* Link:Fr
58	*Graphium eumorphum* Sacc
59	*Graphium putredinis* (Corda)Hughes
60	*Gymnoascella littoralis* (Orr) Currah
61	*Gymnoascella afillamntosa* (Orr and Kuehn) Currah
62	*Gymnoascella citrina* (Massee and Salmon) Orr, Ghosh and Roy
63	*Gymnoascella dankaliensis* (Castellani) Currah
64	*Gymnoascella dervoreyi* (Orr) Currah
65	*Gymnoascella haylinospora* (Kuen, Orr and Ghosh) Currah
66	*Gymnoascus intermedius* Orr
67	*Gymnoascus reessi* Baranetzky
68	*Malbranchea aurantiaca* Sigler and Carmichael
69	*Melanospora* caprina (Fn . ex Hornem) Sacc.
70	*Melanospora zamiae* Corda
71	*Microascus cinereus* (Emile-Weil &Gaudin)

	Curzi
72	*Microascus trigonosporus* Emmons &Dodge
73	*Microsporum fulvum* Uriburi
74	*Microsporum* gypseum (Bodin) Guiart & Grigorakis
75	*Microsporum nanum* Fuentes
76	*Microsporum persicolor* (Sab.) Guriart and Grigorakis
77	Mucor hiemalis Wehmer
78	*Mucor racemosus* Fr
79	Myceliophthora anamorph of corynascus sepedonium (Emmons) v.Arx
80	*Myceliophthora vellerea* (Sacc. & Speg.) Van Oorschot
81	*Nannizziopsis vriesii (Apinis) Currah, source mycotaxon p165*
82	*Oidiodendron setiferum* Udagawa et Toyazaki
83	*Paecilomyces variotii* Bain.
84	*Papulaspora equi* Shadomy & Dixon
85	*Papulospora viridis* Matsushima
86	*Penicillium chrysogenum* Thom
87	*Petriella sordid (*Zukal)Barron&Gilman
88	*Phoma medicaginis* Malbr. & Roum. Var pinodella (L.K.Jones)Boerema
89	*Pseudallesheria boydii* (Shear) McGinnis *et al*
90	*Rhizoctonia solani* Kuhn
91	*Rhizopus oryza* Went & Prinisen Geerligs
92	*Rhizopus stolonifer* (Ehrenb.:Fr)Vuill
93	*Scedosporium apiospermum* Sacc
94	*Scopulariopsis asperula* (Sacc.)Hughes
95	*Scopulariopsis brevicaulis* (Sacc) Bainier
96	*Scopulariopsis brumptii* Salvanet-Duval
97	*Scopulariopsis flava* (Sopp)Morton and G.Smith
98	*Scopulariopsis koningii* (Oud.) Vuilli
99	*Scytalidium hyalinum* Campbell and Mulder
100	*Sepedonium nevieum* Massee et Salmon
101	*Sphaeroides levita* (Udagawa & Cain) D.Garcia , Stchigel & Guarro
102	*Stachybotrys bisbyi* (Srinivasan) Barron
103	*Thielavia terricola* (Gilman & Abbott) Emmoms
104	*Trichoderma viridis* Pers. Ex Gray
105	*Trichophyton equanium* (Matruchot & Dassonville) Gedoelst
106	*Trichophyton mentagrophytes* (Robin) Blanchard
107	*Trichophyton terrestr* Durie and Frey

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108	*Trichophyton verrucosum* Bodin
109	*Trichosporon beigelii* Auctt
110	*Tritirachium oryzae* (Vincens) deHoog
111	*Ulocladium botrytis* Preuss(dr Mustafa)
112	*Uncinocarpus ressii* Sigler and Orr

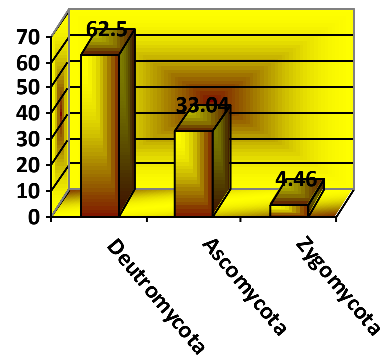
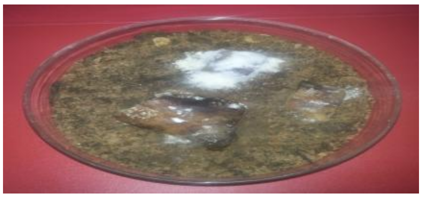


Fig (1): percentage of fungal species isolated during study

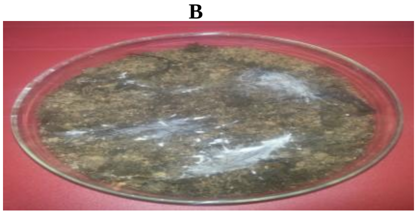
Nature Keratinous sources

Choice three keratinous sources as keratine baiting: horns hedgehog quills and hairs used for isolation keratinophilic and keratinolytic fungi by method keratine baiting on soil .



