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Isolation and Identification of microbes (Fungi and Bacillus species) From Soil and Evaluation of Their Antimicrobial Properties.

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ABSTRACT

Five species of bacteria were isolated from different areas from the faculty science soil in addition to four species of fungi and they were tested for the production of the antibacterial substances. The bacterial species were identified by the microscopic examination and the standard biochemical tests. The bacterial isolates were diagnosed as Bacillus spp and were classified as : Bacillus megaterium, Bacillus. licheniformis, Bacillus firmus, Bacillus pumilus, and Bacillus sphaericus while the fungal species, some of them were pre-defined in previous study included: Aspergillus terrrus, Aspergillus niger ,Penicillium chrysogenum and in this study only Fusarium solani was isolated from the studied area. The antimicrobial activity of the isolated bacteria and fungi was tested against the pathogenic bacteria included: Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella choleraesuis arizonae and Salmonella sp . The results showed that Fusarium. solani and Aspergillus niger had the highest antimicrobial effect against the gram negative bacilli bacteria, while their effect was less on the gram positive cocci bacteria ,in the contrast Aspergillus terrrus and Penicillium chrysogenum were more effective against the gram positive cocci bacteria. Bacillus spp. had a wide broad spectrum effect on the pathogenic bacteria as most the isolates of Bacilus mgaterium and Bacillus licheniformis had a broad spectrum effect on gram-negative and gram-positive bacteria because of their ability to produce antibacterial substances in varying proportions. However the isolates of Bacillus Firmus, Bacillus pumilus and Bacillus sphaericus had a high effect against the gram negative bacteria while their antibacterial effect on Staphylococcus aureus was extremely limited. The antimicrobial substances of the most effective microbes were extracted using the organic solvent after fermentation of fungi and bacterial culture and then the antimicrobial activity of the extracted compounds was tested against the pathogenic bacteria.

عزل وتعريف الميكروبات (أنواع من الفطريات والبكتيريا العصوية الموجبة Bacillus spp) من التربة وتقييم خصائصها المضادة للميكروبات.

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الكلمات المفتاحية:
اختبارات كيموحيوية
استخلاص
بكتيريا
تضاد
فطريات

الملخص

عزلت خمسة أنواع من البكتيريا من مناطق مختلفة من تربة كلية العلوم اضافة إلى اربعة أنواع من الفطريات لاستخدامها في انتاج مواد مضادة لنمو بعض من البكتيريا الممرضة. شخصت الأنواع البكتيرية وذلك بإجراء بعض الاختبارات التشخصية التي شملت الفحوصات المجهرية و الاختبارات الكيموحيوية و كانت جميعها تنتمي إلى جنس Bacillus و التي شخصت في الأنواع التالية: Bacillus megaterium، Bacillus licheniformis، Bacillus firmus، Bacillus pumilus و Bacillus sphaericus. اما الانواع الفطرية منها ما كان معرفاً في دراسة

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سابقة و منها ما تم عزله من منطقة الدراسة و شملت هذه الفطريات: Aspergillus terrrus، Aspergillus niger، Penicillium chrysogenum و Fusarium solani. تم اختبار التأثير المضاد لهذه الفطريات و البكتيريا الموجبة الجرام ضد اربعة أنواع من البكتيريا الممرضة تمثلت في: Staphylococcus aureus, Pseudomonas aeruginosa Salmonella cholera.esuis arizonae و Salmonella sp.، تبين أن لفطري Fusarium solani و Aspergillus niger التأثير الأكبر على البكتيريا العصوية السالبة، بينما كان تأثيرها اقل على البكتيريا الكروية الموجبة، و على النقيض من ذلك كان لفطري Aspergillus terrrus و Penicillium chrysogenum التأثير الأكبر على البكتيريا الموجبة بالمقارنة مع السالبة. اغلب أنواع بكتيريا Bacillus spp. كانت ذات تأثير ذو طيف واسع على البكتيريا المختبرة حيث اغلب عزلات Bacillus mgaterium وBacillus licheniformis كانت ذات تأثير ذو طيف واسع على البكتيريا السالبة والموجبة الجرام وكان لها القدرة لإنتاج المواد المضادة وبنسب متفاوتة بينما الأنواع Bacillus firmus، Bacillus pumilus وB.acillus sphaericus كان لها التأثير الأعلى على البكتيريا العصوية السالبة ولم يكن لها أي تأثير يذكر على البكتيريا Staphylococcus aureus. تم استخلاص المواد المضادة باستخدام المذيب العضوي بعد تخمير المزرعة الميكروبية للفطريات و البكتيريا التي اعطت افضل تأثير مضاد ومن ثم اختبر تأثيرها المضاد للبكتيريا الممرضة.

