Research Article

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GC-MS Analysis of The Compounds Produced from Two Species of *Penicillium*

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Abstract

Penicillium is a genus of the one the most common fungi have located in distinctive and suitable surroundings (temperature, humidity, Ph), *Penicillium* species produced extracellular enzymes that play a necessary position in the microbial decomposition of natural substances. The study is aimed to detect chemicals made by two species of *Penicillium* using a synthetic media that contained a powder made from the leaves of the *Conocarups* tree. In this study, two species of *Penicillium* had used in a fermentation process using synthetic medium contained a powder of the Conocarpus tree leaves. That isolated from water of the Hammar marsh areas in south Iraq particularly in Thi-Qar province.The isolated *Penicillium* observed on beginning of cultural, microscopic and morphological characteristics. Molecular identification of *Penicillium* results proved that all strains were belonging to *Penicillium* genera. According to the β-tubulin sequence, the isolated were divided into 2 species, as *Penicillium brevicompactum* and *Penicillium expansum*. GC-MS analysis appeared different compounds with various retention times (RTs) were produced by the crude extracts of *P. brevicompactum* and *P. expansum*. Similar compounds are (benzyl alcohol, benzyldimethylsilyl ether), (cyclotetrasiloxane, octamethyl), (trolamine), (cyclooctasiloxane, hexadecamethyl-), (methyl stearate), (hexadecanoic acid, methyl ester), (n-hexadecanoic acid), (9-octadecenoic acid, methyl ester,(E-), (9-octadecenoic acid, methyl ester), (heptadecanoic acid, 16-methyl-, methyl ester), (methyl stearate), (9-octadecenoic acid, (E)-), (oleic acid), (methyl 10-trans,12-cis-octadecadienoate), (9,12-octadecadienoic acid (Z,Z)-,methyl ester), (dl-.alpha.-tocopherol), (vitamin E), (gamma.-sitosterol), and (beta.-sitosterol).

Key words: ***Penicillium*, GC- MS, leaves.**

Introduction:

Many *Penicillium* species produced one of a kind chemical sort of secondary metabolites, whilst some of them are important in field of medicine (1). Others are used for the production of Mycotoxins, important pills and some of the *Penicillium* species can reason infection of bread, grains and processed meals (2). For this reason, it is important to identify and classify *Penicillium* at species level and the ability to predict its structure information and biochemical composition in order to distinguish types of *Penicillium* precisely (3). Taxonomy of fungi such as *Penicillium* at the types stage relies upon on morphological attribute (colony feature, hypha, the affiliation of spore) purpose fungal diversity, so morphological acquire about is the most analytic and normal strategies (4-6). The majority of the essential *Penicillium spp*. morphological characteristics discovered by microscopic identification were specifically based on macroscopic morphological traits discovered by naked eye, such as colony type, colony color, size, and structure, and microscopic attribute affiliation of great *Penicillium spp*. spores and conidia discovered under a basic microscope (7). The majority of the necessary morphological characteristics of *Penicillium* spp. determined by microscopic identification strategy have been based on macroscopic phenotypic features like colony type, colony color, size, and shape that had been placed using the naked eye and microscopic characteristic affiliation of spores and conidia of excellent *Penicillium spp*. determined under a compound microscope (7-8). *Penicillium brevicompactum*, one of the main species in the *Penicillium* genus, is a filamentous fungus with significant therapeutic value. Since it is the most significant fungal strain producing MPA, *P. brevicompactum* is frequently used in the fermentation process for MPA synthesis (9). *P. brevicompactum* can be isolated from soil, rotting vegetables, food, cereals, textiles, colors, and a variety of other unusual sources. It is widely distributed across the herbal domain (9). One of the most prevalent and economically significant postharvest fruit rot disorders is blue molds, which is produced by *Penicillium expansum* and other *Penicillium spp* (10). It causes blue mold, a deterioration that can cause significant financial losses at some storage locations and affect fruit intended for treatment due to the production

of the carcinogenic mycotoxin patulin (11). The purpose of the study was to detect chemicals made by two species of *Penicillium* using a synthetic media that contained a powder made from the leaves of the Conocarups tree.