A

B



C

Fig (2): A: fungal growth on horns put on soil
B: fungal growth on hedgehog quills put on soil
C: fungal growth on hairs put on soil

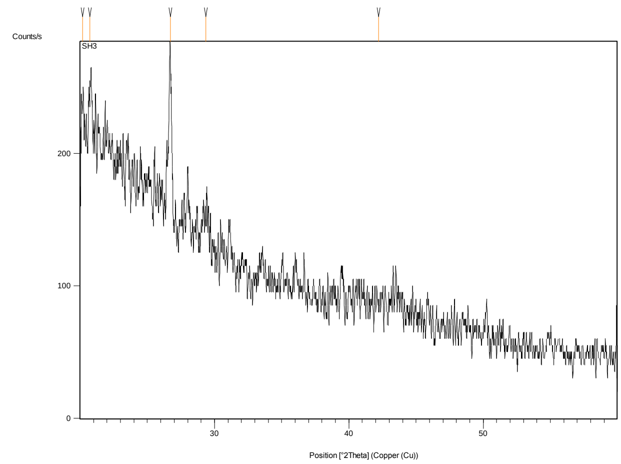


Fig (3) : X-ray diffraction of horns Powder

Table(3) :Peak List: (horns)

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
20.1647	36.31	0.2362	4.40375	78.92
20.7315	36.03	0.9446	4.28461	78.32
26.6996	46.00	0.1574	3.33890	100.00
29.3480	21.04	0.5510	3.04335	45.74
42.2225	7.20	4.6080	2.13865	15.65

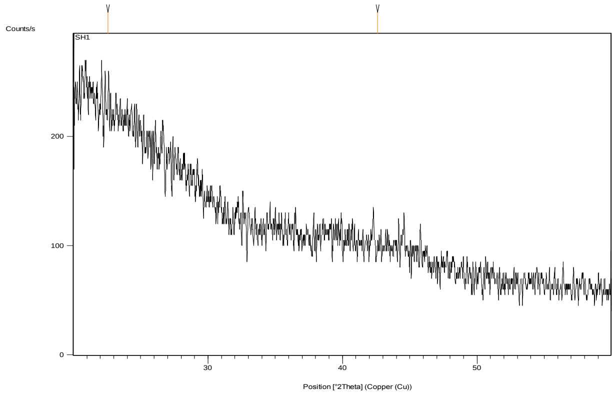


Fig (4) : X-ray diffraction of hedgehog quills Powder

Table(4): Peak List: (*quills*)

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
22.5650	33.17	5.0381	3.94046	100.00
42.6220	8.23	9.2160	2.11953	24.80

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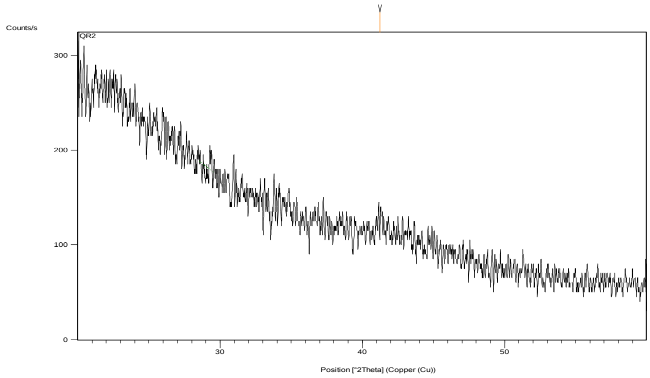


Fig(5) : X-ray diffraction of hairs Powder

Table (5):Peak List: (hairs)

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
41.2283	2.16	3.0720	2.18790	100.00