1.Introduction

Soil is one of the richest and most diverse environment as it is inhabited by many microbial and non-microbial communities, it contains many different microbial populations, and the number of microbes varies greatly in the same land because they are affected by many factors which makes the soil a dynamic medium [1,2]. Relationships between soil microbes could be beneficial or competitive, beneficial relationships include symbiosis, syntrophism, and commensalism while the competitive relations including: Parasitism, Competition, Predation, and Antagonism. Which is defined as the ability of a living organism to suppresses or interferes with the growth and activity of another organism in order to preserve its life and existence, usually among the antagonism chemical substances are secreted into the environment, which is hinder the growth and development of another organism [3]. The phenomenon of antibiotic production among microbes is common especially among soil microbes, which has a prominent role in Microbial equilibrium in the soil, however there are more than 500 antibiotics are discovered every year and about 60% are extracted from the microbes of soil [4]. There are many microbes that can secrete antibiotics, including bacteria such as Bacillus and Pseudomonas in addition to numerous species of fungi including Penicillium, Aspergillus, Fusarium, Trichoderma and Yeasts [5,6]. Antibiotics are low molecular-weight (non-protein) molecules produced by microorganisms as secondary metabolites that able in low concentrations to inhibit or suppress the growth of other organisms [7]. The production of antibiotics by microbes is affected by many factors, These factors include: temperature, humidity, acidity and alkalinity of the medium. In the early forties of the last century the first attempts to produce antibiotics began by utilizing the technique of fermentation and microbial industries. Globally the health care centers facing a major problem of increase the incidence of infectious diseases because of the emergence of multidrug resistance pathogens [8]. Many factors have caused this scenario such as extensive and inappropriate utilize of antibiotics, poor hygienic conditions, immigration of travelers, immunocompromised patients, the late diagnosis of infections [9]. Recently, researchers are interested in studying antibiotic-producing microbes in soil which is considered as the most suitable habitats microbial growth [10]. therefore, the aim of the research is to isolate and define some microbes (bacteria and fungi) from the natural source such as soil, cultivate and purify them, and try to extract their metabolites of antibiotics and display their antimicrobial effects against the pathogenic bacteria.

2.Materials and Methods

2.1. Sample collection

Five soil samples were collected from different regions of the Science College in October 2019 (two samples were collected from the garden of college, a sample from the herbarium, a sample from the college yard and another sample from the courtyard of the college registrar). Approximately 10 grams of the samples were collected from a depth of 10-15cm from the surface of the soil using clean, dry and sterile polythene bag with a sterile spatula, then the bags were transferred directly to the Microbiology Laboratory of the Botany Department for isolation and identification of microbes [11].

2.2. Isolation and Identification of fungi

Fungi were isolated from the soil as described by [12] using the Serial dilution plating method. 1 gram of each sample was suspended in a test tube containing 9 ml of sterile distilled water and then the tubes were vortexed gently to make the suspensions of the five samples. After that successive serial dilutions were prepared by transferring 1 ml of aliquots to the second test tube containing 9 ml of sterile water and in this way dilution up to 10^{-3} were made. An aliquot of 0.1 ml of each dilution was streaked on Potato dextrose agar and Sabouraud dextrose agar media (SDA) then the plates were incubated at $28C^0$ for 5 days. The isolates of Fungi were identified based on their morphological characteristics and the nature of their growth on the plate in addition to the morphology of the mycelium and spores under the microscope. The characters of fungal isolates were compared with the identification books and the classification keys [13]. In addition to the fungi that were isolated from the soil, three isolates were previously identified by the microbiology laboratory at the Botany department – faculty of science- Sebha University also included in this study; *Aspergillus terrrus*, *Aspergillus. niger* and *Penicillium chrysogenum*.

2.3. Isolation and identification of Bacillus spp

Soil sprinkle technique was used to isolate antibiotic producing bacillus spp. whereas about 20-30 particles of soil were sprinkled on the surface of nutrient agar plates which was prepared according to the instructions of the manufacturer (Oxoid). The plates were incubated at 30° C for 24 hours. The isolates of *Bacillus* spp. were diagnosed by the morphological characteristics of the colonies on Nutrient agar medium (color, shape, texture, colony edge). The suspected colonies were stained by Gram and malachite green staining methods. The Gram-positive, bacilli, spore-forming bacteria were selected for additional identification biochemical tests; including: catalase, Oxidase, Voges-Proskauer VP, citrate hydrolysis blood hemolysis, nitrate reduction, starch and sugar utilization and gas production from glucose were performed according to the Bergey's Manual of Determinative Bacteriology [14,15].