Material and Methods

Isolation and identification of *Penicillium* species: Sample preparation of water from Hammar marsh areas in south Iraq particularly South Thi-Qar Province. The sample inoculated in dishes of Czapex Dox medium containing 5% of ampicillin at 25°C for one week. The isolated were sub-cultured on the potato dextrose agar (PDF), malt extract agar (MEA). When Penicillium growth the colony were observed on cultured media (12).

Micromorphology and macromorphology identification of *Penicillium* isolates: After completion of one week fungi culture, morphological characteristics performed of preparing slides using Lactophenol cotton blue and observed with optical microscope. It was observed that feature stipe's, the number of tips, branching of individual hyphae, conidiophores, ornamentation of conidia, conidium shape. Macromorphology colony characters and diameters on unique media are essential elements for species identification (13).

Molecular Identification of Fungal Species (Kits, Primers, and Instruments)
1- DNA extraction and PCR amplification: *Penicillium* genomic DNA extract via the use of G-spin DNA extraction package in accordance to producer protocol (14). Oligonucleotide primers (forward and revers) were designed for *Penicillium genes* such as β-tubulin Oligonucleotide primers (forward and reverse) (F= 5`GGT AAC CAA ATC GGT GCT GCT TTC3`) and (R= 5` ACC CTC AGT GTA GTG ACC CTT GGC 3`**)** 550 bp size in accordance to (15).
2- Preparation of PCR master mix: PCR reaction organized of the usage of Maxime PCR premix kit. The combine is organized in accordance to the company directions as illustrated in the table (1).

PCR amplification: PCR system performed of the utilization the advocated current cycling settings that defined in table (2). PCR machine have been visualized by way of agarose gel stained via Ethidium bromide dye (Biometra, Germany).

3- Isolated Penicillium DNA sequencing The PCR product was sent to the Korean company Macrogen for sequencing. Phylogenetic analysis is done using NCBI-

Blast alignment information to identify (MEGA 6.0 version).

4- Fermentation Process: The fermentation process done using a medium has prepared from leaves of Conocarpus tree (Fig. 1) and other substances and metals. Leaves of the tree are washed in tab water, then distilled water and left in a room temperature for dryness. A small mill used to pulverize dried leaves for getting the powder. Five mg of the powder were dissolved in the distilled water and filtered by a filter paper in which 5 ml of the filtrate had mixed with 20 ml of the trace metal solution consisted of the ammonium molybdate, cobalt nitrate, iron (II) sulfate, manganese sulfate , zinc sulfate, and copper sulfate composed of 0.01, 0.01, 0.1, 0.01, 0.161, and 0.015 gram, respectively. The solution of the trace metals and leaves filtrate was added into 975 ml of distilled water contained potassium dihydrogen phosphate, magnesium sulfate, calcium chloride, yeast extract, peptone, and glucose 0.87, 0.5, 0.5, 2. 20, and 50 grams, respectively. Thus, all components of medium dissolved in one liter of the distilled water and adjusting pH of medium at 5.5. Two flasks (1 liter capacity) were used in which each flask contained 250 ml of the medium. One flask inoculated by two discs (6 mm in diameter) for 10 days all *P. brevicompactum* colony grew on the PDA and second flask was once additionally inoculated through two colony discs however from *P. expansum*. The inoculated flasks have been incubated at 25˚C for 10 days.

5- Extraction and GC- MS Analysis of Crude Extracts: After ending period of the fermentation process, mycelia were separated from filtrate by a filter paper. The filtrate was treated with a same volume of the chloroform using separator funnel that the bellow layer has selected and evaporated at a room temperature until getting a fungal solid crude extract which was dissolved in the absolute methanol (10 ml). Amount of the methanol extract that subjected into GC-MS analysis in which Gas chromatograph: Utilizing an Agelint HP-5ms ultra-fine needle (30 m size x 250 m diameter x 0.25 m internal diameter), an Agelint (7820A) USA GC mass spectrometer analytical column, a stress of 11.933 psi, a GC inlet line temperature of 250 °C, aux heateres temperature of 310 °C, company gas of helium 99.99%, an injector temperature of 250 °C, Ramp 1: Maintain 60°C for three minutes; Ramp 2: Maintain 60°C-180°C for seven minutes; Ramp 3: Maintain 180°C-280°C for eight minutes; and Ramp 4: Maintain 280°C for three minutes.