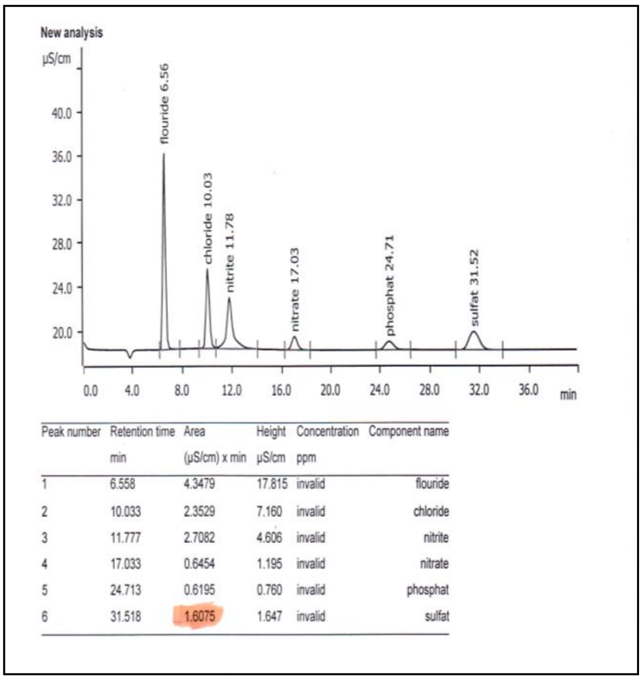


Fig (6): Standard curve

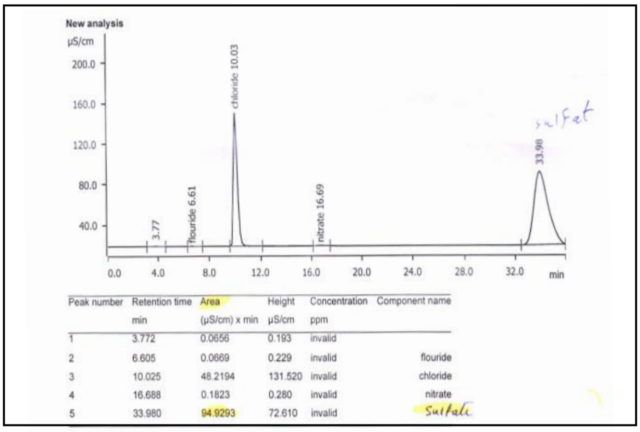


Fig (7): Ione exchange of horns Powder

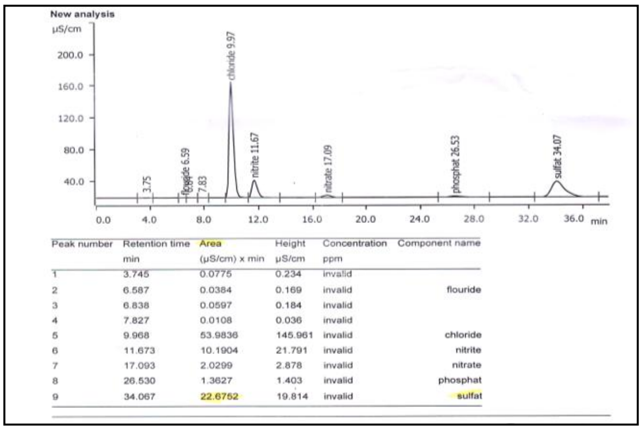
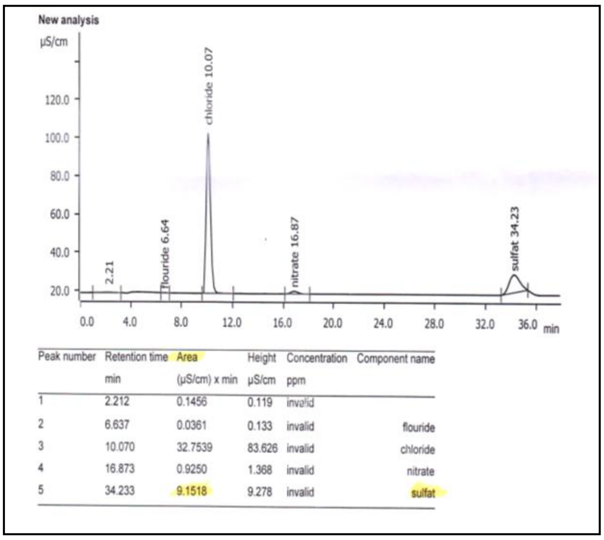


Fig (8): Ione exchange of quills Powder



Fig(9):Ione exchange of hairs Powder

Table (6): sulfur percentage of three materials

series	materials	Sulfur percentage
1	Horns powder	587 mg/gm
2	hedgehog quills powder	139.5 mg/gm
3	Hairs powder	85.1 mg/gm

Table (7) enzymatic activity of 15 fungal isolates

S.	Fungal isolates	Protease	lipase
1	*Arachinotus rubrua*	+	+
2	*Aspergillus tamari*	-	+
3	*Chrysosporium demonbrorium*	-	+
4	*Chrysosporium merdarium*	+	+
5	*Fusarium oxysporum*	+	+
6	*Fusarium solani*	+	+
7	*Gymnoascella*	+	+