2.4. The test pathogenic bacteria were used in the study

In this study three species of gram-negative bacilli bacteria and an isolate of gram positive cocci bacteria were obtained from the reference medical laboratory of Sebha. they were tested against the fungi and *Bacillus* spp. The pathogenic bacteria included *Pseudomonas aeruginosa*, *Salmonella* sp. ,*Salmonella choler-*

.arizonae and *Saphylococcus aureus.*

2.5. Preliminary screening of the antimicrobial activities

This test was conducted to show the ability of antimicrobials production by the fungal and *Bacillus* species against the growth of the pathogenic bacteria using the methods were performed by[7]. The antimicrobial effect of fungal isolates was performed by inoculating Nutrient agar plated with 1 ml (containing approximately 10^{-5}/bacterial cell/ml) of the tested organim. Then the plates were left until the bacterial suspension was absorbed, then a disk of fungi was taken by using penetrating cork (5mm diameter) from the edge of the 5-day-old fungi colony and they were placed in the center of the Petri dish with 3 repetitives three other plates containing only a disk of fungi without bacterial suspension were used as a control. The anti-bacterial effect of Bacillus spp was conducted almost in the same way of screening the antimicrobial effect of fungi; However, the incubation temperature was 30˚C for 48 hours and the filter paper discs method was utilized (with a diameter of 0.5 cm)as the disk was inoculated with the suspension of the Bacillus spp and then they were placed on the surface of plates of Nutrient agar medium pre-inoculated with the test pathogenic bacteria. Bacillus sp. isolates were recorded as isolates have an antibacterial effect by increasing their growth diameter or an inhibition zone around the filter paper disc [7,16]. The results were recorded by calculating the mean of fungal and bacterial growth diameters, The percentage of antimicrobials production was calculated as mentioned by Abbott's equation:

Corrected%= [1-(n in T after treatment)/ (n in Co after treatment)]*100 Where: n= population, T= treated(inhibition zones), Co= control [17].

2.6. Extraction and purification of antimicrobial compound from fungal isolates

The inoculum of fungal isolates were prepared by sub-cultured them on potato dextrose agar medium and then the plates were incubated at 30˚C for 3-5 days to obtain the spores that were used for antibiotics production test. The fungi spores was suspended in the Potassium phosphate buffer at pH 7. The suspension of spores was standardized at 530 nm with an absorbance of 0.48. After preparing the fungal inoculum, the medium for the antibiotic production was prepared (g/ 50ml): Ammonium acetate (6), $NaSO_3$ (0.5), $ZnO_4.7H_2O$ (0.02), $MgSO_4.7H_2O$ (0.25), KH_2PO_4 (6) $FeSO_4$.$7H_2O$ (0.02), Phenylalanine (0.5) and (10) Lactose (pH6). All the ingredients of the medium were placed in 250ml flask and 50 ml of distilled water was added to the flask and then were sterilized. The flasks then were inoculated with 5% suspension of spores for each fungus separately. The flasks were incubated with continuous shaking (120rpm) at 28˚C for 21 days, then the antibiotic was purified from the medium by filtering it with No.1 filter paper, then the pH of the filtrate was adjusted to 2 only, then 200 ml of chloroform solvent was added to the filtrate. The flasks were shaken well and then the solvent was separated from the filtrate using a separating funnel and the residual solvent and water were evaporated by using the oven at 100˚C. 20 ml of sterile distilled water was added then to 0.5 g of the fungal extracts powder [18].

2.7. Extraction and purification of antimicrobial compound from Bacillus spp.

The inoculum of the maximum activity Bacillus isolates against the test pathogenic bacteria was prepared in tubes containing 5ml of Tryptic Soy Broth at a concentration of (10^{-5}/bacterial cell/ml) and they were incubated at 30˚C for 72 hours. Then the medium for the antibiotic extraction was prepared (g / 50 ml): L-glutamic acid (0.5), K_2HPO_4 (0.5), $MgSO_4.7H_2O$ (0.2), $MnSO_4.7H_2O$ (0.1), NaCl (0.01), $FeSO_4.7H_2O$ (0.01) , $CuSO_4.7H_2O$ (0.01), $CaCl_2.2HO$ (0.015) and (10) Glucose (pH7). These components were mixed with 50 ml of distilled water in a 250 ml flasks, The medium was then autoclaved. After that a concentrated glucose solution previously sterilized by 0.2 µm pore size filter, was added to give a final concentration of 1% in the medium. Then the medium was inoculated with 1 ml of *Bacillus* spp. suspension (10^{-5}/bacterial cell/ml) and the flasks were incubated at 30˚C on a Shaker incubate at 120rpm for 24 hours. After incubation, the medium containing Bacillus spp. suspensions was centrifuged to obtain cell free supernatant and then they were further used for the screening of the antimicrobial activity [7] .

2.8. Screening of antimicrobial activity of fungal species and *Bacillus* spp by Agar diffusion assay

Agar well diffusion method was used to check the ability of organisms for the secretion of antimicrobial substances. Twenty-four hours of the test bacterial suspension was prepared with a concentration of (10^{-5}/bacterial cell/ml); *Pseudomonas aeruginosa*, *Salmonella* sp. ,*Salmonella choler .arizonae* and *Staphylococcus aureus*. Nutrient agar plates were inoculated with 1ml of the test bacterial suspension. Wells were made in the inoculated plates using sterile penetrating cork (5mm diameter). About 100 µl of fungal extracts were added in the wells(3 repetitives). Chloroform solvent (positive control) and sterile distilled water (negative control) were added to three wells (repetitions). In the same way, the extracts of Bacillus spp were tested Then the plates of fungal and bacterial extracts were incubated at 37ºC for 24 hours. After 24 hours, the zones of inhibition were observed. The diameter of the inhibition zones was recorded in mm [7].

