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Isolation and Identification of Soil Fungi from Ghemins, Libya

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Abstract: ***Soil microscopic fungi have a great economic importance, as the metabolic activity of many of them is auxiliary factor in soil fertility, On the other hand, some of them may cause damage for plants such as vegetables and fruit trees. Various species of soil fungi are plant pathogenic, and some of them can produce toxins that transfer through the food chain to herbivores and man. The most important feature of some of these fungi is their ability to produce antibiotics, enzymes, and other organic compounds that are important to humans. In this research paper, nine genera of fungi (Phyllactinia, Fusarium, Penicillium, Alternaria, Mucor, Aspergillus, Chaetomium, Phytophthora and Rhizoctonia) were isolated and identified from nine regions in Ghemins, which located on the eastern coast of Libya, in the south of Benghazi.***

Keywords: Soil fungi, Symbiosis, Saprophytism, Parasitism

1.Introduction

The soil is considered a habitat of several organisms includes fungi that differ in their phenotype and genotype, more to their various economic and ecological importance. Fungi are one of the most diverse kingdoms of eukaryotes. Most of them are spore-producing, heterotrophic organisms because they do not contain plastids (Alexopoulos, Mims and Blackwell, 1996). Fungi are like other organisms, especially animals, in that they need organic compounds as a source of food (Baxter et al., 1999). The diversity in fungal nutrition methods whether symbiosis, saprophytism or parasitism, has given them great importance for influencing all terrestrial ecosystems, including plant communities (Martin et al., 2011). Fungi are classified according to the structure, where the fungal body may be either unicellular or more commonly filamentous, in addition to life cycles, taking into account the mode of sexual and asexual reproduction (Hugo and Russell, 1993). Glucans, chitin and glycoproteins are characteristic compounds of fungal cell wall structure. This makes it an excellent target for antifungal therapy, since fungal cell wall components are not present in humans (Rubio et al., 2020).

Genera and species of fungi in soil are related to diverse chemical and physical factors, for instance, oxygen, pH, moisture and organic compounds (Yakop, Taha and Shivanand, 2019). Henderson, 1961isolated several groups of soil fungi including yeasts from various places using an enrichment technique with vanillin additional to p-hydroxybenzaldehyde. Henderson study focused on identification the isolated fungi and explanation their morphological characters according to their ability to grow in different suitable enrichment media that were experimented as sole sources of carbon in the research. According to Gaddeyya et al., 2012 fungal growth varies among the seasons, where multiple environmental factors affect on fungal genera and their rate in soil. The group used soil dilution technique and soil plate technique, and different fungal genera were isolated such as *Aspergillus* spp. Similar research on different rice fields soil samples was carried out by Kumar *et al.*, 2015, and by applied the same previous methods, a high number of Deuteromycotina and few of Zygomycotina were isolated and identified.

Seth, Alam and DN, 2016 examined soil fungi in samples that were collected from three different wheat fields during a year, and by using soil dilution plating technique thirteen different genera were isolated, like *Aspergillus* and *Penicillium* that were obtained from the all three study area. As mentioned above, fungi groups are differ according to the kind of soil that live in, where several genera of fungi which belong to three classes, Zygomycetes, Ascomycetes and Deuteromycetes were obtained from different areas in Western Georgia by using Waksman soil dilution and direct inoculation method (Kutateladze et al., 2016). The aim of this paper is to isolate and identify of soil fungi from nine areas in Ghemins region, and the main purpose is to storage the isolated fungi and examine their capacity to produce antibiotics or chemical compounds in general in future studies.

2.Materials and Methods

2.1 Soil Sample Collection

Ghemins region was divided into nine parts, from the center of the area to the seashore in the west (1-2-3), toward Benghazi in the north (4-5), towards the main road that separates Ghemins and Suluq in the east (6-7), and towards Ajdabiyato the south (8-9). Each region was divided into three parts on the basis of the depth. Surface area symbolized by number (1), depth of 5 cm symbolized by number (2), and depth of 10 cm symbolized by number (3). Soil samples were collected from the nine regions under free microbial contamination conditions, i.e. Samples were

collected in sterile bags according to the method of Baxter *et al.*, 1999, then the chemical, physical and microbial analysis were conducted.