Table (1): Components of Maxime PCR Premix kit reaction

		Maxime PCR Premix Kit	Volume
1		DNA template	10 µl
2		Mastermix	25 µl
3	β-tubulin	F. primer (10Pmol)	2 µl
		R. primer (10 Pmol)	2 µl
6		Nuclease-free water	11 µl
7		Total volume	50 µl

Table (2): PCR program setting for Penicillium isolates.

Step	Temperature	Time	Cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	45 sec	30
Annealing	55°C	45 sec	
Extension	72°C	1 min	
Final extension	72°C	5 min	1
Hold	10	10 min	

Fig. 1: **Tree of Conocarpus species**

Results

1- Macroscopic and Microscopic Features Isolated Penicillium: The isolated fungi observed on beginning of cultural, microscopic and morphological characteristics. Figure (2). *Penicillium brevicompactum* and *P. expansum* appeared various colonies on three culture media. On the CDM, *P. brevicompactum* produced colony had white center and yellowish white edges beside yellow reverse. No aerial growth and the colony were poor as well as no exudates. Contextually, PDA manifested good growth of the colonies had white edges and yellow reverse. Green Colonies wrinkled and grooved. Exudates were observed on the colonies that are irregular. As well as no aerial growth. Also, *P. brevicompactum* appeared sparse colonies are similar to that on PDA but small in size, no exudates, and brownish yellow reverse. Regarding to *P. expansum*, this fungus produced poor growth on CDM in which brownish yellow colonies had white edges when they were old but the young colonies were white. No exudate and aerial growth were observed. In addition, the colonies are irregular and reverses liked the colonies. In this context, growth of *P. expansum* is good appearance in which the colonies were irregular and they were bluish green with white edges. Colonies were wrinkled and grooved. Old colonies possessed brownish yellow centers and presence of the exudates on the edges in addition, dark yellow reverse. In the same context, this fungus appeared irregular colony was bluish green with white edges. Aerial growth and exudates were not observed and reverse was brownish yellow.

