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Diversity and Biotechnological Applications of Some Fungi that Isolated from Unusual Soil Samples in Egypt

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Abstract: Thirty fungal isolates, which include nineteen species and belong to five genera, were isolated from unusual soil samples from Assiut and Wadi El-Natrun, Egypt. All fungal cultures were cultivated on YES medium for secondary metabolites production; the culture filtrates were separately subjected to solvent extraction by: Ethyl Acetate, Petroleum Ether, Dichloromethane and Dichloromethane: Methanol (2: 1); the fungal mat was extracted by Dichloromethane: Methanol (2: 1) only. The obtained Ethyl Acetate extracts of all fungal isolates were tested for their antimicrobial activity against six different pathogenic bacterial strains; three of them were gram positive bacteria (*Staphylococcus Aureus*, *Bacillus Cereus*, and *Streptococcus Pneumonia*); the others were gram negative bacteria (*Sertatia Marcescens*, *E. Coli* and Salmonella *Typhi*). In addition, against five pathogenic fungal strains (*Aspergillus Candidus*, *Aspergillus Flavus*, *Aspergillus Niger*, *Aspergillus Terreus* and *Geotrichum Candidum*), the tested Ethyl Acetate fungal extract showed marked antimicrobial activity against most of the tested strains. On the other hand, the highest antibacterial activity was recorded in case of the *Penicillium Chrysogenum* extract, but the highest antifungal activity was recorded in case of the *Penicillium Aurantiogriseum* extract. The secondary metabolites of all fungal isolates were separated on TLC plates, and the most four active strains were completely identified as *Aspergillus Candidus*, *Aspergillus Terreus*, *Penicillium Aurantiogriseum* and *Penicillium Chrysogenum*. Furthermore, the antimicrobial, anti-insect, anti-herbal and anti-tumor activities of its extracts were evaluated.

Keywords: Fungi, Diversity, Biotechnological applications, Secondary metabolites, TLC, Anti-bacterial, Anti-fungal, pesticide, herbicide and Ant-itumor.

1 Introduction

The current study addresses the biotechnological application of biological systems and organisms, including their technical and industrial processes **[3].** In this regard, many industrial processes have used the different types of fungi, such as the production of enzymes, vitamins, polysaccharides, polyhydric alcohols, pigments, lipids and glycolipids. Thus, the production of some of these products was com-metrically, while other types are potentially valuable in the field of Biotechnology.

The treatment of infected wounds and cuts in ancient Egypt through the application of moldy bread is considered to be the first recorded medical use of fungi **[23].**

As for the biological activity, we find that it is present in about 70-80% of the secondary metabolites which have been isolated from fungi **[10].** In addition, the increasing number of the secondary metabolites isolated from the different varieties of fungi indicates that they are a rich and abundant source of bioactive compounds with therapeutic potential **[11]**. Thus, they could be considered as a valuable source for various pharmaceutical applications with highly complex structures, due to their various biological activities, which in turn makes it difficult to supply them economically by chemical synthesis **[5].**

Moreover, a wide range of natural products with high therapeutic value could be produced out of the different types of fungi, such as antibiotics, cytotoxic substances, insecticides, compounds that inhibit growth, attractors, repellents, etc. The natural products produced from fungi vary in production, function and specify to a particular fungus; thus, the different fields of medicine and industries

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have been using these metabolites **[16].**

There is a wide range of diverse sources for fungi, so are the compounds themselves and their biological functions that are usually highly specific. The fungi which have been isolated from the soil environment have shown that there is a massive potential as suggested by the diversity of secondary metabolites **[5].**

Taking into consideration its nature as a special ecosystem, a soil could supply plenty of fungal resources, yielding various secondary metabolites with novel structures and interesting biological activities **[4, 14].**

2 Materials and Methods

Ten contaminated soil samples were collected from Wadi El-Natrun, Behira Governorate, about 150 Km from Cairo. The soil samples were collected at a depth of 30.0 cm from the soil surface. After collecting the soil samples, the plant rests were separated, and the soil samples were dried, grounded and sieved, then they were used for isolating the fungi.

2.1 Isolation of soil fungi

The fungi were isolated by the Direct Plating method **[26, 6]** on a Czapek's Dox agar medium (mentioned hereafter) plates amended with an antibacterial agent (Chloramphenicol, 0.5 g/L). Then, the autoclaved, Czapek's Dox agar medium was poured in sterilized Petri dishes, after solidification, 0.5g of the soil samples, which are inhabited by fungi, which were placed directly over the surface of the solidified Czapek's Dox agar medium. The plates were then incubated at 28°C ±2 for 7–15 days**.** Every single fungal colony exerted was purified, identified and saved by transferring to a pure slant containing the same medium; hence, after the incubation period, the slants were stored at 4°C, for further testes. A light microscopic examination of the obtained fungal isolates was used in order to check up the purity of the isolates. Preliminary identification of these fungal isolates was made in order to exclude the repeated genera and species.

2.3 Media used for isolation, purification and identification of fungi

Czapek's Dox Agar Medium: This medium contains (g/L) the following: Sucrose 30.0, Sodium Nitrate (NaNO3) 3.0, Di-Potassium Hydrogen Orthophosphate (K2HPO4) 1.0, Potassium Chloride (Kcl) 0.5, Magnesium Sulfate (MgSO4.7H2O) 0.5, Ferrous Sulfate (FeSO4.5H2O) 0.001, and Agar-Agar 15; and these components were dissolved and completed to one liter by distilled water [1]. The pH of the medium was adjusted at 5.5-6.0 by Lactic Acid (10%, v/v).

Malt Extract Agar (MEA) Medium: According to **[22],** this medium contains (g/L) the following: Malt Extract 20.0, Peptone 1.0, Glucose 20.0, Agar-Agar 20.0 and distilled water up to 1 L. The pH of the medium was adjusted to 5.5, then autoclaved at 121°C for 20 min.