2.2 Chemical and Physical Analysis for Soil Samples

PH Measurement

The soil sample pH was measured by weighing 200 g of soil for each sample and then placed in a flask (500 ml), and 200 ml of distilled water was added to it. The samples were placed in a shaker at a rate of (200 shakes per min) for 5 min until the samples were homogeneous. The samples were left for half an hour outside the shaker to obtain a clear solution, and then the reading was taken by using a pH-meter device (Allison *et al*, 1954).

Moisture Measurement

The soil sample in which moisture was to be measured was passed through a sieve with a capacity of each hole is 9 micron.10 grams of sifted soil were dried inside the oven for 12 hours. The soil was re-weighed after it was dried to determine the moisture of soil. The percentage of moisture was calculated by the following equation:

$$\text{Moisture rate} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100$$

Salinity Measurement

A Total dissolved solids TDS device was used to determine the salinity of the soil samples.

Organic Compounds Estimating

The organic compounds were estimated by simple direct estimation, where 5 grams of soil were put in an oven for 48 hours at 200 m (burning process (Allison, 1960)). After that the samples were weighed again, where the Percentage of organic compounds was obtained by the following equation:

The percentage of organic compounds = (weight of soil before burning - weight of soil after burning) x 100

Isolation of Fungi

The isolation of fungi from soil samples was applied by using Spread Plate Method and dilution technique (1g soil + 10ml sterile distilled water, and 1ml of suspension was inoculated to each plate) (Waksman, 1922). Sabouraud Dextrose Agar SDA, Potato Dextrose Agar PDA and Malt Extract Agar MEA were used for cultivation of fungi, and the plates were incubated at 25°C.

Identification of Fungi

The growing fungi were isolated in pure cultures for easy identification and determine their characteristics in terms of shape and colour. The microscopic detection was conducted for them after staining the slides with Lactephenol. The identification of isolated fungi to species level as possible was on the basis of macro morphological characteristics, for instance time of colonies growth and the colour, additional to the micro morphological characteristics such as the fungal hyphae and their reproductive structures. The macro and micro morphological characters of isolated fungi were compared with several reference books (Domsch, Gams and Anderson, 1980; Alexopulos, Mins and Blackwell, 1996, Brooks, Butle and Morse, 1998 & Carlile, Watkinson and Gooday, 2001).

Rates of the isolated fungi from the tested soil fractions, as well as the chemical and physical properties of the soil were represented graphically using Excel system histogram. Using SPSS Statistics, Version 23, the International Business Machines Corporation (IBM) to find out if there are a correlation between the factors of organic compounds, salinity, humidity and pH, and the isolated fungi performed Chi-Square Test.

3.Results

3.1 Chemical and Physical Analysis for Soil Samples

Results of the organic compounds analysis show that the highest ratio was in region 8 with a depth of 10 cm (7.4072), and the lowest was in region 7 with a depth of 10 cm (2.1404). Region 3, the surface part, is the highest in salinity rate (6.1672), while region 7 with a depth of 10 cm is the lowest in salinity rate (0.0420). Region 5 with a depth of 5 cm displays the highest moisture content (4.5099), while the lowest is observed in region 7 with a depth of 10 cm (0.8693). The highest pH was recorded for region 7 with a depth of 5 cm (9.10), and the lowest pH was recorded for region 4 with a depth of 10 cm (7.80) (Table 1) (Images 1, 2, 3 & 4).