2.9. statistical analysis

One way Analysis of variance (ANOVA) test was used by SPSS (23 Version) To test the efficiency of antimicrobial substances production by fungal species and *Bacillus* spp. where the different means were significantly distinguished among them at a probability level of (0.05).

3.Results and Discussion

3.1. Isolation and identification of fungi

Among 5 samples only one species of fungi was isolated which identified as *Fusarium solani* based on the morphological characteristics such as growth pattern, hyphae, the color of the colony, surface texture, margin character, morphology of mycelium, sporulation and the size and coloration of the conidia using standard identification manuals **Fig.1** [13,19]. *Fusarium solani* is frequently isolated from soil because it acts as decomposers, but some are putative parasites which cause diseases in the plants, insects, humans, and animals [20]. On the top of that, the presence of this fungus is common in the study area, where it was isolated several times from the soil of the Faculty Science by previous studies. In this study, the secretion of antimicrobial substances of previous identified fungal species by the laboratory of Botany department was studied as well. They were isolated from soil and classified as *Aspergillus terrrus*, *Aspergillus. niger* and *Penicillium chrysogenum.*

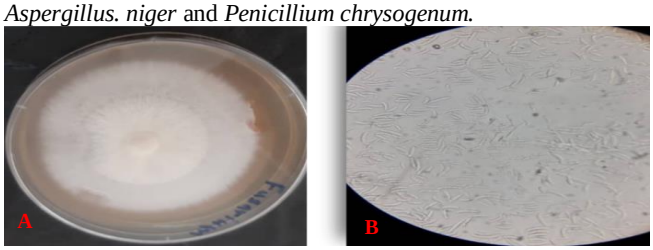

Figure 1. (A) *Fusarium solani* on SDA medium **(B)** chlamydospores and conidiospores of *Fusarium solani*.

3.2. Isolation and identification of Bacillus spp.

The identification of Bacillus spp. in this study was based on the morphological and biochemical characteristics according to Bergey's manual of bacteriology [15]. Five species of Bacillus were obtained in this study where their morphological biochemical properties were in agreement with the others results mentioned in this field [14, 21]. All the isolates of Bacillus spp. were brown white, round, opaque, with an irregular edge, gram positive bacilli and spore-forming bacteria in addition to the ease growth on the simple media such as Nutrient agar **Fig .2.** [21,22]

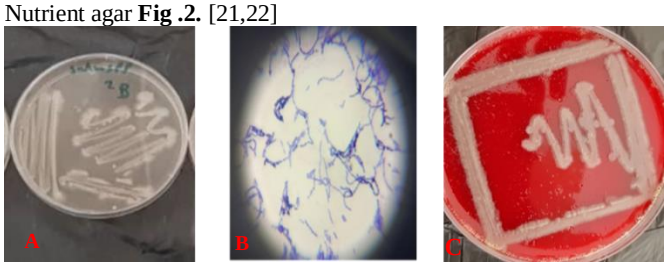

Figure 2. Morphological and biochemical characteristics of *Bacillus* spp. **(A)** white irregular colonies of *Bacillus* spp. on Nutrient agar **(B)**

gram positive bacilli cells under the microscope **(C)** colonies of *Bacillus* spp. on blood agar.

Bacillus spp were positive for catalase enzyme and non-hemolytic, and their response to other tests were varied **Tab .1 Fig 2.** These results are similar to the previews study of [14]. Regarding Catalase, is expected a structural respiratory enzyme and therefore it is used by aerobic bacteria for the dispose of hydrogen peroxide (H_2O_2), which is common in aerobic bacteria, including Bacillus spp.

while oxidase, it is differential enzyme, which most of Bacillus spp. are able to produce it. These enzymes are important for the normal cellular metabolism and the diagnosis of unknown bacterial isolates. Regarding the hydrolysis of starch and the utilization of citrate, the results were diverse and agreed with the study of [14,23] **Tab .1**. The use of these two compounds is prominent for bacteria because they are sources of carbon, which is one of the major elements that microbes need it in large quantities.

Table 1. The Morphological and biochemical characteristics of Bacillus spp.

Morphological and Biochemical tests	*B. megaterium*	*B.licheniformis*	*B. firmus*	*B. pumilus*	*B.sphaericus*
Grams staining	*+	+	+	+	+
Shape	Bacilli	bacilli	bacilli	bacilli	cocco bacilli
Spore formation	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	+	+	-	-	-
Starch hydrolysis	+	+	-	+	+
Citrate utilization	+	+	-	+	+
Nitrate reduction	-	+	+	-	+
Voges-Proskauer (VP)	-	+	-	+	-
blood hemolysis	-	-	-	-	-

* (+) positive results, (-) negative result.