2.4 Cultivation of isolates for production of secondary metabolites

As for the production of secondary metabolites, I used yeast-extract sucrose (YES) in the form of liquid medium. The YES medium contains (g/L) the following: Yeast Extract 20.0, Sucrose 150.0, ($MgSo_4.H_2o$) 0.5 and distilled water up to 1.0 L. The medium was distributed in two 250 ml Erlenmeyer flasks, each containing 100 ml of the medium. The pH was adjusted to 6.5 ±0.2. Then, the medium was autoclaved at 121ºC for 15 min. Five liters were used for each fungal strain. The YES medium (100 ml) was inoculated with 1 ml spore suspension of the studied fungus, and incubated at 25ºC for 21 days **[8].**

2.5 Extraction of extracellular secondary metabolites

By the end of each incubation period, the inoculated flasks were collected. The culture filtrates were separately subjected to solvent extraction as previously described by **[27]** with slight modifications as follows:-

For each fungus the whole broth (5 L) was filtered through Whatman Filter Paper No. 1 in order to separate the culture filtrate and mycelia. The culture filtrates were mixed with Ethyl Acetate in a separating funnel, shaken vigorously and well and left at least six hours until the complete separation **[17].**

2.6 Antimicrobial activity assay

2.6.1 Screening for antibacterial activity assay

A test was conducted for the antibacterial activity of the obtained Ethyl Acetate extract, with regard to all fungal isolates; and that is against six different pathogenic bacterial strains; three of which were gram positive bacteria, specifically: *Staphylococcus Aureus*, *Bacillus Cereus and Streptococcus Pneumoniae*; in addition, three of them were gram negative bacteria, specifically: *Sertatia Marcescens, E. Coli and Salmonella Typhi*. Then, the tested bacterial cultures were inoculated in Petri dish, then poured with sterilized nutrient agar medium, then allowed to solidify. After that, the filter paper discs were saturated with fungal crude extract and transferred into the Petri dish. Thus, all plates were incubated at 37 ºC for 24 h; after the end of the incubation period, all plates were observed for formation of inhibition zone around the discs [9, 18].

2.6.2 Screening for antifungal activity assay

A test was conducted for the antifungal activity of the obtained Ethyl Acetate extract, with regard to all fungal isolates; and that is against five pathogenic fungal strains, specifically: *Aspergillus Candidus*, *Aspergillus Flavus*

Aspergillus Niger, *Aspergillus Terreus* and *Geotrichum Candidum*. Then, the tested fungal cultures were inoculated in sterilized Petri dishes, then poured with sterilized Czapkes medium, then allowed to solidify. After that, wells of 0.5 cm were made in the medium using sterilized cork borer. Then, 150 µl of the fungal crude extract was transferred into the wells. Thus, all plates were incubated at 28 ºC for 3-5 days; after the end of the incubation period, all plates were observed for formation of inhibition zone around the wells [9, 18].

2.7 Further studies based on selected isolate

The most active strain for antimicrobial efficacy was selected for further studies, in order to evaluate its antitumor activity, Seed Germination-Influencing Bioactive Secondary Metabolites on lettuce seeds, and the effect of the extracellular secondary metabolites of fungi on the death of larvae *Musca Domestica*.

2.8 Identification of the most potent bioactive isolates

Assuit University, Mycology Centre (AUMC) performed this identification based on morphology and microscopic characters **[19].**

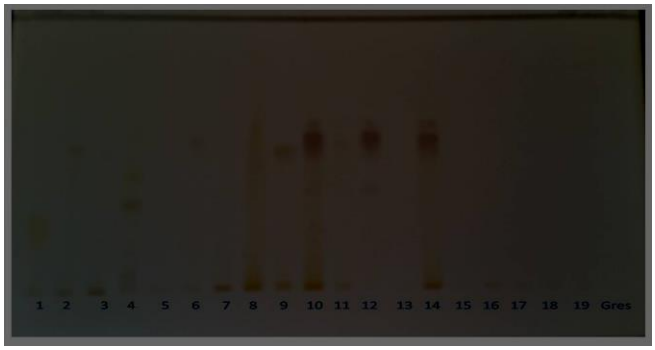


Fig. (1): Separation of secondary metabolites on TLC plate in visible light. .

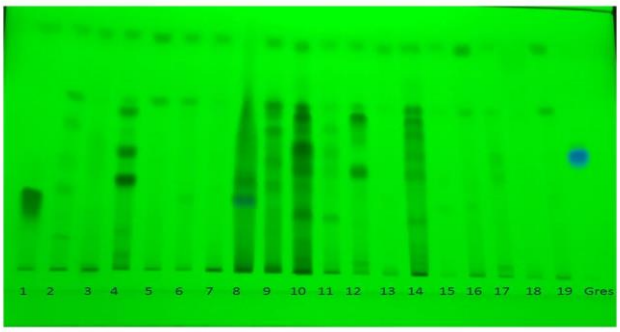


Fig. (2): Separation of secondary metabolites on TLC plate Band under short wave length UV light.

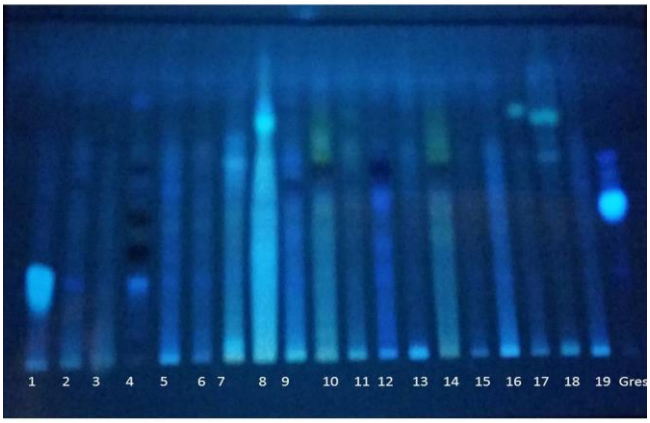


Fig. (3): Separation of secondary metabolites on TLC plate under long wave length UV light.

2.9 Identification of extracellular secondary metabolites on TLC

"Extracted samples from all fungi were applied on Thin Layer Chromatography (TLC) plates (silica gel G-60 aluminum sheet - Merck, Germany), using CAMMAG LINOMAT 5 application system" (TLC scanner unit at RCMB, Al-Azhar University); and Griseofulvin was used as a reference standard. The plates were developed for 17 cm using Toluene, Ethyl Acetate, 90% (w/v) formic acid (5:4:1, v/v/v) (TEF) as a solvent system. The TLC plates were removed from the solvent system and dried at the room temperature. Then, the plates were examined in visible light, under long UV light (365 nm), short UV light (254 nm) and back under long UV light (365 nm).