Table 1: Value of the organic compounds, salinity, moisture and pH in soil

Region	Org Com	Salinity	Moisture	pH
1	2.8471	0.0708	2.5275	8.15
2	3.1597	0.2232	2.0788	8.29
3	2.4982	0.0756	0.9683	8.23
4	2.3828	2.1636	3.4984	8.82
5	3.0937	2.1092	1.5824	8.83
6	2.5053	1.3672	1.7576	8.87
7	5.2888	6.1672	3.8303	8.34
8	6.5347	1.22	2.68	9.95
9	3.217	0.0898	2.7764	8.77
10	5.4863	1.4032	2.8606	8.94
11	2.8329	1.3716	1.8707	8.9
12	2.842	1.372	1.788	7.8
13	3.6502	0.326	1.0705	8.28
14	7.151	2.9048	4.5099	8.61
15	3.374	0.3668	1.2085	8.69
16	3.844	3.2408	2.612	8.06
17	3.6071	0.1152	2.1175	8.1
18	2.6971	0.915	1.9406	8.15
19	2.4399	1.5604	1.8513	8.93
20	2.1889	1.5732	1.4512	9.1
21	2.1404	0.042	0.8693	8.2
22	2.7979	0.9252	2.8505	8.12
23	2.797	0.915	2.8406	8.11
24	7.4072	0.148	1.555	7.95
25	3.4086	1.1508	2.0833	8.26
26	3.4086	1.1508	2.0833	8.37
27	2.9202	3.7272	2.4924	8.13

