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Ecological Studies on Microorganisms Producing Antimicrobial Agents from Different Soil Types.

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ABSTRACT

In the present study, a trial was done to find out the distribution of antimicrobial agent producing bacteria from different soil types collected from different regions of Egypt governorates. Physical and chemical properties of soil samples were determined and isolation of different bacterial colonies from different soil types was also carried out. All the isolated bacterial colonies were then screened for their antimicrobial activity against the human pathogenic bacteria (Gram negative and positive), plant pathogen bacteria and fungi. The highest number was from Tanta, Sheben El-Kom and El-Mansora (33.33%) and El-Tahrir and Ismailia (27.78%) followed by Sedi-Salem and Kafr-El-Sheikh (16.67%), EL-Tall El Kebeer (16.67%) and the least isolates active from Borg El-Arab and El-America (5.56). According to the spectrum of 18 active bacteria, it was found that only three bacterial isolates SOF12, SMF4 and AMF11 showed the highest antimicrobial activity against all the tested microorganisms with high potential inhibitory activity against both filamentous fungi. This study indicates that microorganisms isolated from Salt affected soil from Kafr-El-Sheikh, sandy loam soil from Ismailia and alkaline soil from EL-Tall El Kebeer, respectively could be an interesting source of antimicrobial bioactive substances.

Keywords: Antimicrobial agent, Bacterial isolates, ecosystem ecology, Pathogenic microbes.

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INTRODUCTION

Ecology is the scientific study of relationships in the natural world. It includes relationships between organisms and their physical environments (physiological ecology); between organisms of the same species (population ecology); between organisms of different species (community ecology); and between organisms and the fluxes of matter and energy through biological systems (ecosystem ecology).

Soil is considered one of the most suitable environments for microbial growth [1], for that the microorganisms which have been isolated from the soil having leading in this area. Soil as a living system inhabits assorted cluster of living organisms, both micro flora (fungi, bacteria, algae and actinomycetes) and micro-fauna (protozoa, nematodes, earthworms, moles, ants). The density of living organisms in soil is exceptionally high i.e. as much as billions per gm of soil, usually density of organisms is less in cultivated soil than uncultivated or virgin land and population decreases with soil acidity [2].

In nature, multiple ecological interactions take place; which can be negative or positive for the organisms involved. The organisms and the physical-chemical conditions present in an ecological niche will delimit the type of interactions that can be observed. Competition is an interaction encountered in all habitats since the prevailing organisms need to do so in order to survive. Also, it is known that when various communities in an ecological niche utilize the same type of substrates they must compete [3]. Both theoretical and empirical studies suggest that in plant and animal communities, spirited interaction is the key determinant of species abundance and diversity [4]. As part of physiological and metabolic processes, communities, which are found colonizing definite areas provide the production of intracellular or extra-cellular low molecular weight components such as alcohols, fatty acids, secondary metabolites and some antimicrobial agents [4-6]. The substances secreted to the environment can be harmful or toxic to the surrounding organisms acting as a competitive advantage for the secretor. Amensalism or antagonism is the term used in the classification of ecological interactions where one component has the competitive advantage of producing and secreting substances that have inhibitory effects on other populations [7,8]. The substances must alter the habitat in a disadvantageous style so that the interaction may be categorized as antagonism or amensalism.

Antibiotic is a drug used to treat infections caused by bacteria that can cause illness to humans, animals and plants. Antibiotic functions to inhibit or destroy the bacterial cells that cause certain disease [9]. In fact, antibiotic is secondary metabolite produced by bacteria [10] to maintain their niche and territory. There are few groups of microorganisms that can be used as sources for clinically useable antibiotics. As stated by Cooke and Gibson [11], only antibiotics that have an effect on pathogenic cells but not the host cells are categorized as useful antibiotics. To date, over 100 different antibiotics are available to cure minor and life-threatening infections. Antibiotic resistance occurs when the effectiveness of drugs and chemicals designated to cure diseases are reduced [12].

Scientists are continuously searching for novel antibiotic producing microbes because drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics [4]. Consequently, a numbers of antibiotics that can fight against pathogenic bacteria had been discovered. According to Roberts [13], it is important to discover new antibiotics as the emergence of new diseases and reemergence of multiple-antibiotic resistant pathogens have caused current antibiotics ineffective. There are many sources where antibiotics can be discovered, however, soil is the most important source for the discovery of novel antibiotics. According to Dulmage and Rivas [14], soil microorganisms have continually been screened for their useful biological active metabolites, such as antibiotics since long ago.