2.10 Effect of Extracellular secondary metabolites of fungi on the death of larvae Musca domestica

2.10.1 Sample collection

After three days of fermentation, chicken viscera were used as bait for collecting adult-stage flies in an open area through hand catch by a net, which was performed on May 2016. The process began in mornings and continued until a sufficient number of specimens for the colonization process, was captured. The samples were caught from different places including gardens and around livestock, in Al-Azhar University, Assiut; and they were sent to the laboratory (Zoology Department) in the Faculty of Science, in appropriately labeled tubes.

2.10.2 Rearing of flies in the laboratory:

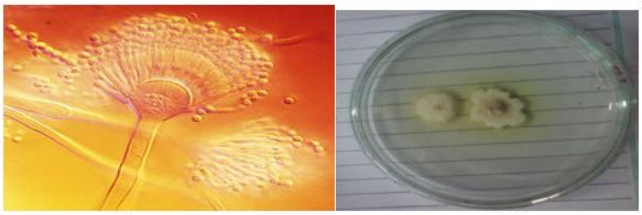
An experimental study was performed on May 2016. The study included adult insects, 40 flies: (25 females and 15 males). The adult insects were kept in 40 × 40 × 40 cm cages. The flies were maintained in the laboratory under controlled conditions of mean temperature of 30±4°C, relative humidity of 80±10%, and daily light /dark period of 12 h, protected

with an external net curtain to avoid the entry of other insect species. The flies were fed on 150 g of diets placed in the Petri dish (15×15 cm), 20 ml carbohydrate-rich source, and 30% sucrose in a Petri dish (10×10 cm) containing cotton. After the eggs were laid and hatched, during the larval stage developments, I took 10 individuals from the second larvae stage, and put them in a Petri dish (10×10 cm) containing 100 g of meat as a control group; then, I made replicates of three Petri dishes (10×10 cm) containing 10 individuals from the larvae stage, and 100 g of meat for four fungi extracellular secondary metabolites of *Asperillus Candidus, Asperillus Terreus, Penicillium Chrysogenum and Penicillium Aurantiogriseum*. Then, I injected 1 ml from the extracellular secondary metabolites fungi. Supervising the rearing cages was essential; in addition to taking a count number of the second stage larvae at a visit every four hours. Intervals of larvae were made for 24 hours; and I recorded the larvae that were dead in each extraction.

2.11 Effect of Fungal Strains on Germination of lettuce seeds

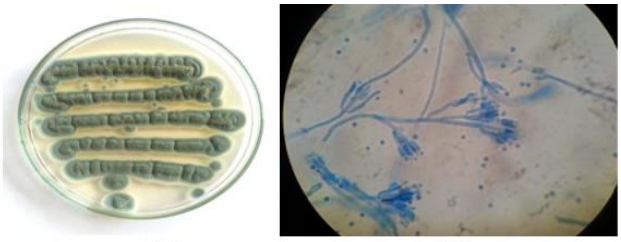
2.11.1 Collection of seed samples

The lettuce seeds samples were collected from the commercial lots that were used for sowing in the seed samples lettuce farms; then, I sterilized the seeds by soaking for 5 min in a 1% Sodium Hypochlorite solution. Then, the seeds were kept dry on Whatman Filter Paper No. 1 under a laminar flow hood; and that is after rinsing with sterile distilled water.



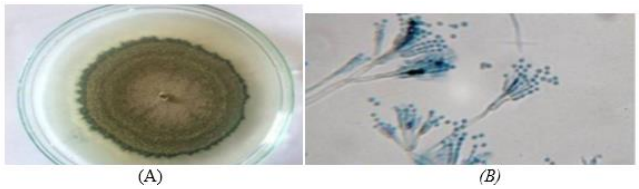
(B) (A)

Fig. (4): (A) I Cultivated of *Aspergillus terreus on Czapek-Dox agar medium, (B) under light microscope X40, C-X 100)*



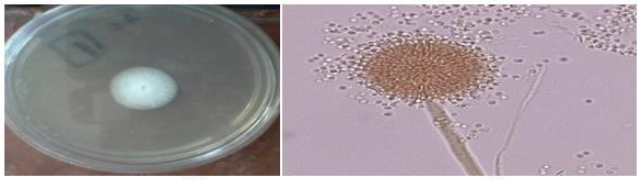
(A) (B)

Fig. (5) : (A) I Cultivated of *P. chrysogenum* Thom AUMC 10510 on Czapek-Dox agar medium, (B) under light microscope with cotton blue stain X40, C-X 100)



(A) (B)

Fig. (6): *(A)* I Cultivated *Penicillium aurantiogriseum on Czapek-Dox agar medium, (B) under light microscope with cotton blue stain X40, C-X 100)*



(A) (B)

Fig. (7): (A) Cultivated *Aspergillus candidus* Link *on Czapek-Dox agar medium, (B) under light microscope).*

After that, I put the lettuce seeds in water, and I cut the glass fiber filter papers (GF/C) discs (8 mm diameter); in order to sterilize and prepare them for further use, they were wetted thoroughly with water and placed in an oven at 100 ºC for 1 h. Then, I used 5 ml of distilled water to moisten the sterilized discs, which were placed in 9 cm petri dishes and lined with the glass fiber filter papers (GF/C). Then, I injected 1 ml from the filtrate extracellular secondary metabolites fungi with different concentrations of filtrate (100%, 50%, 25%, 12.5% & 6.5%); and I used treatment sterile distilled water with the control group. Culture filtrates of *Asperillus Candidus, Asperillus Terreus, Penicillium Chrysogenum and Penicillium Aurantiogriseum* were significantly inhibitory towards the germination of the lettuce seeds. I placed thirty seeds in 24 petri dishes containing three layers of blotters moistened with 5 ml of distilled water, set to germinate at 20±2 °C in the incubator; and I observed them daily for 6 days for germination. The germination percentage was recorded daily from one to six days**.**