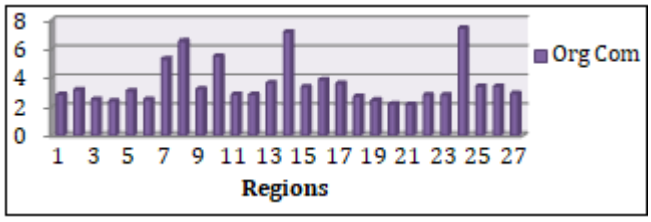


Image 1: Value of the organic compounds in soil

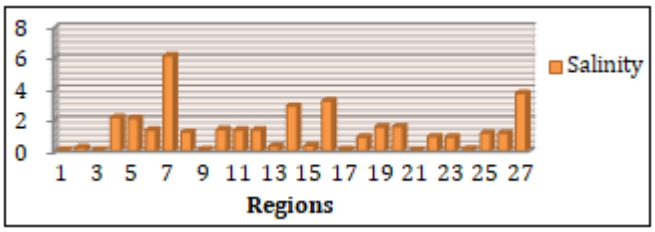


Image 2: Rate of salinity in soil

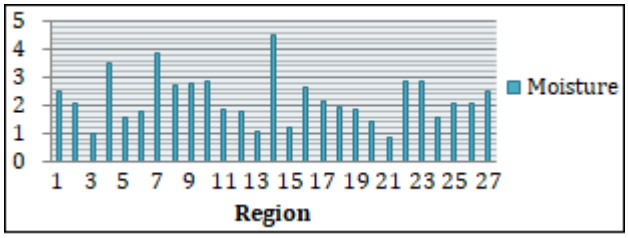


Image 3: Rate of the soil moisture

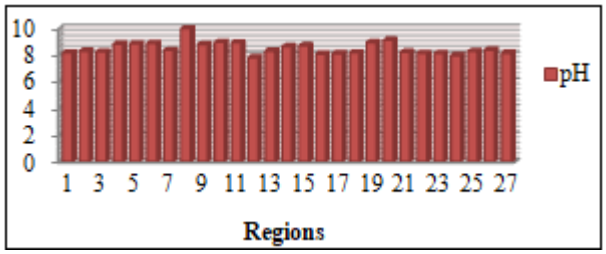


Image 4: Rate of soil pH

3.2 Isolation of Fungi

Several fungi were observed to grow in the nine soil regions of Ghemins, and according to dividing each region into three parts according to the depth (Table 2 & Images 5, 6, 7, 8, 9, 10, 11, 12, 13) (ratios of the recorded fungi are approximate). This study found that the almost most fungal growth are in regions; 4 in all its parts (*Fusarium sporotrichioides*, *Phytophthora infestans*, *Penicillium digitatum*), 5 with a depth of 10 cm (*Fusarium oxysporum*), 6 with a depth of 5 cm (*Fusarium oxysporum*) and 10 cm (*Alternaria alternata*), and at the surface of regions 7 (*Fusarium graminearum*) and 8 (*Alternaria tenuissima*). There is no any fungal growth appeared in region 2 with a depth of 5 cm. The almost lowest fungal growth are found in regions; 3 with all its parts (*Aspergillus clavatus* and *Alternaria tenuissima*), especially at the depth of 10 cm, the surface part of region 6 (*Aspergillus candidus*), and the two parts of region 9 at the depth of 5 cm (*Aspergillus ochraceus*), followed by the depth of 10 cm (*Alternaria alternate* and *Fusarium oxysporum*).

Chi-Square Test results show a huge significant association between organic compounds, salinity, humidity and pH with the percentage of isolated fungi.

Table 2: The Isolated fungi list

Region	*Fungus*	*Ratio*
1	*Phyllactinia rigida, Fusarium oxysporum*	10%, 50%
2	*Penicillium chrysogenum, Alternaria alternate*	5%, 40%
3	Penicillium chrysogenum, *Fusarium oxysporum*	5%, 30%
4	*Penicillium canescens*	10%
5	Non	Non
6	*Penicillium canescens, Mucor piriformis*	5%, 75%
7	*Aspergillus clavatus, Alternaria tenuissima*	5%, 5%
8	*Aspergillus clavatus, Alternaria tenuissima*	5%, 5%
9	*Aspergillus clavatus*	5%
10	*Chaetomium bostrychodes, Fusarium sporotrichioides, Fusarium oxysporum*	40%, 90%, 5%
11	*Penicillium digitatum, Phytophthora infestans*	50%, 90%
12	*Fusarium oxysporum, Penicillium digitatum*	40%, 90%
13	*Aspergillus candidus, Aspergillus niger, Aspergillus ustus, Rhizoctonia solani*	20%, 5%, 20%, 10%
14	*Aspergillus niger, Fusarium oxysporum*	70%, 5%
15	*Penicillium digitatum, Fusarium oxysporum, Aspergillus niger*	5%, 90%, 10%
16	*Aspergillus candidus*	5%
7	*Fusarium oxysporum, Aspergillus niger, Mucor piriformis, Alternaria alternata, Penicillium digitatum*	90%, 5%, 40%, 40%, 5%
18	*Alternaria alternata, Fusarium sporotrichioides, Penicillium digitatum, Fusarium oxysporum*	90%, 5%, 5%, 5%
19	*Fusarium graminearum, Aspergillus fumigatus, Penicillium digitatum*	90%, 5%, 10%
20	*Fusarium graminearum, Mucor piriformis*	60%, 50%
21	*Fusarium graminearum, Mucor piriformis*	60%, 50%
22	*Fusarium sporotrichioides, Penicillium digitatum, Alternaria tenuissima*	20%, 80%, 90%
23	*Fusarium sporotrichioides, Alternaria tenuissima*	20%, 80%
24	*Fusarium sporotrichioides, Fusarium oxysporum*	50%, 60%
25	*Aspergillus flavus*	10%
26	*Aspergillus ochraceus*	5%
27	*Alternaria alternata, Fusarium oxysporum*	5%, 5%