The results of sugars consumption by *Bacillus* spp. in this study are supported with the results of [14] **Tab. 2**. Many heterotrophs microorganisms use multiple organic sources to obtain energy. These sources include carbohydrates, organic acids, fatty acids and amino acids. However, the preferred compounds for the species of Bacillus are carbohydrates, especially Glucose.

Table 2. The fermentation of carbohydrates by Bacillus spp.

sugar	*B. megaterium*	*B. licheniformis*	*B. firmus*	*B. pumilus*	*B. sphaericus*
Glucose	*+	+	+	+	+
Sucrose	+	+	+	+	+
Dextrose	+	+	+	+	+
Lactose	+	+	-	+	-
Galactose	+	+	-	+	+
fructose	+	+	+	+	-

* (+) A positive result and the color of the medium changed to yellow. Negative result There is no change in the color of the medium.

Among 5 soil samples, only 11 isolates of Bacillus spp. were obtained. These bacteria were classified in five spices including *Bacillus megaterium* (55%), *Bacillus licheniformis* (18 %), *Bacillus firmus* (9%) , *Bacillus pumilus* (9%) and *Bacillus sphaericus* (9 % **).** The results demonstrated that *Bacillus megaterium* were the most abundant species of Bacillus spp. **Fig. 3**. This is may be explained by the fact that this bacterium possesses a multi-enzymatic system, as the isolates of Bacillus *megaterium* were positive for most of the studied tests.

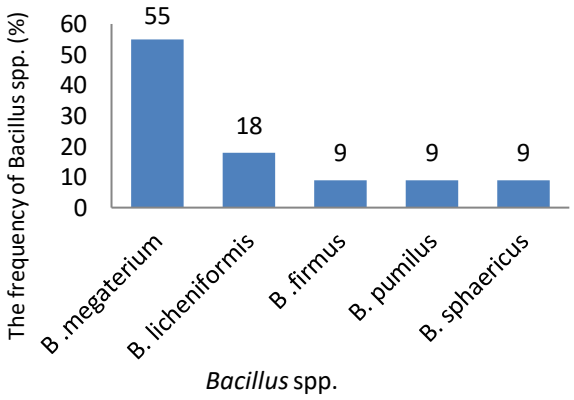

Figure 3. The percentage of *Bacillus* spp frequency in soil samples.

3.3.The antimicrobial effect of fungal species

The antimicrobial effect of fungal species: *Fusarium solani Penicillium chrysogenum ,Aspergillus terrrus* and *Aspergillus. niger* was tested against gram positive and negative bacteria represented in *Staphylococcus aureus*, and *Salmonella choler .arizona, Salmonella sp and Pseudomonas aeruginosa.* The tested gram positive and negative bacteria are associated with many diseases and infections such as skin infections, sepsis, inflammations of heart and abdomen, meningitis and urinary tract infection [9]. The previous studies indicated that the tested fungal species in this study have the ability to produce many antimicrobial substances [24],[25],[26],[27]. **Tab .3** Demonstrates the mean diameters of the fungal species growing in the medium inoculated with the tested bacteria compared with the control . The percentage of antimicrobials production by each fungus was calculated using Abbott's equation [17].

The results of antagonism test showed that *F. solani* had the highest antibacterial activity against *Staph. aureus, Salm.choler.arizonae, Salmonella* sp. *and P.aeruginosa* as the mean diameters of *F. solani* growth on the inoculated medium with the pathogenic bacteria was calculated by 81.67, 45.67 and 31 51.80 mm respectively. The Comparison of the mean diameters of *F. solani* growth on the

inoculated medium with *Salm.choler.arizonae*, *Salmonella* sp. *and P.aeruginosa* with the control showed statistical significance at a probability value of 0.05 **Tab. 3, Fig. 4**.This indicates that *F. solani* have a broad spectrum antimicrobial effect against Gram-negative and Gram-positive bacteria, which was also indicated by [24] as their study showed the antimicrobial effect of *F. solani* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at a concentrations of 12.5 µg/ml and of 25-100 µg/ml against *Salmonella typhi*. *Aspergillus niger* had a significant antimicrobial effect at P-value 0.05 against *Salm.choler.arizonae* and *Salmonella* sp. as the mean diameters of *A. niger* growth on the inoculated medium with the pathogenic bacteria were recorded at 13.67, 22 mm, respectively, compared with the mean diameter of the control at 66 mm **Tab. 3, Fig. 4** which is consistent with the studies of [25,26]. The calculation of antimicrobial production percentage of fungi using Abbott's equation showed that *F. solani* and *A. niger* had the maximum antimicrobial activity against Gram negative bacteria while the percentage of antimicrobial substances against the Gram positive bacteria was limited. The secretion percentage of antimicrobial substances by *A. niger* against *Salm.choler.arizonae*, *Salmonella sp.* and *P. aeruginosa* was recorded at 79.28%, 66.66%, and 24.44% respectively.