2.12 Determination of antitumor activity

The crude extract of *P. Chrysogenium* was tested for its inhibitory against three tumor cells lined against hepatic cellular carcinoma (HEPG2); taking into consideration that the Human Breast Cancer cell line is (MCF-7), and the human colon carcinoma cells are (Caco-2 cells). The crude extract of *P. Chrysogenium* was dissolved in 1 ml of DMSO (10 mg/ml). Then, after the confluent sheet of cells was formed, a growth medium was decanted from 96 well micro titer plates. After that, a wash media was used to wash the cell monolayer twice. A RPMI medium was used to made two-fold dilutions of extract (CAISSON RPMI 1640 Medium) with 2% serum (as a maintenance medium). Then, I tested 0.1 ml of each dilution in various wells leaving 6 wells as a control group, and receiving only the maintenance

medium. I incubated the plate at 37°C, and I examined it frequently for up to 3 days. I checked the cells for any physical signs of toxicity, (e.g., partial or complete loss of the monolayer, rounding, shrinkage or cell granulation). Then, I prepared MTT solution (5mg/ml in PBS) (BIO BASIC CANADA INC.); and I added 20 ul of the MTT solution to each well. It was placed on a shaking table, (150 rpm for 5 minutes), to thoroughly mix the MTT into the media. After that, it was incubated (37C, 5% CO2) for 1-5 hours to allow the MTT to be metabolized, and then dumped off the media. I used a dry plate on paper towels in order to remove the residue when necessary. Then, the formazan was re-suspend (MTT metabolic product) in 200 ul DMSO, and placed on a shaking table, (150 rpm for 5 minutes), in order to mix the formazan thoroughly into the solvent. Finally, I read the optical density at 560 nm and I subtracted the background at 620 nm; thus, the optical density shall be directly correlated with the cell quantity **[21].**

3 Results and Discussion

As for the isolation of fungal cultures from different sources and the preliminary identification for these isolates, in the present investigation, thirty fungal isolates were isolated from different sources. The sources of the samples were from soil samples of two coastal environs along Assiut and Behira governorates at Wadi El-Natrun. The samples were inoculated on the Czapek´s agar solid medium, and amended with antibacterial agent; and the fungal colony was encountered daily within the incubation period. Thirty isolates comprising 19 species belonging to 5 genera were isolated and identified in this study (Table 1). Of all these, *Aspergillus* was the most dominant genus with 7 species represented by 14 isolates (46.66%) namely: *Aspergillus Flavus* (3), *Aspergillus Niger* (4), *Aspergillus Candidus* (1), *Aspergillus Terreus* (2), *Aspergillus Ustus* (1), *Aspergillus Ochereus* (2) *and Aspergillus Clvatus* (1); followed by *Penicillium* with 8 species represented by 10 isolates (33.33%) namely: *Penicillium Chrysogenium* (2),

Table (1): Isolation of fungal cultures from different sources and their identification.

No.	Fungi	Sources of isolation			
		Soil Contaminated with Sewage	Garbage waste soils	Benzene contaminated soil	Hypersaline soil of Wadi El-Natrun
1	*Aspergillus flavus var. flavus Link*	1	1	-	1
2	*Aspergillus niger VanTieghem*	1	1	-	1
3	*Aspergillus terreus var. terreus Thom*	-	1	-	1
4	*Aspergillus ustus (Bainier) Thom & Church*	-	-	1	-
5	*Aspergillus candidus Link*	-	1	-	-
6	*Aspergillus clavatus Desm*	-	-	1	-
7	*Aspergillus ochraceus G. Wilh*	1	-	-	-
8	*penicillium chrysogenium Thom*	1	1	1	-
9	*Penicillium citrinum Thom*	1	-	1	-
10	*Penicillium purpurogenum Flerov*	-	1	-	-
11	*Penicillium aurantiogriseum Dierckx*	1	-	-	1
12	*Penicillium brevicompactum Dierckx*	1	-	-	-
13	*Penicillium duclauxii Delacr*	-	1	-	-
14	*Penicillium crustosum Thom*	-	1	-	-
15	*Penicillium verrucosum Dierckx*	-	-	1	-
16	*Trichoderma sp Persoon*	-	1	-	1
17	*Fusarium solani (Mart.) Sacc.*	1	-	-	1
18	*Fusarium oxysporum Schltdl*	-	1	-	-
19	*Cladosporium herbarum (Pers.) Link*	-	-	-	1

Table (2): In vitro antimicrobial l activities of the ethyl acetate extracellular extracts obtained from all fungal tested isolates at 25 mg/l.

Tested org. / Isolates	Gram positive bacteria			Gram negative bacteria			Fungi strains				
	B. cereus	*S. aureus*	*S.pneumoniae*	*S. typhi*	*E. coli*	*S. marcescens*	*A. candidus*	*A. flavus*	*A niger*	*A terreus*	*G candidum*
Aspergillus flavus var. flavus Link	13.4±0.9	0	0	12.3±0.5	12.3±0.4	0	0	14.5±0.6	0	0	0
Aspergillus niger VanTieghem	0	0	0	11.6±0.7	13.0±0.6	0	10.2±0.7	9.2±0.9	0	0	0
Aspergillus terreus var. terreus Thom	12.3±0.4	0	0	16.4±0.5	13.5±0.4	16.1±0.5	0	9.0±0.7	10.6±0.8	12.3±0.5	0
Aspergillus ustus (Bainier) Thom &	0	0	0	0	0	0	0	0	0	0	0