Most of the antibiotic producers used today are the soil microbes. Antibiotics are one of the most significant commercially exploited secondary metabolites produced by the microorganisms and employed in a wide range. Bacterium, fungal strains and actinomycete members are extensively used in industrial antibiotic production and are easy to isolate, culture, maintain and to improve their strains. Over 5,000 antibiotics have been identified from the cultures of Gram positive and Gram negative organisms, and filamentous fungi, but only about 100 antibiotics have been commercially used to treat human, animal and plant diseases [15].

That soil is rich in microorganisms capable of antibiotic synthesis is well accepted, but the frequency with which synthesis occurs at ecologically significant levels in nature has been much less clear. Microorganisms synthesize a variety of antibiotics, even under field conditions, in the rhizosphere (that portion

of the soil enriched in carbon and energy resources released by plant roots). These antibiotics can contribute to microbial competitiveness and the suppression of plant root pathogens, and the bacteria that produce them are therefore of considerable interest as a practical means of plant disease control. More generally, the techniques used to understand the role of antibiotics in the rhizosphere are applicable to other habitats where mechanisms of microbial antagonism or the production of bioactive metabolites are of interest. The quantity and quality of nutrients available and the ability to compete successfully for them are major determinants of microbial population size and metabolic activity, both of which are integrally linked to the regulation of antibiotic synthesis. Nutrients are not dispersed uniformly throughout soil, but rather, are localized in the spermosphere and rhizosphere of plants, and in and around plant debris, wounds, lesions, and fungal propagules.

According to the previous studies it was found that the physiochemical prosperities and nutritional status of the different soil types could affect on the distribution of antimicrobial agent producing microorganisms in the soil so the aim of this study is the ecological studies on microorganisms producing antimicrobial agents from different soil types.

MATERIAL AND METHODS

Soil sample collection

Rhizosphere soils samples where any plant has flourished were collected from different locations in Egypt representing different soil conditions texture and classes, namely:

- Calcareous soil from Borg El-Arab and El-America
- Sandy loam soil from South El-tahreer and Ismailia.
- Salt affected soil from Sedi Salem and Kafr El-Sheikh.
- Alkaline soil from El-Tal El-Kebeer.
- Silty clay loam from Tanta, Sheben El-Kom and El-Mansora.

Plant samples were removed from each site with a block of soil, placed in a polyethylene bag and stored at 4 °C until examination. Some physical and chemical properties of soil samples were determined after air drying and sieving in a 2mm sieve. Particle size distribution was determined by Bouyoucus [16] hydrometric method. Carbonate, phosphorus, potassium and pH were determined by the methods described by Page et al. [17]. pH was by 1:2.5 soil-water suspension [18], organic matter by modified Walkley Black method [19], available phosphorus by the methods of Olsen et al. [20], sodium, and calcium in an extraction of neuter ammonium acetate [21]. Nitrogen and phosphorus were determined by Kjehldahl [22] and vanadomolibdo phosphoric acid yellow color methods [23], respectively.

Isolation of antibiotic producing organisms

About 10 ml of water was added to one gram of each soil sample, it was well shaken and 1 ml added to the Luria-Bertani (LB) agar medium. The agars were poured into sterile Petri dishes and allowed to set. The plates were incubated at 25°C and 37°C up to seven days with daily observation. Colonies on the agar plates with clear zones around them were suspected of showing antagonistic activities. These colonies were picked with the help of a sterile toothpick and purified by streaking on the surface of a set LB agar medium. The pure colonies were then transferred with the help of a sterile platinum loop into tubes containing 10 ml sterile LB broth medium and incubated at 37°C for 72 hours.

Microorganisms used in this study (Tested organisms)

The following test pathogenic organisms were used as indicator microorganism in all assays for determination of the screening isolates and metabolites produced (antibiotic). The test organisms, namely, the bacteria Gram negative (*Agrobacterium tumefaciens* (plant pathogen), *Erwinia carotovora* (plant pathogen), *Escherichia coli*, *Klebsiella pneumonia*) and Gram positive *(Staphylococcus aureus, Enterococcus faecalis, Micrococcus luteus, Methicillin-resistant Staphylococcus aureus (MRSA)* and the fungi *Fusarium solani* (plant

pathogen), *Fusarium oxysporumnd* (plant pathogen), *Candida albicans* were all from the culture collection in the Department of Microbiology, National Research Centre, and included both human and plant pathogens.