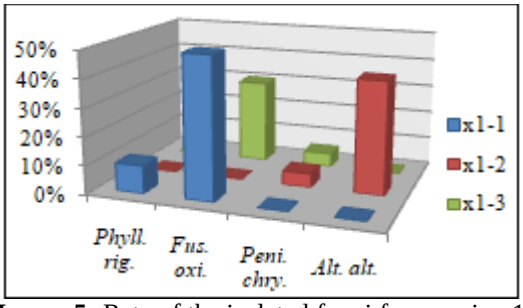


Image 5: Rate of the isolated fungi from region 1

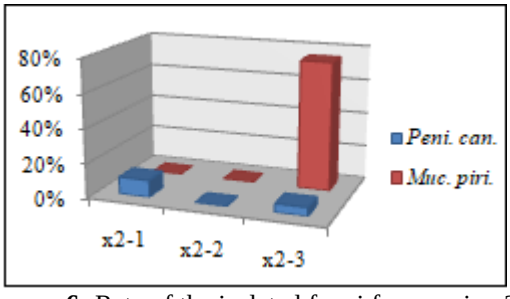


Image 6: Rate of the isolated fungi from region 2

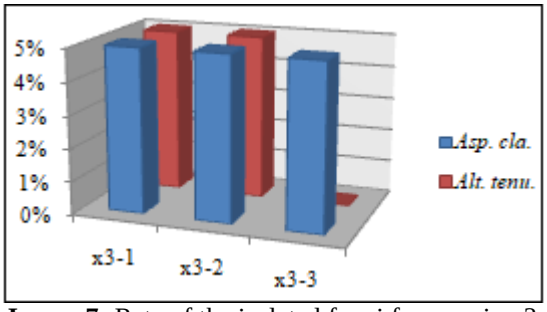


Image 7: Rate of the isolated fungi from region 3

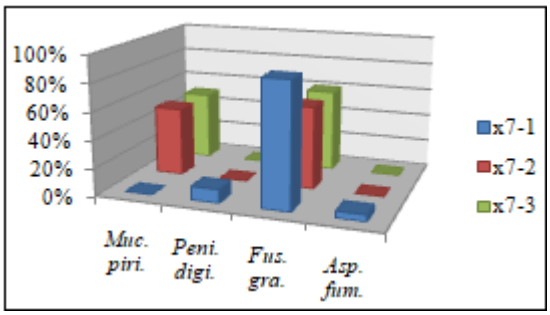


Image 11: Rate of the isolated fungi from region 7

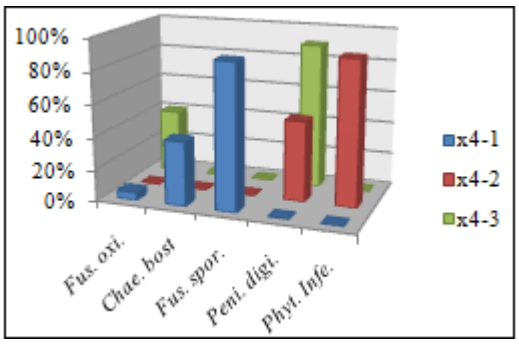