Church											
Aspergillus candidus Link	12.2±0.5	11.3±0.6	11.1±0.4	12.6±0.9	14.2±0.4	0	0	9.0±0.7	9.0±0.7	0	10.2±0.8
Aspergillus clavatus Desm	13.0±0.4	0	0	0	0	0	0	0	11.0±0.7	0	0
Aspergillus ochraceus G. Wilh	13.2±0.5	0	11.2±0.5	15.2±1.1	0	12.5±0.5	0	0	0	12.0±0.5	0
penicillium chrysogenium Thom	14±0.7	13±0.7	13±0.4	14.6±0.9	13.1±0.6	11.0±0.4	0	13.3±0.4	0	10.5±0.7	0
Penicillium citrinum Thom	13.2±0.5	0	0	11.1±1.0	0	11.4±0.6	0	12.2±0.7	9.2±0.4	0	0
Penicillium purpurogenum Flerov	13.1±0.4	0	0	0	0	0	12.5±0.7	0	0	0	0
Penicillium aurantiogriseum Dierckx	12.3±0.4	11.4±0.4	11.6±0.4	12.4±0.9	13.1±0.4	0	11.3±0.8	13.2±0.5	13.2±0.5	0	0
Penicillium brevicompactum Dierckx	0	13.4±0.8	0	0	0	12.6±0.4	0	0	0	0	0
Penicillium duclauxii Delacr	13.2±0.4	10.1±0.7	0	0	0	13.2±0.5	0	12.5±1.3	0	10.4±1.0	0
Penicillium crustosum Thom	11.6±0.5	13.1±0.5	0	12.1±0.5	11.4±0.5	11.8±0.4	9.2±1.2	0	0	9.2±0.8	0
Penicillium verrucosum Dierckx	13.1±0.5	0	0	0	0	0	13.5±0.4	0	0	0	13.6±0.7
Trichoderma sp Persoon	13.2±0.8	0	12.7±0.9	0	13.4±0.4	14.0±0.6	0	0	13.2±0.4	10.4±0.7	0
Fusarium solani (Mart.) Sacc.	0	13.4±0.4	0	13.6±0.6	13.0±1.0	12.5±0.5	9.2±0.4	9.5±0.7	0	0	0
Fusarium oxysporum Schltdl	13.2±0.6	11.3±1.0	0	12.5±0.9	12.6±1.1	13.1±0.4	0	0	0	0	0
Cladosporium herbarum (Pers.) Link	0	13.4±0.5	0	13.0±0.5	11.4±0.9	0	0	13.5±0.7	0	0	10.2±0.4
*References standard	27.5±0.6	22.4±0.7	22.6±1.0	25.4±0.6	24.0±1.1	30.4±0.7	22.5±0.4	24.5±0.7	25.5±0.8	22.4±0.8	30.2±0.4

Chloramphenicol , Streptomycin ,Clotrimazole and Dermatin were used as standard drugs against the tested fungal ,Gram positive and Gram negative bacteria. Respectively.

Table (3): In vitro inhibitory activity of the of the ethyl acetate fraction from P. *chrysogenum* Thom AUMC 10510 against the three tumor cell lines tested.

sample	**Cell lines**	**MCF-7**		**Caco-2**		**HepG-II**	
	Sample Conc.(µg/ml)	viability %	cytotoxicity	viability %	cytotoxicity	viability %	cytotoxicity
Cell control	**……**	100	0	100	0	100	0
sample	500	0	100	0	100	0	100
	250	0	100	0	100	0	100
	125	0	100	0	100	0	100
	62.5	0	100	0	100	0	100
	31.25	1.590	98.409	0	100	0.347	99.65
	15.65	2.272	97.727	0	100	0.694	99.30
	7.87	3.282	96.717	0	100	1.388	98.61
	3.93	4.040	95.959	2.252	97.747	5.208	94.79
	1.96	9.343	90.656	20.870	79.129	15.277	84.72
	0.98	35.606	64.393	52.102	47.897	47.916	52.08
	0.49	82.070	17.929	92.042	7.957	87.5	12.5
	0.24	98.484	1.515	98.484	0	100	0

Penicillium Citrinum (2), *Penicillium Purpurogenum* (1), *Penicillium Aurantiogriseum* (1), *Penicillium Brericompactum* (1), *Penicillium Duelauxi* (1), *Penicillium Crustosone* (1) and *Penicillium Verrucosum* (1); followed by *Fusarium* with 2 species represented by 3 isolates (10.0 %) namely: *Fusarium Solani* (2) and *Fusarium Oxysporum* (1); followed by *Trichoderma Harzainum* (1) and *Cladosporum Herbamum* (1). Similarly, **[2]** new biologically active metabolites were obtained from 26 fungal isolates, collected from different locations of Alexandria coast, Egypt with *Penicillium* as the most frequently genus isolated. **[20]** A total of 64 colonies of different *Keratinophilic* fungi were isolated from 48 soil samples.

3.1 Screening of the antimicrobial activities of the fungal isolates.

In the present investigation, the extracellular metabolites obtained from the fungal isolates were extracted by Ethyl Acetate, obtained from the fungal isolates. The crude solvent extracts were concentrated to dryness then tested for antimicrobial activities, using the well diffusion method. The resulted inhibition zones were measured (in terms of mean diameter by mm), after the incubation of the tested microorganisms with 1 mg/ml concentration from each extract obtained from the tested fungal isolates. The results were summarized in Table (2). Out of the represented data, it was found that most of the fungal secondary metabolites (extracts) have antimicrobial activity, where the highest antimicrobial activity were recorded in case of *Aspergillus Terreus, Aspergillus Candidus, Penicillium Aurantiogriseum and Penicillium Chrysogenium;* while, no antimicrobial activity was recorded in the case of *Aspergillus Ustus;* hence, it has no activity at all against the bacterial or fungal tested organisms. Similarly, the fungi of the genus *Penicillium* are considered as promising objects in the search for new biologically active compounds [7, 25]**.**

3.2 Effect of Extracellular secondary metabolites of fungi on the death of larvae Musca Domestica

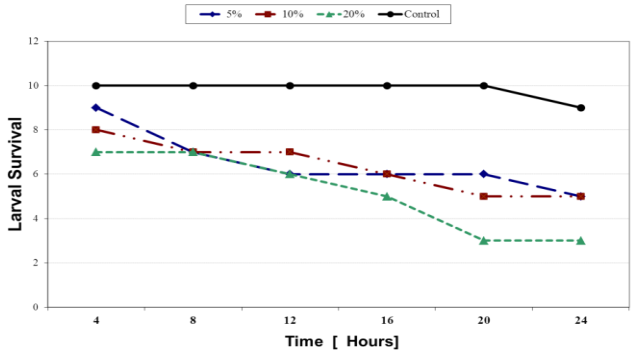


Fig. (8): Effect of extracellular filtrate *P. chrysogenium* on larvae of *Musca domestica.*