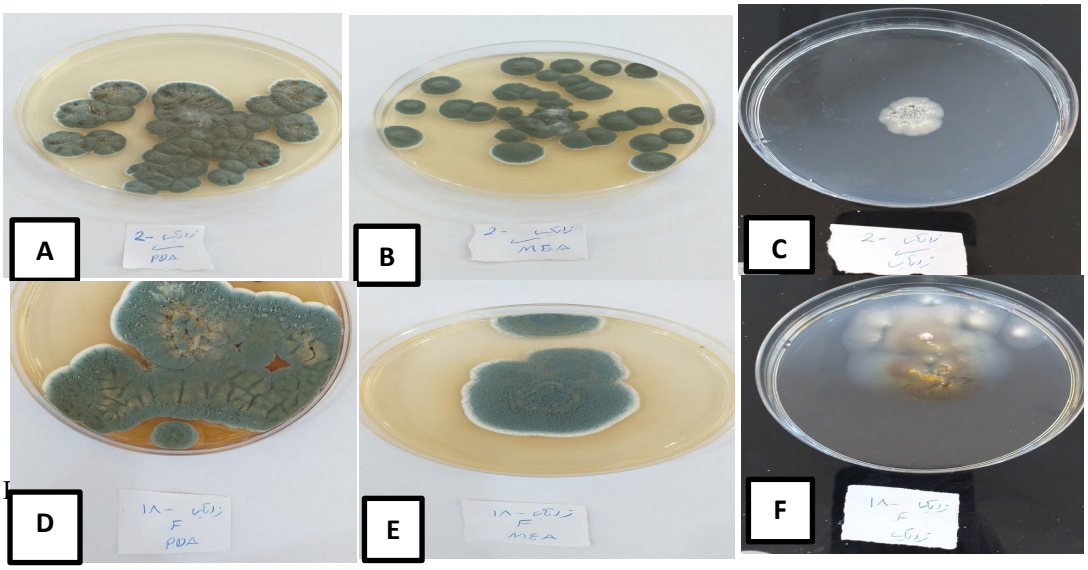

Fig. (2): **A- Growth of *P. brevicompactum* on PDA, B- Growth of *P. brevicompactum* on MEA, C- Growth of *P. brevicompactum*, D- Growth of *P. expansum* on PDA, E- Growth of *P. expansum* on MEA, F- Growth of *P. expansum*.**

2- GC- MS Analysis

The GC-MS analysis appeared in different compounds with various retention times (RTs) that produced of fermentation process of *Penicillium brevicompactum* and *P. expansum*. Some of these compounds recoded to be similar compounds of both fungi. Similar Compounds are (benzyl alcohol, benzyldimethylsilyl ether), (cyclotetrasiloxane, octamethyl), (trolamine), (cyclooctasiloxane, hexadecamethyl-), (methyl stearate), (hexadecanoic acid, methyl ester), (n-hexadecanoic acid), (9-octadecenoic acid, methyl ester,(E), (9-octadecenoic acid, methyl ester), (heptadecanoic acid, 16-methyl-, methyl ester), (methyl stearate), (9-octadecenoic acid, (E), (oleic acid), (methyl 10-trans,12-cis-octadecadienoate), (9,12-octadecadienoic acid (Z,Z) ,methyl ester), (dl-.alpha.-tocopherol), (vitamin E), (gamma.-sitosterol),and (beta.-sitosterol). (Figures: 3) and (Tables 3).

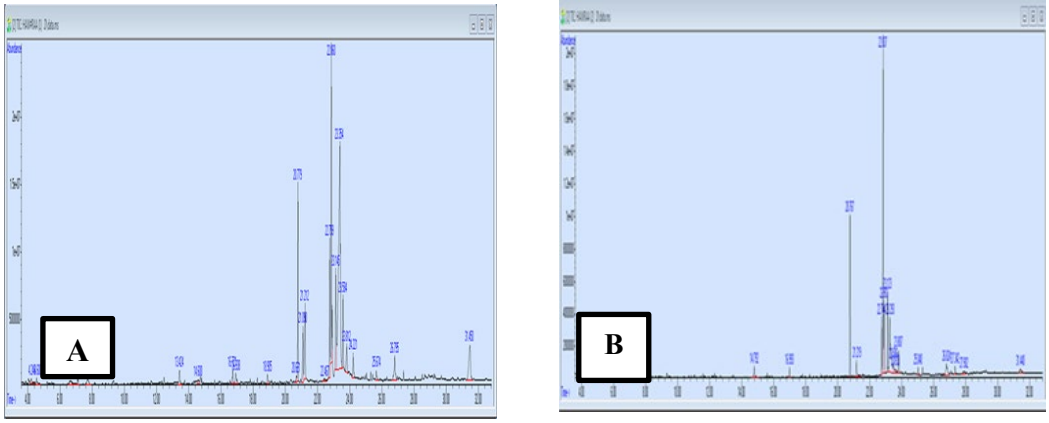

Fig (3): **A- GC-MS evaluation of the crude extract produced from *P. expansum*, B- GC-MS evaluation of the crude extract produced from *P. brevicompactum***

Table (3): **GC-MS evaluation of the crude extract produced from *P. expansum* and *P. brevicompactum***