Image 8: Rate of the isolated fungi from region 4

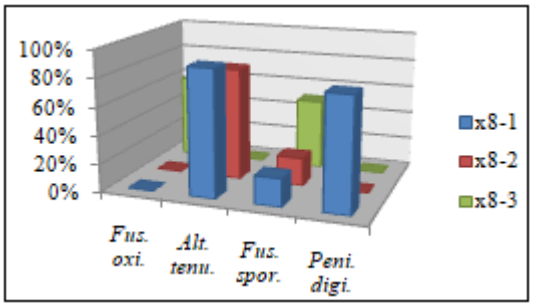


Image 12: Rate of the isolated fungi from region 8

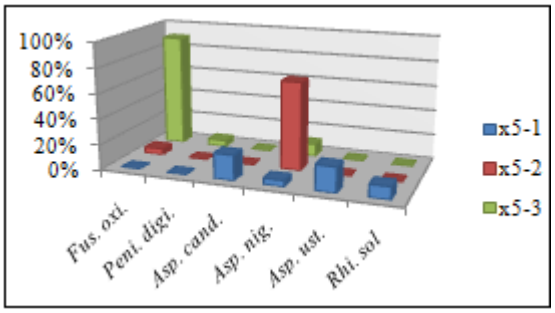


Image 9: Rate of the isolated fungi from region 5

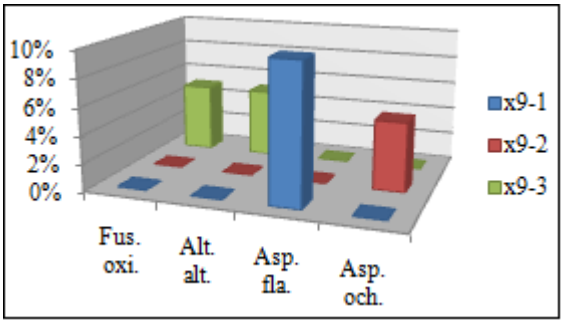


Image 13: Rate of the isolated fungi from region 9

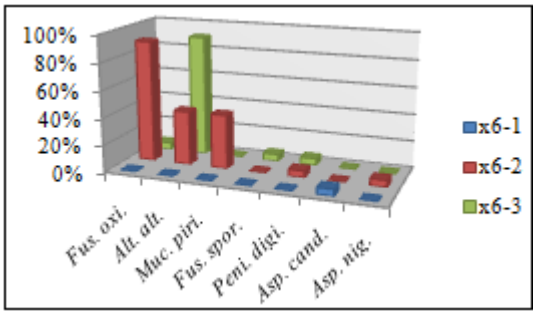
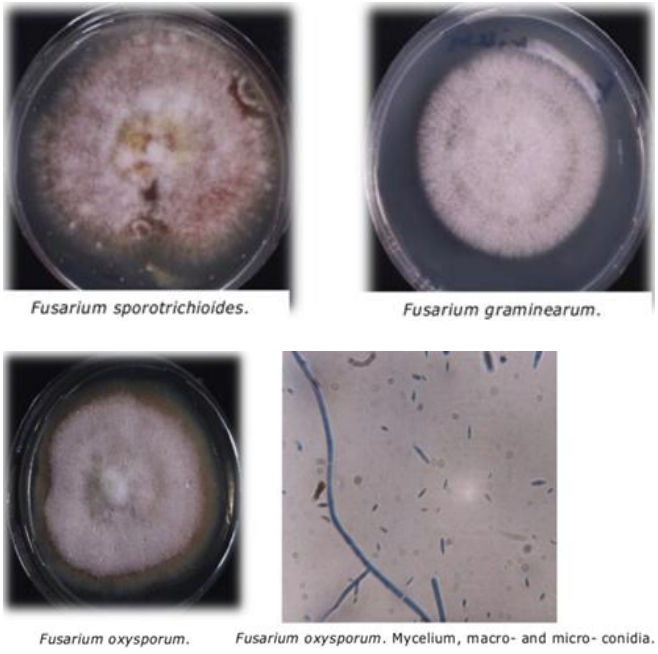
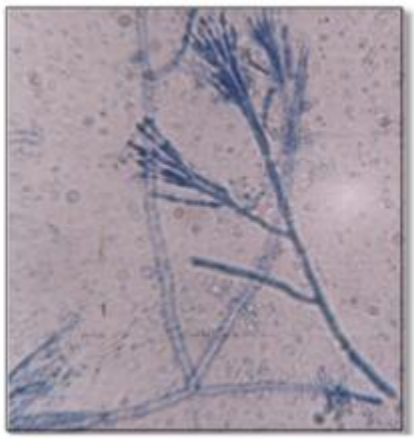