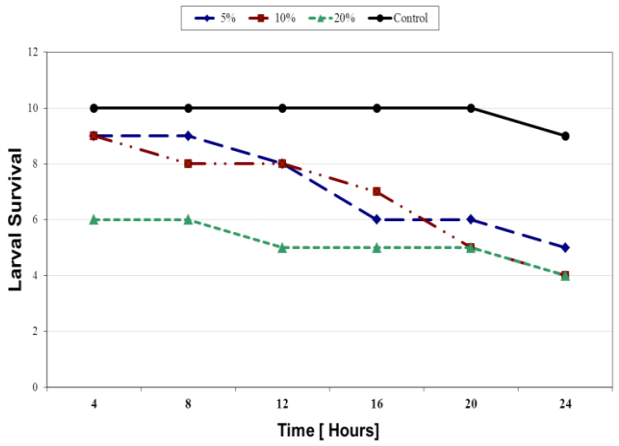


Fig.(9): Effect of extracellular filtrate of *P aurantiogriseum* on the death of larvae Musca *domestica.*

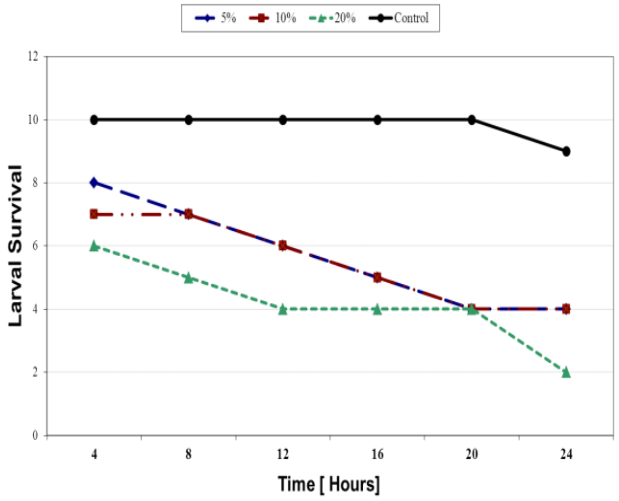


Fig. (10): Effect of filtrate *A terreus* on the larvae of Musca *domestica*

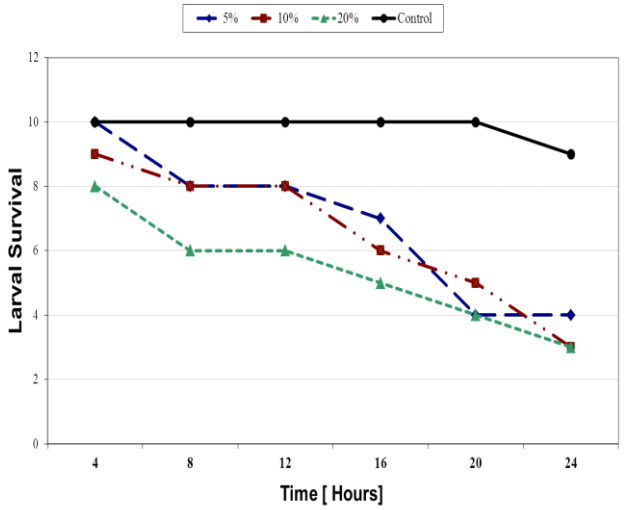


Fig. (11) :Effect of filtrate *A candidus* on the death of larvae *Musca domestica*

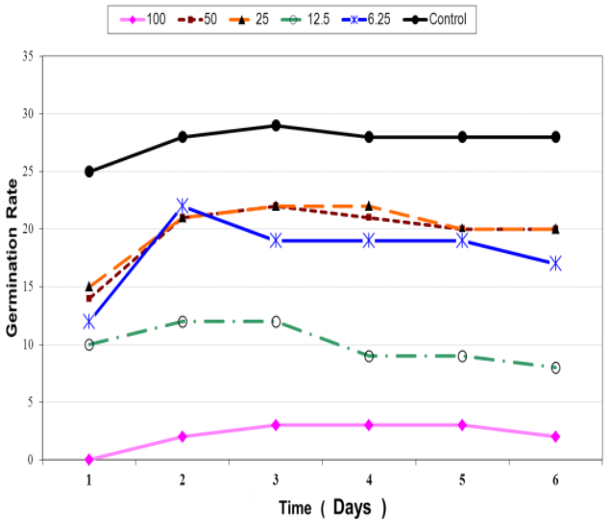


Fig. (12): Effect of pointillism *chrysogenium* strains at different concentrations on lettuce seeds germination.

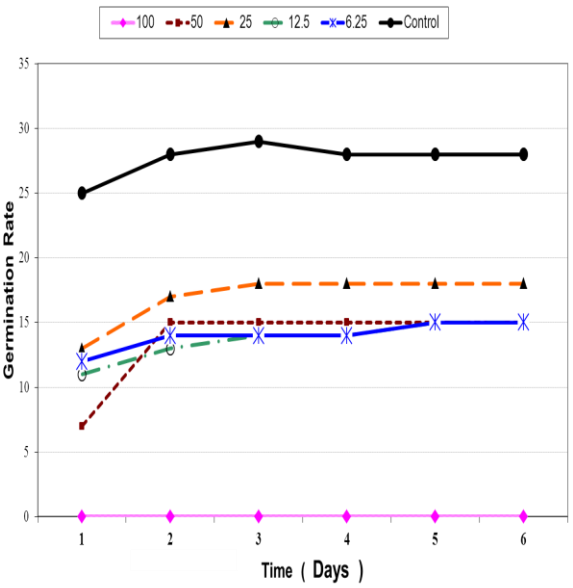


Fig. (13): Effect of filtrate *Aspergillus candidus* strains on at different concentrations on lettuce seeds germination.

This study addresses the effect of four filtrate fungal strains (*Asperillus Candidus, Asperillus Terreus, Penicillium Chrysogenum and Penicillium Aurantiogriseum)* on the death of larvae *Musca Domestica.* Thus, after being inoculated with 1.0 ml of filtrate fungal strains, the death of the larvae according to the control group was identified. Among all fungal strains, the highest on death of larvae are *Asperillus Terreus (58.34%)* (Fig. 10) followed by *Penicillium Chrysogenum* (48.34%), *Penicillium Aurantiogriseum* (48.30%) and *Asperillus Candidus (46.67)* as recorded in figures (8, 9, 10 & 11).