Table 3. The Percentage of antimicrobials production by fungi

Treatment	*F. solani*	*P. chrysogenum*	*A. niger*	*A.terreus*
Control	*82.67	24.67	66	64
Staph. aureus	81.67	15.33	65.33b	31
	**12%	37.85%	1.01%	51.56%
Salm.choler.arizonae	45.67	0	13.67a	0
	44.75%	0	79.28 %	0
Salmonella sp.	31	16	22	0
	62.50%	35.14%	66.66 %	0
P. aeruginosa	51.8	14.4	50	19
	37.34%	41.62%	24.44 %	70.31%
P-value	0.000	0.000	0.004	0.000

*The mean diameter of 3 replications (mm).
**Percentage

while the production percentage by *F. solani* against the same Gram negative bacteria was obtained at 44.7%, 62.50% and 37.34%, respectively. *P. chrysogenum* and *A. terreus* had a noticeable effect on *staphylococcus aureus* and some species of the tested Gram-negative bacteria where the percentage of the antimicrobials that were secreted by *P. chrysogenum* against *Staph. aureus*, *Salmonella sp*. and *P. aeruginosa* was calculated at 37.85%, 35.14% and 41.62% with mean diameters of 15.33, 16 and 14.40 mm respectively, comparing these mean diameters with the control demonstrate statistical significance at a P- value of 0.05. In contrast *P. chrysogenum* was unable to produce antimicrobial substances against *Salm.choler.arizonae* **Tab. 3, Fig. 4**. These results agreed with the results of [14,27]. *A. terreus* had a statistically significant effect on *staphylococcus aureus* and *P. aeruginosa* with mean diameters of 31 and 19 mm, respectively and the production rate of antimicrobials was calculated at 51.56% and 70.31% against *Staphylococcus aureus* and *P. aeruginosa* respectively. while *A. terreus* was unable to produce antimicrobials against the other pathogenic bacteria these results are consistent with the findings of [28].

4. The antimicrobials effect of *F. solani* and *A. terreus* extracts

All the studied fungi had the ability to produce antimicrobial substances against the gram positive and negative bacteria with different proportions. *F. solani* showed a significant antimicrobial effect on Gram-negative bacteria while *A. terreus* had a noticeable antimicrobial effect on *Staphylococcus aureus*, and *P. aeruginosa* therefore they were selected to extract their antimicrobial substances. **Tab 4.** demonstrated the effect of *F. solani* and *A. terreus* extracts against the pathogenic bacteria represented in the mean diameters of inhibition zones where the effect of *F. solani* extract on *P. aeruginosa* was a statistically significant at a P- value of 0.005 with a mean diameter of 36.33 mm compared to the mean diameter of the control **Tab. 4 ,Fig. 5**.

the significant effect of *F. solani* extract on Gram-negative and Gram-positive bacteria is evidence that this fungus secret broad-spectrum antimicrobials. Although the previous studies indicated the ability of *A .terreus* isolated from the soil to produce antimicrobial substances such as terreic acid and butyrolacton [25]. In this study the effect of *A. terreus* extract on the Gram positive and negative bacteria was not statically significant at the P-value of 0.05 **Tab .4**.

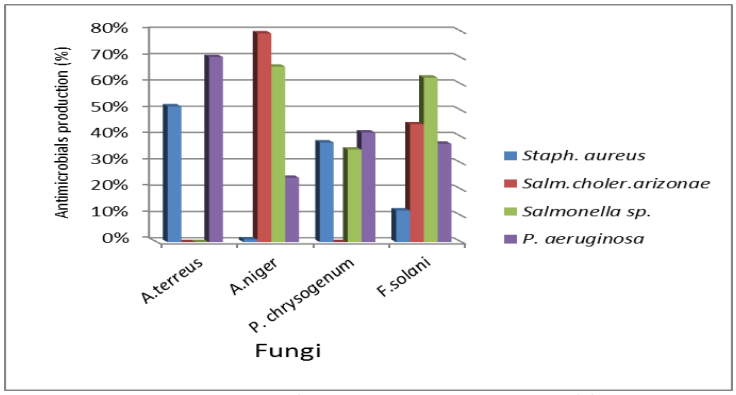

Figure 4. The percentage of antimicrobials production of fungi

Table 4. The inhibitory effect of fungal extracts on the pathogenic bacteria.

Treatment	*Staph. aureus*	*Salm.choler.arizonae*	*Salmonella sp.*	*P.aeruginosa*
Control	*35.33	36	22.33	20
F. solani	28	20.33	23	36.33
	(4.35)	(1.15)	(1.00)	(6.42)
A. terreus	25	35.67	24.33	22.67
	(2.64)	(9.23)	(2.51)	(1.52)
P-value	0.218	0.156	0.433	0.004

*The mean diameter of 3 replications (mm), () Standard deviation.