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	darkanensis		
8	***Malbranchea aurantiaca***	+	+
9	***Melanospora caprina***	+	+
10	***Microsporum fulvum***	+	+
11	***Microsporum persicolor***	+	+
12	***Sphaerodes levita***	+	+
13	***Trichophyton equanium***	+	+
14	***Trichophyton terrestre***	+	+
15	***Tritirachium oryza***	+	+

85.1 mg/gm Table (6) Figure (6 , 7 , 8 , 9) This is consistent with a study [29] , which pointed to the high percentage of sulfur in horn as amino acid cystein which reach to the ratio 22% of solid keratin followed by quills and hair which have flexible keratin which reach

IV. DISCUSSION

The present study included the collection (50) soil sample from different locations in the province of Basra , during which isolated and identificated (112) species of fungi belonging to 58 genera, Table (1) Characterized Deutromycota dominance over the rest of the phylum as accounted percentage % (62.5) of the total species, followed by Ascomycota accounted for% (33.04) Then zygomycota has accounted % (4.46) Figure (1). The reason for the dominant of deutromycota compared with the rest of the phylum for being able to produce the conidia in large numbers, small size, these conidia have the ability to spread over long distances of up to hundreds of kilometers as this led to increased number it This is agreement with the study of [27].The result showed in Table (2): that Chrysosporium genus more occurrence the percentage reach100% may reason backed to use tow of keratinous materials beside of hairs it is consider saprophytic and keratinolytic Fungi wider spread in world soilor may be it has active enzymatic system and Scopulariopsis 56% the followed by Apanoascus 46%, the proportion varied occurrence of other genera and lower rates[28]. also used three keratinous biting to isolate fungi figure (2) and was the best baits horns as the number of genera which reach 58 genus then hedgehog quills 26 genera, followed by horse hair 20 genera , table (2)may be reasons backed to different type of α-keratin in horns solid but the type which found in quills and hairs flexible X-Raythe horns showed higher reflection at (2ϴ = 0.1574) (d=3.33890 A°) ,Table (3) and Figure(3) which it represent appropriate direction of the crystal growing there for may be crystal growing of horns and solidify permit to growth of the keratinophilic and keratinolytic positive result for the secretion of the culture, which consisted of 15 fungal isolation during the enzyme protease and lipase except types Aspergillus tamari and

while Also X- Ray of the hedgehog quills and hair powder showed Table(4,5) and Figure (4,5) that these have non crystal structure there for retarder growth of fungi so the fungal which isolated by quills and hairs less advantage in isolation of fungi , Also used ion exchange chromatography technique to determine the percentage of sulfur in the three keratinous baiting and found that the horns containing the highest percentage of sulfur 587 mg / gm followed by quills 139.5 mg / gm and hair the ratio of cystein 14-10 % fungi and stay for many year on this keratinous baitng and it resistance enzymatic activities of this fungal Enzymatic activity on solid culture Observed in a study current study Table (7) isolated from different locations of the soil it gave a

Chrysosporium demonbrorium gave a negative result for the secretion of the enzyme protease The reason for the inability of tow species on production the protease in the current study, may be backed to the weakness and inactivity or physiological factors such as pH, incubation temperature and incubation time, and biological factors such as the genetic nature of the organism influences the metabolic and biochemical nature of the microbial strain effect on enzyme production [31] The potential 13 fungal isolates to produce protease denote the consumption of protein from before tested fungi because fungi either keratinophilic or keratinolytic fungi her ability to secrete enzymes for the analysis of complex protein and this susceptibility uncertain because of these fungi need for protein as nutrition [32] so produce Lipase by all isolates tested because they either keratinophilic or keratinolytic fungi where exploit the fat that covers keratin tissues as a source of carbon[30]

V. CONCLUSION

Appeared that the horns practically excellent to isolate fungi from the soil so as to note the intensity growth of mycelium on cut horns and produce perfect and imperfect state, and contain the horns a high percentage of sulfur, allowing fungi to take advantage of it for biological activity and survival longer on this bait compared quills hedgehog and hair which has less sulfur.