Compounds *P. expansum*	RTs	Peaks	*Compounds P. brevicompactum*	RTs	Peaks

Benzaldehyde, 3-(4-fluorophenoxymethyl)-4-methoxy-	4.246	1	Dimethyl sulfoxide	4.011	1
Benzothiazole-6-carboxylic acid			Dimethyl sulfoxide	4.206	2
1,2-Benzenedicarboxylic acid, 4-hydroxy-, dimethyl ester			Silane, trimethyl(phenylmethoxy)-	4.552	3
Benzyl alcohol, benzyldimethylsilyl ether	4.598	2	Benzyl alcohol, benzyldimethylsilyl ether		
Alpha-benzamido-3,4-dimethoxyacetophenone			6H-Purin-6-one, 2-amino-1,7-dihydro-1-methyl-		
Benzamide, o-(2-hydroxy-2,2-diphenylethyl)-			4,5,6,7-Tetrahydro-benzo[c]thiophene-1-carboxylic acid (4-fluoro-phenyl)-amide	4.676	4
1-Tributylsilyloxytridec-2-yne	6.691	3	1-(4-Nitrophenyl)piperazine		
Cyclotetrasiloxane, octamethyl			1,6-Dimethyl-7-oxo-1,2,3,7-tetrahydroimidazo[1,2-a]pyrimidine		
Trolamine	7.109	4	Benzene, 1,1'-(2,2-dichloroethylidene)bis[4-ethyl-	5.250	5
1,3-Butanediol, 2-methyl-			1,3-Dibenzyl-1,1,3,3-tetramethyldisiloxane		
Glutaric acid, 3-phenylpropyl tridecyl ester			3-Amino-5-(3-indolyl)-4-pyrazolecarbonitrile		
Tridecane	7.722	5	Oxirane, 2,2-diphenyl-	5.576	6
Hexadecane			Benzenamine, 3-(2-phenylethenyl)-,		
Dodecane			Carbazole, 1,4-dimethyl-		
Cycloheptasiloxane, tetradecamethyl-	13.422	6	Cyclotetrasiloxane, octamethyl-	6.678	7
Pentasiloxane,			Trolamine		

dodecamethyl-					
Cycloheptasiloxane, tetradecamethyl-	14.603	7	Pyridine, 4-(4,5-dihydro-2-oxazolyl)-	7.122	8
Pentasiloxane, dodecamethyl-			Ethyl 3-oxobutan-2-yl carbonate		
Cyclooctasiloxane, hexadecamethyl-	16.722	8	Cycloheptasiloxane, tetradecamethy l-		
Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester			3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris(trimethylsiloxy)t etrasiloxane	14.792	9
Cyclooctasiloxane, hexadecamethyl-	16.937	9	Cyclooctasiloxane, hexadecamethyl-		
Silane,[[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bi s[trimethyl-			Silane, [[4-[1,2-bis[(trimethylsil yl)oxy]ethyl]-1,2-phenylene]bis(ox y)]bis[trimethyl-s	16.996	10
Cyclononasiloxane, octadecamethyl-	18.907	10	Hexadecanoic acid, methyl ester	20.766	11
Triacontanoic acid, methyl ester	20.681	11	n-Hexadecanoic acid		
Nonadecanoic acid, methyl ester			l-(+)-Ascorbic acid 2,6-dihexadecanoate	21.222	12
Methyl stearate			9,12-Octadecadienoic acid (Z,Z)-,methyl ester	22.742	13
Hexadecanoic acid, methyl ester	20.779	12	Methyl 10-trans,12-cis-octadecadienoate		
n-Hexadecanoic acid	21.092	13	9-Octadecenoic acid, methyl ester,(E)-		
Pentadecanoic acid			cis-13-Octadecenoic acid, methyl ester	22.840	14
n-Hexadecanoic acid	21.209	14	9-Octadecenoic acid, methyl ester		
Pentadecanoic acid			9-Octadecenoic acid, methyl ester,(E)-		
9-Octadecenoic acid (Z)-, 9-hexadecenyl ester, (Z)-	22.468	15	cis-13-Octadecenoic acid, methyl ester	22.905	15
1,3-Dioxolane, 4-ethyl-5-octyl -bis(trifluoromethyl)-, trans-			Methyl stearate		