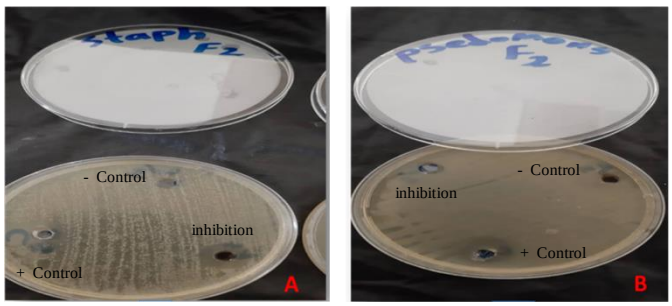

Figure 5. (A) the effect of *F. solani* extract on *Staphylococcus aureus*, **(B)** the effect of *F. solani* extract on *P.aeruginosa*.

5. The antimicrobial effect of Bacillus spp

Bacillus species are the dominant soil bacteria because of their resistant-endospore formation and production of prominent antibiotics such as bacitracin [23]. The antibacterial effect of Bacillus species was evaluated against the pathogenic bacteria. all *B. megaterium* isolates showed high ability to produce antibacterial substances against the species of the pathogenic bacteria in varied proportions **Tab. 5, Fig. 6** High percentage of antibacterial substances was secreted by *B. megaterium1* against *Salm.choler.arizonae*, *Salmonella sp.* and *P. aeruginosa* with a rate of 71%, 81.9% and 40% respectively, while only 18% of antibacterial substances were produced against *Staphylococcus aureus.* Statistical significance was obtained at P-value 0.05 when the mean diameter of the control was compared with the mean diameters of *B. megaterium1* growth in the presence of the pathogenic bacteria **Tab. 5**. These findings were in agreement with the results of [29]. *B.*

megaterium2, B. megaterium3, B. megaterium4 and B. megaterium5 showed high efficacy in producing antibacterial substances against the gram-positive and gram-negative bacteria, only *B. megaterium6* isolate was less effective in producing antibacterial substances against the pathogenic bacteria compared to other isolates of *B. megaterium* **Tab. 5, Fig. 6**. High and varied rate of antibacterial materials were produced by *B.licheniformis*1 against *Staphylococcus aureus, Salem.choler arizonae, Salmonella sp* and *P.aeruginosa* with a percentage of 61.23%, 52.72%, 33.32% and 34.88%, respectively. The same efficiency of antibacterial substances secretion was displayed by *B.licheniformis*2 these findings were mentioned by [30] *as well*. Statistical significance was noticed at P-value 0.05 when the mean diameter of the control was compared with the mean diameters of *B.licheniformis* isolates growth in the presence of the pathogenic bacteria **Tab. 5, Fig. 6.** *B.firmus, B.pumilus* and *B.sphaericus* showed a significant and high antibacterial activity against the Gram-negative bacteria, while they were unable to produce anti-bacterial substances against *Staphylococcus .aureus*. Also, the effect of the antibacterial substances produced by these species was statistically significant at a P-value of 0.05 **Tab. 5, Fig. 6**.these findings are consistent with the study of [31].

Table 5. The percentage of antibacterial substances produced by Bacillus spp

	Bacillus spp (percentage of antibacterial substances production)					
Bacillus spp	Control	*Staph. aureus*	*Salm.choler arionae*	*Salmonella sp*	*P.aeruginosa*	P- value
B.megaterium1	*86.67	71	25	15.67	52	0
		**18%	71%	81.90%	40%	
B.megaterium2	40.67	20.67	19.67	20.67	18.33	0
		49.17%	51.63%	49.17%	54.92%	
B.megaterium3	40.67	18	29.33	21.33	19	0.009
		55.74%	27.88%	47.55%	53.28%	
B.megaterium4	36.67	23.67	17.67	18	22.33	0.004
		35.45%	51.81%	50.91%	39.10%	
B.megaterium5	42.67	22.67	21.33	27.33	24.33	0.037
		46.87%	50.01%	35.95%	42.98%	
B.megaterium6	33.33	24.33	20.33	31	21.33	0.001
		27%	39%	6.99%	36%	
B.licheniformis1	43	16.67	20.33	28.67	28	0.003
		61.23%	52.72%	33.32%	34.88%	
B.licheniformis2	61.33	38	25	23.33	23.67	0
		38.04%	59.23%	61.95%	61.40%	
B.firmus	82.33	83	17.67	28	18.33	0
		0.81%	78.53%	65.99%	77.73%	
B.pumilus	42	0	25	34.33	22.33	0
		0	40.47%	18.26%	46.83%	
B.sphaericus	74.67	0	41.33	27.33	27.33	0
		0	44.64%	63.39%	63.39%	