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Appendix

Table(2) :fungal genus which isolated and number of species, with methods isolated it and% occurrence

S.	Genus	Number of sample	Occurrence %	Species number	Methods isolated it		
					horns	Hedgehog quills	Hairs
1	*Achaetomium*	3	6	2	*Achaetomium*	*Achaetomium*	
2	*Acremonium*	1	2	1	*Acremonium*		
3	*Alternaria*	2	4	1	*Alternaria*	*Alternaria*	
4	*Apanoascus*	23	46	4	*Apanoascus*	*Apanoascus*	*Apanoascus*
5	*Arachinotus*	2	4	1	*Arachinotus*		
6	*Arthrographis*	1	2	1	*Arthrographis*		
7	*Ascotricha*	3	6	1	*Ascotricha*		
8	*Aspergillus*	17	34	12	*Aspergillus*	*Aspergillus*	*Aspergillus*
9	*Auxarthron*	3	6	1	*Auxarthron*		
10	*Beauveria*	1	2	1	*Beauveria*	*Beauveria*	
11	*Bipolaris*	1	2	1	*Bipolaris*	*Bipolaris*	
12	*Botryotrichum*	3	6	1	*Botryotrichum*		
13	*Cephaliophora*	5	10	1	*Cephaliophora*		
14	*Chaetomium*	8	16	4	*Chaetomium*	*Chaetomium*	*Chaetomium*
15	*Chaetomidium*	2	4	1	*Chaetomidium*		
16	*Chrysosporium*	50	100	10	*Chrysosporium*	*Chrysosporium*	*Chrysosporium*
17	*Cladosporium*	5	10	1	*Cladosporium*		
18	*Colletotrichum*	1	2	1	*Colletotrichum*		
19	*Curvularia*	4	8	1	*Curvularia*	*Curvularia*	
20	*Drechslera*	5	10	1	*Drechslera*	*Drechslera*	
21	*Emericilla*	6	12	1	*Emericilla*		
22	*Engydontium*	10	20	1	*Engydontium*	*Engydontium*	*Engydontium*
23	*Eurotium*	2	4	1	*Eurotium*		
24	*Exerohilum*	1	2	1	*Exerohilum*		
25	*Fusarium*	10	20	5	*Fusarium*	*Fusarium*	*Fusarium*
26	*Geotrichum*	3	6	1	*Geotrichum*	*Geotrichum*	
27	*Graphium*	15	30	2	*Graphium*	*Graphium*	
28	*Gymnoascella*	17	34	6	*Gymnoascella*	*Gymnoascella*	*Gymnoascella*
29	*Gymnoascus*	13	26	2	*Gymnoascus*	*Gymnoascus*	*Gymnoascus*
30	*Malbranchea*	2	4	1	*Malbranchea*		
31	*Melanospora*	12	24	2	*Melanospora*	*Melanospora*	*Melanospora*
32	*Microascus*	7	14	2	*Microascus*	*Microascus*	*Microascus*
33	*Microsporum*	20	40	4	*Microsporum*	*Microsporum*	*Microsporum*

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34	*Mucor*	7	14	2	*Mucor*		*Mucor*
35	*Myceliophthora*	1	2	2	*Myceliophthora*		*Myceliophthora*
36	*Nannizziopsis*	1	2	1	*Nannizziopsis*		
37	*Oidiodendron*	1	2	1	*Oidiodendron*		
38	*Paecilomyces*	5	10	1	*Paecilomyces*		
39	*Papulaspora*	4	8	2	*Papulaspora*		
40	*Penicillium spp*	10	20	1	*Penicillium spp*		*Penicillium*
41	*Petriella*	2	4	1	*Petriella*		
42	*Phoma*	1	2	1	*Phoma*		
43	*Pseudallesheria*	2	4	1	*Pseudallesheria*		
44	*Rhizoctonia*	2	4	1	*Rhizoctonia*		
45	*Rhizopus*	7	14	2	*Rhizopus*	*Rhizopus*	
46	*Scedosporium*	1	2	1	*Scedosporium*		
47	*Scopulariopsis*	28	56	5	*Scopulariopsis*	*Scopulariopsis*	*Scopulariopsis*
48	*Scytalidium*	4	8	1	*Scytalidium*	*Scytalidium*	
49	*Sepedonium*	1	2	1	*Sepedonium*		
50	*Stachybotrys*	1	2	1	*Stachybotrys*	*Stachybotrys*	
51	*Sphaeroides*	10	20	1	*Sphaeroides*	*Sphaeroides*	*Sphaeroides*
52	*Thielavia*	2	4	1	*Thielavia*		
53	*Trichoderma*	1	2	1	*Trichoderma*		*Trichoderma*
54	*Trichophyton*	18	36	4	*Trichophyton*	*Trichophyton*	*Trichophyton*
55	*Trichosporon*	1	2	1	*Trichosporon*		
56	*Tritirachium*	2	4	1	*Tritirachium*		*Tritirachium*
57	*Ulocladium*	5	10	1	*Ulocladium*		
58	*Uncinocarpus*	3	6	1	*Uncinocarpus*	*Uncinocarpus*	*Uncinocarpus*
Sum	58				58	26	20