3.3 Effect of Extracellular secondary metabolites of fungi on germination of lettuce seeds

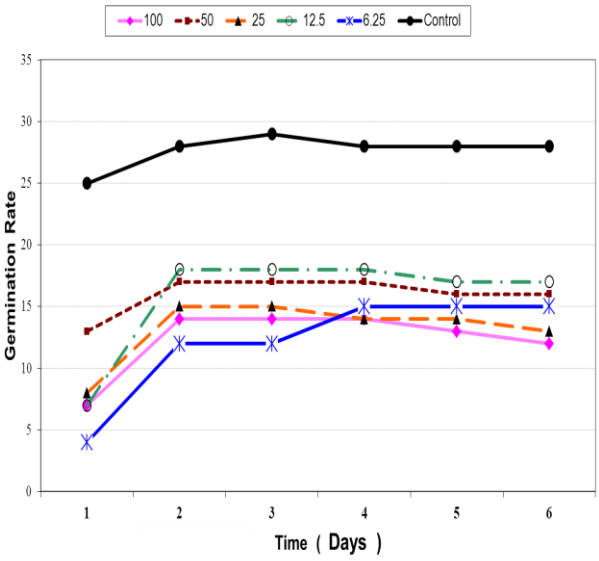


Fig. (14): Effect of *Aspergillus terreus* strains at different concentrations on lettuce seeds germination.

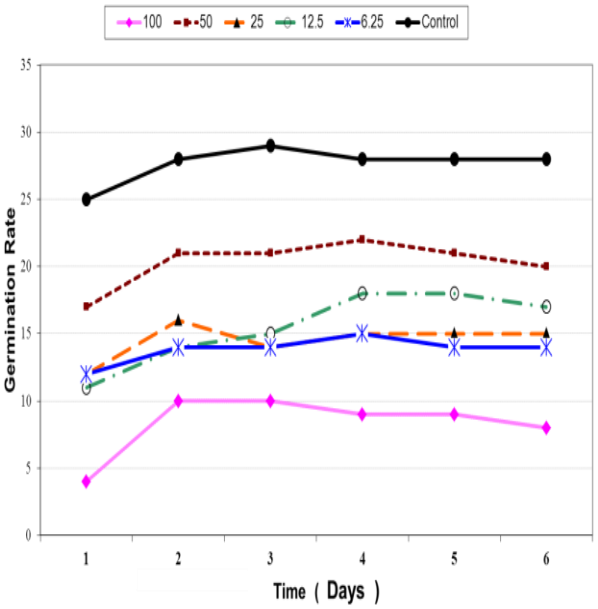


Fig. (15): Effect of filtrate *Penicillium aurantiogriseum* strains at different concentrations on lettuce seed germination.

This current research addresses the effect of four filtrate fungal strains (*Asperillus Candidus, Asperillus Terreus, Penicillium Chrysogenum and Penicillium Aurantiogriseum) on lettuce* seeds with a germination percentage of *lettuce* seeds, distinguished based on the treatments and their concentration. The highest germination percentage was observed in the control group (92.22%). In addition, after being inoculated with Asperillus *Candidus, Asperillus Terreus, Penicillium Chrysogenum and Penicillium Aurantiogriseum*, the *lettuce* seeds showed reduced germination according to the control group. The

highest inhibitory effect on the germination of lettuce seeds among all fungal strains was *Penicillium Chrysogenum (7.22%)* at 100%. Nonetheless, I did not record any germination (0.0%) when I treated the seeds with *Aspergillus Candidus* at 100%, as shown in figures (12, 13, 14 & 15). Likewise, [12] the percentage of germination of the maize seeds soaked in *A. Niger* filtrate (65.33%) is significantly different from its counterpart seeds soaked in *P. Chrysogenum* filtrate (79.6%). A percentage of 100% seed germination was recorded in the control experiment. It was also noticed that the fungal filtrate of *A. Niger* has inhibited the seed germination to 65.33%; and this result was supported by **[13]** who had observed that *A. Niger* and *A. Flavusand Alternaria Alternate* had inhibitory power to reduce the seeds germination. Moreover, the percentage of seeds germination was decreased by 20.33% with *P. Chrysogenum* [15].

3.4 Screening for antitumor activity

IC50=8.0 µg/m

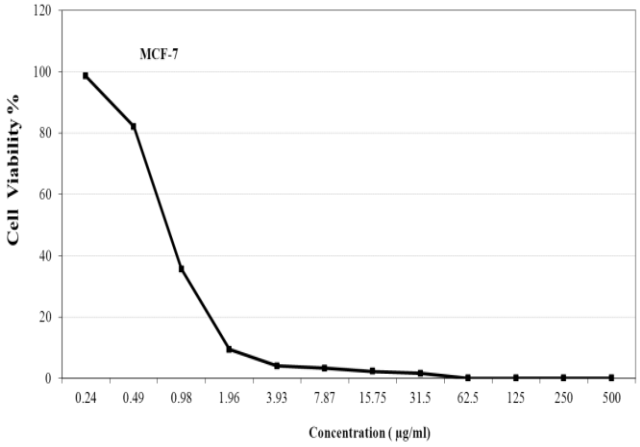


Fig. (16): Effect of different concentrations of sub fraction of *P chrysogenium* on viability of cancer liver cells (human breast cancer cell line (MCF-7)

The obtained crude extract of *P. Chrysogenium* was evaluated for potential cytotoxicity, against three tumor cells lined against hepatic cellular carcinoma (HEPG2); taking into consideration that the Human Breast Cancer Cell Line is (MCF-7), and the Human Colon carcinoma cells are(Caco-2 cells). The cells line was treated with serial concentrations of 500, 250, 125, 62.5, 31.5, 15.75, 7.87, 3.93, 1.96, 0.98, 0.49 and 0.24 µg/ml. The results are displayed in Figures (16, 17 & 18) with IC50% of 8.0µg/ml respectively; while the minimum concentration 500, 250 & 125 killed 100% of the viable cells. Similarly, **[24]** isolated *Auranomide B.* from the marine derived fungus *Penicillium Aurantiogriseum*, which exhibited the most potent inhibitory effect against the human myelogenous leukemia HEPG2 cells, with an inhibitory effect of 73.28**.**