Screening of microbial isolates for antimicrobial metabolite production

The antibacterial activities of tested microorganisms were investigated by disc diffusion LB agar plates were prepared, sterilized and solidified [24]. After solidification pathogenic microorganisms cultures were swabbed on these plates. The sterile disc was dipped in the broth culture of each isolate and placed in the agar plate and kept for incubation at 37°C for 24 hrs. Zone of inhibition was measured and recorded. The ratios of clear zone to colony were calculated by dividing the area of the clear zone by the area of the colony. Zone of inhibition was measured and compared with control disc negative control treatment with cell-free water extract. The experiments were repeated thrice and mean values of zone diameters were presented. Isolates showing promising activities were selected for further studies.

Separation of microbial cells from soluble metabolites produced

About one millilitre (1 ml) broth cultures of the selected isolates were separately inoculated into 10 ml LB broths and incubated at 37°C for 72 hours. They were then centrifuged at 4500 *rpm* for one hour to precipitate the microbial cells from the metabolite solutions. The resulting supernatants were decanted and filtered through Whatman No. 1 filter paper into clean sterile test tubes and tested for antimicrobial activities by the agar disc diffusion method as described previously, using pathogenic microorganisms showed previously as the test organism. The diameters of inhibition zones were measured and recorded. The isolates which have high diameter of inhibition zone were selected for further investigation in this study. Selection was based on sizes of inhibition zones greater than 3 mm on at least two or more test organisms.

Statistical analysis

All the experiments were carried out in five replicates and mean values were presented. The data presented in graphs and tables corresponding to mean values + SEM and the statistical significant ($P < 0.05$) was established by using GraphPad prism 5 software.

RESULTS

For the work described here, the rhizosphere soil samples were collected from different locations, including, calcareous soil from Borg El-Arab and El-Ameria, sandy loam soil from South El-Tahrir and Ismailia, salt affected soil from Sedi Salem and Kafr El-Sheikh, alkaline soil from El-Tal El-Kebeer and silty clay loam soil from Tanta, Sheben El-Kom and El-Mansora (Table 1). On the whole 20 rhizosphere soil samples were collected, among them three from calcareous; five from sandy loam agricultural farm lands, including corn, onion and sugar cane fields (Table 2). Four from salt affected soil including canola, faba bean and rice field while two from alkaline soil. Six from silty clay agricultural farm lands, including wheat, onion and rose fields. The rhizosphere soil samples were collected batch wise at different times and different seasons in sterile plastic bags. Later the soil samples were treated physically and chemically, for the selective isolation of antibiotic producing microorganisms. The selective media used for this purpose is LB agar medium. A preliminary antimicrobial activity test was performed at this stage and only the bioactive strains were selected for further work.

From the all 20 soil samples of different origin (Table 2) 112 bioactive bacterial isolates were obtained on the basis of clear zones suspected of showing antagonistic activities. The whole bacterial isolates collection were screening for metabolites produced (antibiotic) against the bacteria Gram negative, Gram positive and hyphal growth inhibition and divided into five major groups on the basis of origin of the strains from different soils.

Group 1 includes 19 strains that were isolated from calcareous soil of Common Bean and Bean fields. The actual number of the bacterial strains obtained from these 3 soil samples was high, among the strains of group 1, the isolates CBF3, CBF4, BF8, and BF11exhibited potent activity against Gram positive, Gram negative and hyphal growth inhibition against of plant pathogenic filamentous fungal and were finally selected for further characterization and antibiotic screening.

Group 2 includes 30 strains isolated from sandy loam soils of a maize, pea and wheat fields, among these strains the isolates, SMF4, SMF7, SMF9, SMF12, SWF1, SWF7 and SWF9 were active against both Gram positive, Gram negative test bacteria and hyphal growth inhibition, while rest of the strains showed activity either against Gram positive or Gram negative test bacteria and were selected for further analysis.