5.beta.,6.beta.-Epoxy-7.alpha.-bro mocholestan-3.beta.-ol			Heptadecanoic acid, 16-methyl-, methyl ester	23.120	16
9,12-Octadecadienoic acid (Z,Z)- methyl ester	22.762	16	9-Octadecenoic acid, (E)-		
9,12-Octadecadienoic acid (Z,Z)- methyl ester			Oleic acid	23.290	17
9-Octadecenoic acid, methyl ester,(E)-	22.859	17	Oleic acid	23.505	18
11-Octadecenoic acid, methyl ester			cis-9-Hexadecenal		
9-Octadecenoic acid, methyl ester			2-Methyl-Z,Z-3,13-octadecadienol	23.635	19
Heptadecanoic acid, 16-methyl-, methyl ester	23.146	18	Z,E-2,13-Octadecadien-1-ol		
9-Hexadecenoic acid, methyl ester,(Z)-			8,11-Octadecadienoic acid, methyl ester		
Methyl stearate			7,10-Octadecadienoic acid, methyl ester	23.805	20
9-Octadecenoic acid, (E)-	23.355	19	Methyl 10-trans,12-cis-octadecadienoate		
Oleic acid			cis-11-Eicosenoic acid, methyl ester		
Octadecanoic acid	23.583	20	Methyl 9-eicosenoate	25.038	21
Oleic acid			cis-13-Eicosenoic acid, methyl ester		
trans-13-Octadecenoic acid			Vitamin E		
9,12-Octadecadienoic acid (Z,Z)-,methyl ester	23.812	21	dl-.alpha.-Tocopherol	26.825	22
Methyl 10-trans,12-cis-octadecadienoate			alpha.-Tocopheryl acetate		
9,12-Octadecadienoic acid (Z,Z)-,methyl ester			Methyl 20-methyl-heneicosanoate	27.340	23
9,12-Octadecadienoic acid (Z,Z)-	24.222	22	Docosanoic acid, methyl ester		
9,12-Octadecadienoic			Stigmast-4-en-3-one		

acid, methyl ester					
9-Octadecenamide, (Z)-	25.677	23	(2,3-Dichlorophenyl) carbamic acid 4-methoxyphenyl ester	27.901	24
8-Methyl-6-nonenamide			Pyrazine, 2-methoxy-3-methyl-		
alpha.-Tocopherol-.beta.-D-mannoside	26.792	24	gamma.-Sitosterol	31.442	25
dl-.alpha.-Tocopherol			beta.-Sitosterol		
Vitamin E					
gamma.-Sitosterol	31.449	25			
beta.-Sitosterol					

3- Conventional PCR Screening for β-tubulin Gene of *Penicillium*

The molecular identification of *Penicillium*, be dependent on standard PCR for the amplification of a partial gene of β-tubulin Gene with the aid of special primer sequences. Gene used to be as quickly as present with a PCR product dimension of 550 bp , show figure (4). *Penicillium* isolates recognized on the degree of species, tested by means of PCR and nucleotide sequencing of the β-tubulin gene in contrast with on hand sequences in Genbank database the use of the BLAST algorithm software of NCBI. PCR results proved that all strains were belonging *Penicillium* genera. According to the β-tubulin sequence, the isolated were divided into 2 species, as *Penicillium brevicompactum* and *Penicillium expansum*. The outcomes of this study printed that BLAST analysis of the NCBI gene bank gave 99% homology with *Penicillium brevicompactum*, 98% with *Penicillium expansum*.

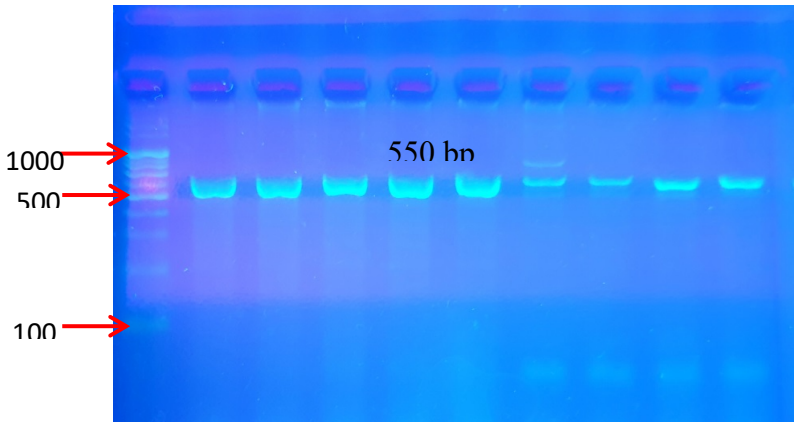

Figure (4): Evaluation of PCR products using *Penicillium* isolates is shown by agarose gel electrophoresis. *Penicillium* isolate's -Tubulin gene has 550 base pairs and is located on lanes 1-5, the Lane M Marker Ladder (1000 bp).