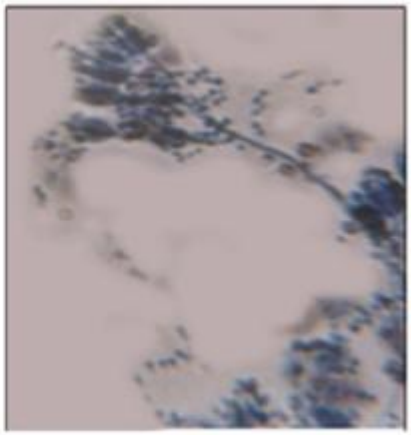
Image 10: Rate of the isolated fungi from region 6



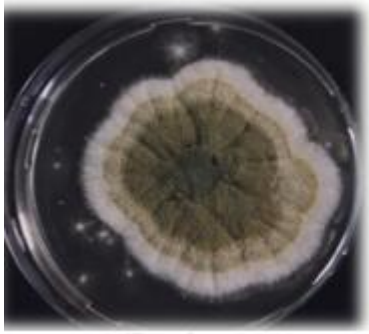
Penicillium chrysogenum.



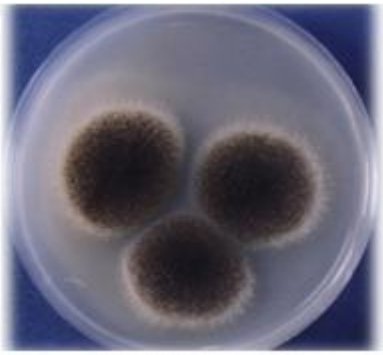
Penicillium chrysogenum. Penicillus with conidial chains.



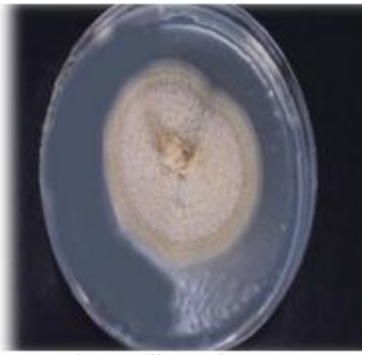
Penicillium canescens. Penicilli and conidia.



Aspergillus clavatus.

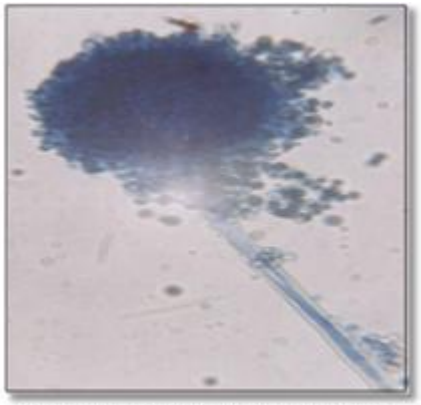


Aspergillus niger.



Aspergillus ochraceus.

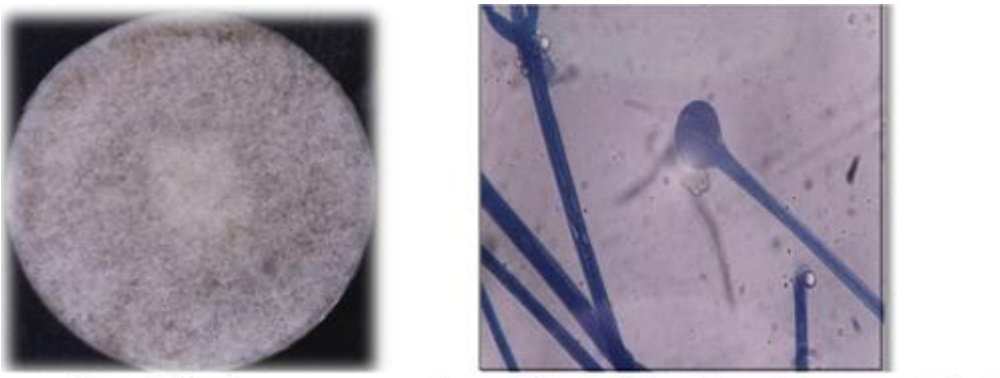
Aspergillus flavus.