Group 3 includes 12 bacterial strains isolated from saline soil of onion field, from this group the isolates, SOF2, SOF4, SOF5, SOF9 and SOF12 were active against both Gram positive, Gram negative test bacteria and hyphal growth inhibition, while rest of the strains showed activity either against Gram positive or Gram negative test bacteria.

Group 4 includes 22 bacterial strains isolated from alkaline soil of a clover and maize fields, from this group the isolates, ACF4, ACF8, AMF5, and AMF11, were selected finally for characterization and antibiotic screening.

Group 5 includes 29 bacterial strains isolated from alluvial soil of a rhizosphere of pea, rose, bean, maize, clover and onion fields, among them 8 representative strains including, PF4, RF8, BF5, MF1, CF3, OF2, OF7 and OF9 were selected finally for characterization and antibiotic screening.

Table (3) summarizes the number of bacterial isolated from various locations in the Egypt. The selected strains were cultivated on LB medium and preserved as slants in the -4 ^{o}C for further analysis.

Primary antimicrobial testing of bacterial isolates by disc diffusion technique and hyphal growth inhibition

A total of 28 isolates of bacterial were tested for antimicrobial activity against bacteria and yeasts by disc diffusion method against human pathogenic bacteria: *S. aureus, methicillin-resistant S. aureus (MRSA), E. coli, P. aeruginosa*, two species of plant pathogenic bacteria *Agrobacterium tumefaciens* and *Erwinia carotovora,* two species of yeasts: *C. neoformans* and *C. albicans*, hyphal growth inhibition against two species of plant pathogenic filamentous fungi: fungi *Fusarium solani* and *Fusarium oxysporum*. Sixty five percents of the isolates showed antimicrobial activity against at least one tested microorganism, 4% isolates were from the calcareous soil, 18% from sandy loam soil, 11% from the alkaline soil, 21% from the alluvial soil and 11% from the salt affected soil (Table 4).

Among them, 10% inhibited only bacteria, 10% inhibited plant pathogenic bacteria, 7% inhibited only yeasts, 14% inhibited only filamentous fungi, 9% inhibited both yeasts and filamentous fungi, and 15% had both antibacterial and antifungal activities (Table 5). The percentage of active bacterial against each test strain and the top 65% active bacterial were shown in Figure 1 and Table 5, respectively.

Isolate SMF4 showed the best inhibitory activity against *S. aureus* (inhibition zone 30.95 mm) and SWF9 against MRSA (inhibition zone 35.17mm) (Table 6). Only 43% and 47% inhibited *E. coli* and *P. aeruginosa*, respectively. BF5 had the best activity against *E. coli* (inhibition zone 32.87 mm) and SOF12 against *P. aeruginosa* (inhibition zone 33.62 mm). On the other hand, 40% inhibited both plant pathogenic bacteria *Agrobacterium tumefaciens* and *Erwinia carotovora* and isolates SWF1 and SOF5 had the best activity against *Agrobacterium tumefaciens* (inhibition zones 40.33 and 32.20 mm, respectively) and *Erwinia carotovora* (inhibition zones 25.26 and 35.56 mm, respectively). For anti-yeast activity, 29% of bacterial isolated inhibited *C. albicans* and SMF4 had the best activity against *C. albicans* (inhibition zone 32.45 mm). 43% inhibited *C. neoformans* and isolates AMF11 and CF3 had the best activity against *C. neoformans* (inhibition zones 34.37 and 28.87 mm, respectively). For antifungal activity against filamentous fungi, *Fusarium solani* and *Fusarium oxysporum*, 39% inhibited *Fusarium solani* and 43 % inhibited *Fusarium oxysporum*. In addition, 2 and 15 isolates showed high inhibitory activity >90% hyphal growth inhibition and 4 isolates strongly inhibited both fungi.