*The mean diameter of 3 replications (mm).
**Percentage

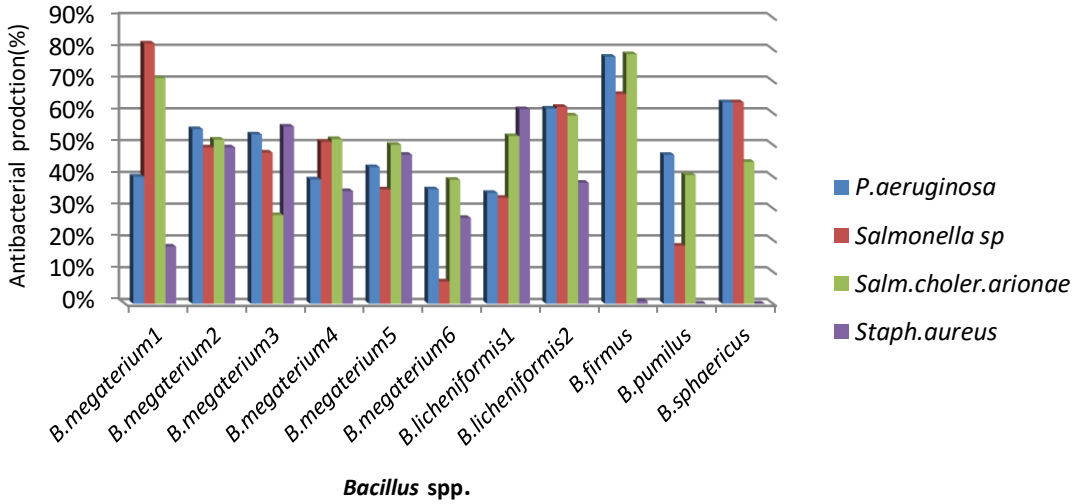

Figure 6. The percentage of antibacterial compounds production of *Bacillus* spp.

6. The antibacterial compound effect of Bacillus spp extracts

All the isolated Bacillus spp. showed high and varied efficiency in the secretion of antibacterial substances, only 5 isolates of Bacillus spp. were selected to extract their antibacterial substances, represented in *B.megaterium1, B.licheniformis1, B.firmus, B.pumilus* and *B.sphaericus* . The results demonstrated that Bacillus spp. extracts had a significant and high effect on gram-negative and gram-positive bacteria species *Staphylococcus aureus, Salm.choler.arizonae, Salmonella sp*. and *P.aeruginosa* **Fig. 7** shows high inhibitory effect of *B.megaterium1, B.licheniformis1* extracts against *staphylococcus aureus* where the effect of antibacterial substances was extremely high therefore measuring the diameter of the inhibition zones was not possible due to the interaction between inhibition zones of Bacillus spp. extracts and the positive and negative control diameters. The antibacterial effect of Bacillus spp. against the Gram-positive and Gram-negative bacteria is related to the ability of Bacillus spp to secrete multiple polypeptide antibiotics, such as polypeptide bacteriocins, as an effective antibiotics against the Gram-positive bacteria [30]. Other compounds are produced by Bacillus spp such as Polymyxin and Colistin which are effective against the Gram-negative bacteria [32,14].

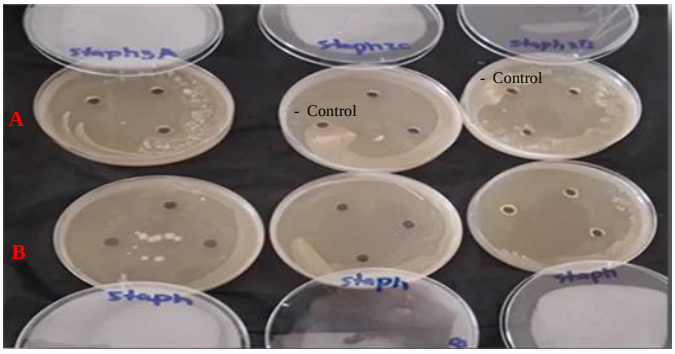

Figure 7. The inhibitory effect of **(A)** *B.megaterium1* **(B)** *B.licheniformis1* extracts on *Staphylococcus aureus*.

Conclusion

Screening of the new antibiotics from natural sources is becoming increasingly prominent for the pharmaceutical industry because of the significant increase of pathogenic bacteria resistance to the commonly used curative agents. some fungi and Bacillus spp. Commonly found in the soil and they have high ability to inhibit the growth of other microorganisms due to its production of antimicrobial substances. The present study was conducted to evaluate the production of antibiotics from some fungal species and *Bacillus* spp. isolated from soil. Among all screened isolates, *Fusarium solani* and *Aspergillus niger* metabolite showed maximum inhibition against the gram negative bacteria while *Penicillium chrysogenum* and *Aspergillus terreus* showed greater inhibitory effect against the Gram-positive bacteria. Bacillus spp. showed high inhibitory effect against the tested bacteria and they had the ability to produce antibacterial compounds in varying proportions. However, through the result of the present study we recommend to study the structure of the active compounds in the extracts of fungi and Bacillus spp. by Phytochemical test of all fungal and Bacillus spp. extracts.

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