IC50=8.0 µg/m

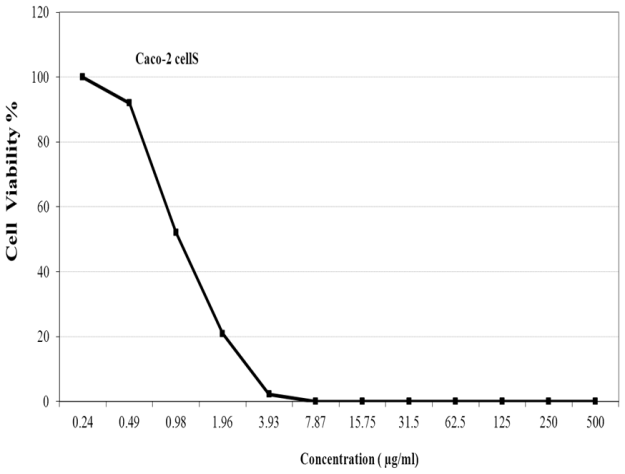


Fig (17): Effect of different concentrations of sub fraction of *P chrysogenium* on viability of cancer liver cells (human colon carcinoma cells (Caco-2 cells).

IC50=8.0 µg/m

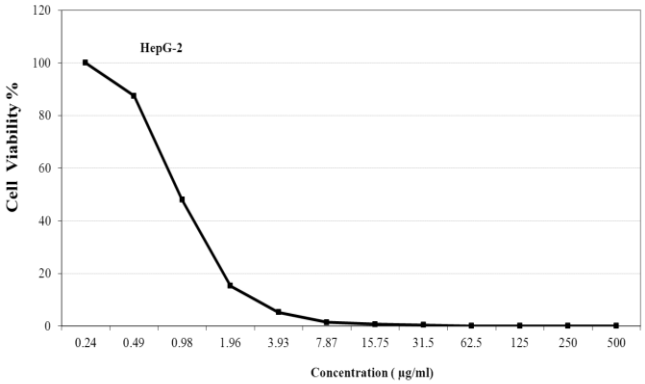


Fig. (18): Effect of different concentrations of sub fraction of *P chrysogenium* on viability of cancer liver cells (Hepatic Cellular Carcinoma).

[17] extracted 11 compounds from *Penicillium Brevicom*pactum, which was isolated from the associated marine alga *Pterocladia Sp.*; and they reported that the maximum concentration of compound 9 (100 µg/ml) killed about 40% of the viable infected liver cells, and also killed about 50% of the viable infected lung cells at a concentration equal to 91.6 µg/ml.

4 Conclusion

The result of this study revealed that a selected isolate is a good producer of safe bioactive compounds which can be used as antimicrobial, anti-insect, anti-herbal and anticancer compounds. Further studies are required in order to separate and identify these compounds in a pure state, and to determine which compound has the biological activity in order to be used in application.

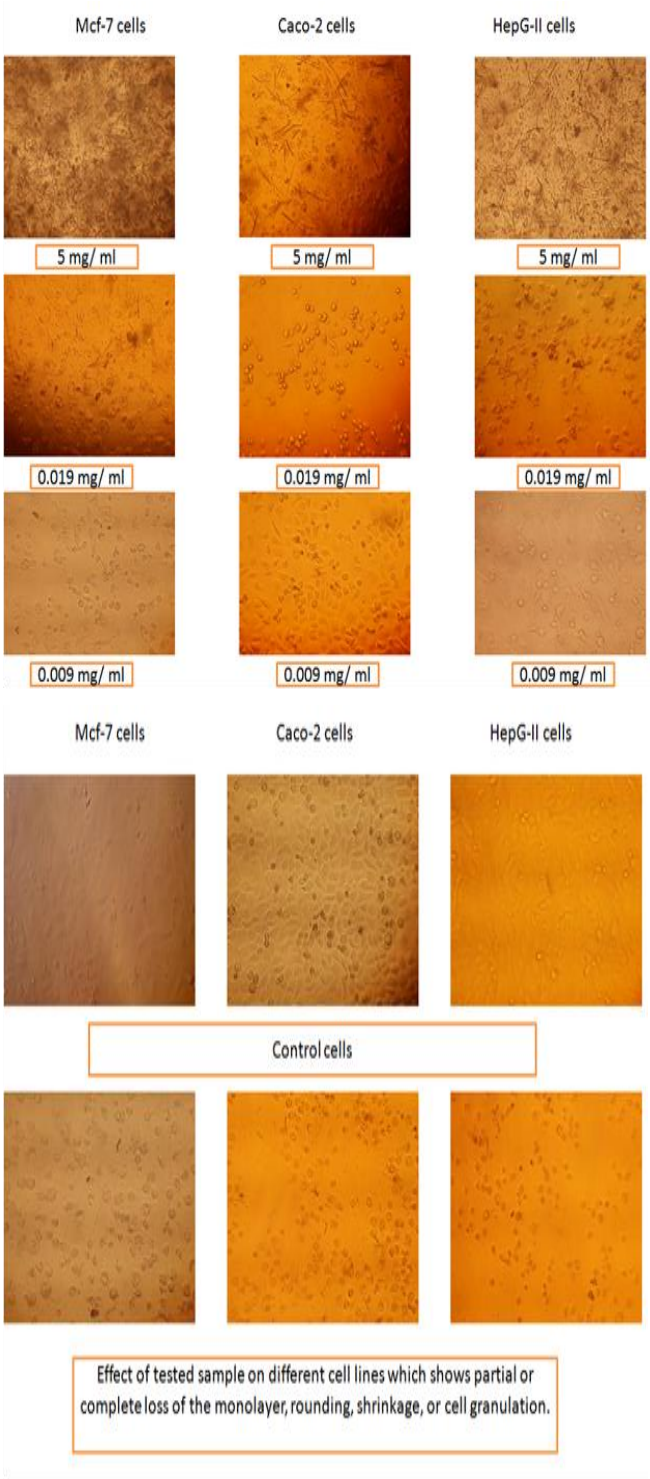


Fig (19). Effect of different concentrations of the Ethyl acetate fraction from *P. chrysogenum Thom* AUMC 10510 against the three tumor cell lines tested.

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