Discussion:

Penicillium is widely distributed in the environment and is inherently segregated from the air and soil. Numerous posts to now have revealed that Penicillium is extensive and frequently found in soil samples from various climatic zones and geographic regions (16). Freshwater provides a wide variety of types of habitation for fungi, including soil, aquatic insects, and aquatic plant living. It is a diverse and challenging environment for microorganisms (17). By utilizing the natural processes of degrading organic matter and creating secondary metabolites, fungus demonstrate a major characteristic in the ecosystem in controlling dietary nutritional vitamins and carbon cycles (18). *Penicillium* is a remarkable group of the largest fungus. It can be found in many habitats, including soil, vegetation, air, indoor spaces, and a wide variety of materials. merchandise (12).

Identification of *Penicillium* is challenging. It is based heavily on morphological elements, in addition to the utilization of a variety of media and regulated research lab settings. Traditional morphological species delineation has consistently been a taxonomic challenge, and at the moment accurate identification requires analysis of DNA in specific based upon sequence data from a mix of excellent genes, primarily ITS, BenA, and CaM. In our research, *Penicillium brevicompactum* and *Penicillium expansum* were isolated as a species. Purified PCR products and a DNA sequencing service were used (Macrogen Inc., Seoul, Korea). The GenBank database's BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST/) was used to search the obtained nucleotide sequences. The resulting -tubulin sequences were discordantly separated from the genuine sequences uploaded by way of For phylogenetic analyses (19) , the reference species *P. piscarium, P. crustosum, P. brasilianum, Penicillium expansum* and *Penicillium piscarium* remoted from freshwater in Korea. As mentioned above, BenA works properly for species identifications *Penicillium*. The growth of a fungus including the fermentation process is affected by different factors especially in the laboratory tests. Examples of these factors are the components of a medium and its precursors, temperature degree, pH, incubation period, aeration etc. in which the growth curve can be affected. In this context, composition of a medium is the most important factor gives significant effect on the fungal growth leading to produce compounds including secondary metabolites from fungi. Medium which is supplemented with metals such as iron, zinc, and magnesium with phosphate plays important role in a biosynthesis of the fungal

metabolites that the phosphate in the magnesium is utilized to be co-factor of the enzymes. While iron and zinc have effects on the cytochrome P450 oxidase and stabilizing protein structures respectively when a fungus grows in a medium contains them (20, 21). Therefore, the current study tested a medium to grow two species of *Penicillium* in the fermentation process for evaluation of the medium effects on the producing secondary metabolites from *P. brevicompactum* and *P. expansum.* The tested medium contained leaf powder of the *Conocarpus* tree as natural source and other compounds were magnesium, iron, zinc, trace metals, glucose etc.

Based on the above, media have influences on a biosynthesis of the secondary metabolites due to the precursors that media possess which have effect on the producing these metabolites from fungi in the fermentation media. Species of *Penicillium* and *Aspergillus* produced different compounds were obtained from the same media (22). This may give a reason that why our tested *Penicillium* species produced different compounds in the same medium and conditions of the incubation, however, similar compounds of them were recorded. Generally, our results were also agreed with (23). Also, fungal secondary metabolites are controlled by genetics and the environmental factors can stimulate the genetics. This relationship between environment and genetics can lead to form different or similar secondary metabolites (24). Results of the present study showed different and similar compounds were given by *P. brevicompactum* and *P. expansum* may be attributed to the effects of medium components on the genetic of the fungi and vice versa that led to get these compounds (Tables 3). By other words, it may be said that a composition of the tested medium led to change in the metabolism and genetic control of both fungus where a presence of the trace metals and other components made these fungi to produce their secondary metabolites that some of them were similar. The similarity of some produced compounds may be attributed to the same ability of the fungi to utilize the medium components to be same precursors for biosynthesis similar compounds of the fungal secondary metabolites. The incidence of infection caused by opportunistic fungi had increased markedly with increasing in frequently of organ transplantation. cancer chemotherapy human immunodeficiency virus infection (25). The early, fast and right identification of the pathogenic fungus is essential for timely, excellent management. The common identification of pathogenic fungi in the clinical microbiology laboratory is mainly based totally on morphological and physiological tests frequently require three or greater days and can additionally be inaccurate in latest years a multiplex PCR method