Aspergillus flavus. A conidial head.

Aspergillus fumigatus.

Aspergillus fumigatus. A conidial head



Images of some isolated fungi

4.Discussion

Range of the organic compound contents is 2.1404-7.4072, the salinity is 0.0420-6.1672, the moisture is 0.8693-4.5099, and pH is 7.80-9.10. El-Said and Saleem, 2008 found that the organic compound contents, the salinity, the moisture and pH values ranged between (0.54-1.71), (0.48-8.89), (3.6-15.5) and (4.5-7.3) respectively. In this study it is evident the great effect of each of the organic compounds, salinity, moisture and pH on the growth of soil fungi, which is shown by the results of the Chi-Square Test, as the results showed a strong significant correlation between them.

The regions of the tested study were distinguished by growth of several fungal genera, *Aspergillus spp., Alternaria spp., Chaetomium sp., Fusarium spp., Mucorsp., Penicillium spp., Phyllctinia sp., Phytophthora sp.* and *Rhizoctonia sp.* That almost similar to result study of El-Said and Saleem, 2008 that conducted in Western region in Libya, in which soil samples were collected from the desert and the Mediterranean sea coast, which showed the growth of *Alternaria, Aspergillus, Emericella, Fusarium, Mycosphaerella, Nectria* and *Penicillium*. Another study conducted in the South of Libya by Altayyar *et al.*, 2016 also isolated *Aspergillus* species (*A. niger, A. fumigatus* and *A. flavus*, additional to *A. nidululans* and *A. terreus*), *Mucor spp* and other genera were not isolated in the current study (*Acremonium spp., Chrysosporium spp., Trichoderma spp. and Microsporum spp.).*

Gaddeyya *et al.*, 2012 isolated15 species belonging to 6 genera of fungi from agricultural fields at Salur Mandal, India (*Aspergillus spp., Penicillium spp., Trichoderma spp., Fusarium spp., Curvularia spp*. and *Rhizopus sp*) by using soil dilution technique and soil plate technique. Kumar et al., 2015 isolated *Aspergillus flavus, A. fumigatus, A. nidulans, A. niger, A. terreus, Penicillium chrysogenum, P. frequentens* in their study on soil samples that collected from different rice fields at Tekkali region of Srikakulam District.

Fungi species belonging to the genera *Aspergillus, Penicillium, Colletotrichum, Mucor, Rhizopus, Cunninghamella, Scopulariopsis* and *Cladophialophora* were isolated from soil samples collected from 20 zones in Loyola college campus, Chennai, and the method that was used is soil dilution method beside staining technique by using Lactophenol and cotton blue (Raja, Praveena and William, 2017). Alwakeel, 2017 isolated *Aspergillus, Penicillium, Thielavia, Fusarium, Emericella, Cladosporium, Scytalidium* and *Alternaria* from seventy-seven samples were collected from coastal regions of Red Sea, and the identification was by using both phenotype and genotype techniques.

Results of a research was conducted in Algeria by Chamekh *et al.*, 2019, on two types of soil, demonstrated that pH values are almost in agreement with the current study (7.2-8.2). In contrast to the results of salinity which ranged between 3.8-46. Furthermore, the same researchers isolated genera of fungi similar to the genera that isolated in the current research, for instance *Aspergillus spp., Penicillium spp., Fusarium spp.* and *Alternaria*. More recently, 41 isolates of actinomycetes (*Streptomyces spp*., *Nocardia spp.* and *Micro-monospora spp*.) were isolated and identified from 11 soil samples collected from different sites in Nepal (Sapkota et al., 2020).

5.Conclusion

Ghemins region, which located on the eastern coast of the Mediterranean Sea, in the south of Benghazi, distinguishes, according to results of the chemical and physical analysis of this research, by its medium fertility soil. There are various genera and species of fungi live in the soil of study area, that probably produce important compounds like antibiotic.

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