was as soon as developed to identify similtaneously more than one fungal pathogen in a single reaction (26).

Conclusion:

The isolation and identification of Penicillium isolated from water of the Hammar marsh areas in the south of Iraq particularly in Thi-Qar province. As the present study showed different and similar compounds were given by *P. brevicompactum* and *P. expansum* may be attributed to the effects of medium components on the genetic of the fungi and vice versa that led to get these compounds.

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Conflict of interest: The authors declare no conflict of interest.

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تحليل GC-MS للمركبات المنتجة من نوعين من البنسليوم

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البنسليوم هو جنس من أكثر الفطريات شيوعًا التي توجد في محيط مميز ومناسب (درجة الحرارة ، الرطوبة ، درجة الحموضة) ، أنتجت أنواع البنسليوم إنزيمات خارج الخلية تلعب دورًا ضروريًا في تحلل الأحياء الدقيقة للمواد الطبيعية. كان الغرض من الدراسة هو الكشف عن المواد الكيميائية التي يصنعها نوعان من البنسليوم باستخدام وسط اصطناعي يحتوي على مسحوق مصنوع من أوراق شجرة كونوكاروبس. في هذه الدراسة ، تم استخدام نوعين من البنسليوم في عملية التخمير باستخدام وسط صناعي يحتوي على مسحوق من أوراق شجرة .Conocarpus النوعان هما *P. brevicompactum* و *P. expansum*اللذان تم عزلهما من مياه مناطق اهوار الحمّار في جنوب العراق خاصة في محافظة ذي قار ، وقد لوحظت البنسليوم المعزول في بداية الصفات المزروعة والميكروسكوبية والمورفولوجية. أثبت التحديد الجزيئي لنتائج البنسليوم أن

جميع السلالات تنتمي إلى أجناس البنسيليوم. وفقًا لتسلسل بيتا-توبولين ، تم تشخيص العزلات إلى نوعين هما *Penicillium brevicompactum* و*Penicillium expansum*. ظهر تحليل GC-MS إنتاج مركبات مختلفة بأوقات احتفاظ مختلفة (RTs)بواسطة المستخلصات الخام من *P. brevicompactum* و *P. expansum*. المركبات المماثلة هي (كحول بنزيل ، بنزيل ميثيل سيليل إيثر) ، (سيكلوتيتراسيلوكسان ، أوكتاميثيل) ، (ترولامين) ، (سيكلوكتاسيلوكسان ، هيكساديكاميثيل-) ، (ميثيل ستيرات) ، (حمض هيكساديكانويك ، ميثيل إستر) ، (حمض هيكساديكانيك ، ميثيل إستر) ، - حمض الأوكتاديسينويك ، إستر الميثيل ، (E-)، 9-octadecenoic acid)، (methyl ester، heptadecanoic acid)، 16-methyl-، (methyl ester، methyl stearate)، 9-octadecenoic acid)، (- (E)،)حمض الأوليك) ، (ميثيل 10-ترانس ، 12-cis-octadecadienoate)، 9)، 12-octadecadienoic acid (Z، - (Z، methyl ester)، dl-alpha.-) tocopherol)،)فيتامين (E و (جاما- سيتوستيرول) و (بيتا-سيتوستيرول).

الكلمات المفتاحية: البنسلين، GC- ms، أوراق.