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Table 1: Physico-chemical properties of different soils samples from Egypt

Soil location	Mechanical analysis			Soil texture	pH	E.C ($dS\ m^{-1}$)	$CaCO_3$	Organic carbon	Total N (%)	Total P (%)	Available P (ppm)
	Clay %	Silt %	Sand %								
	Calcareous Soils										
Borg El-arab	16.8	31.0	52.2	Loamy	7.80	2.8	37.390	0.390	0.056	0.019	8
El-Ameria	5.4	38.2	56.4	sandy loam	7.50	3.1	35.500	0.420	0.060	0.020	7
	Sandy loam soils										
El-Tahrir	17.2	21.0	71.7	sandy loam	8.00	1.8	9.950	0.140	0.038	0.016	5
Ismailia	5.4	18.4	76.1	sandy loam	7.80	2.3	2.700	0.250	0.040	0.020	19
	Salt affected soils										
Sedi –Salem	42.0	36.5	21.5	Clay loam	7.67	5.8	0.350	0.636	0.035	0.021	10
Kafr El-Sheikh	38.7	32.1	28.2	Clay loam	7.78	6.0	1.200	0.418	0.055	0.019	18
	Alkaline soil										
EL-Tall El Kebeer	35.9	47.0	17.1	Silty clay loam	8.90	3.5	1.100	0.270	0.050	0.018	8
	Alluvial soils										
Tanta	40.2	47.1	12.2	Silty clay loam	8.0	3.8	1.350	0.779	0.085	0.022	18
Sheben El-Kom	42.2	48.8	8.0	Silty clay loam	7.40	2.8	1.400	1.136	0.180	0.027	16
El-Mansora	34.6	50.0	15.4	Silty clay loam	7.80	2.8	1.250	0.872	0.096	0.026	18

Exchangeable sodium percent (ESP), 18

Table 2: Rhizosphere soil samples collected for the isolation of antibiotic producing microorganisms

Soil sample No.	Plant sample	Nature of the sample	locality
1	Common bean *(Phaseolus vulgaris)*	Calcareous soil	Borg El-arab
2	Common bean (*Phaseolus vulgaris*)	Calcareous soil	Borg El-arab
3	Bean (*Vicia faba*)	Calcareous soil	El-Ameria
4	Maize (*Zea mays*)	Sandy loam soils	El-Tahrir
5	Pea (*Pisum sativum*)	Sandy loam soils	El-Tahrir
6	Wheat (*Triticum vulgare*)	Sandy loam soils	Ismailia
7	Pea (*Pisum sativum*)	Sandy loam soils	Ismailia
8	Sugar beet (*Beta vilgarius*)	Sandy loam soils	Ismailia
9	Rice (*Oryza sativa*)	Salt affected soil	Sedi –Salem
10	Onion (*Allium cepa*)	Salt affected soil	Sedi –Salem
11	Clover (*Trifolium alexandrium*)	Salt affected soil	Kafr El-Sheikh
12	Rice (*Oryza sativa*)	Salt affected soil	Kafr El-Sheikh
13	Clover (*Trifolium alexandrium*)	Alkaline soil	EL-Tall El Kebeer
14	Maize (*Zea mays*)	Alkaline soil	EL-Tall El Kebeer
15	Pea (*Pisum sativum*)	Alluvial soils	Tanta
16	Rose (*Althea rosea*)	Alluvial soils	Tanta
17	Bean (*Vicia faba*)	Alluvial soils	Sheben El-Kom
18	Maize (*Zea mays*)	Alluvial soils	Sheben El-Kom
19	Clover (*Trifolium alexandrium*)	Alluvial soils	El-Mansora
20	Onion (*Allium cepa*)	Alluvial soils	El-Mansora

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Table 3: Numbers of bacterial isolated from soils various locations in the Egypt

Locations	No. of isolates	Codes
Calcareous soil from Borg El-Arab and El-Ameria	4	CBF3, CBF4, BF8, and BF11
Sandy loam soil from El-Tahrir and Ismailia	7	SMF4, SMF7, SMF9, SMF12, SWF1, SWF7 and SWF9
Salt affected soil from Sedi-Salem and Kafr-El-Sheikh	5	SOF2, SOF4, SOF5, SOF9 and SOF12
Alkaline soil from EL-Tall El Kebeer	4	ACF4, ACF8, AMF5, and AMF11
Alluvial soil from Tanta, Sheben El-Kom and El-Mansora,	8	PF4, RF8, BF5, MF1, CF3, OF2, OF7 and OF9
Total	28	

Table 4: Distribution of bacterial included in this study according to their antimicrobial activity by disc diffusion method and hyphal growth inhibition

Origin of bacteria	Active isolates/Total isolates tested (%)
Calcareous soil	4%
Sandy loam soil	18%
Alkaline soil	11%
Alluvial soil	21%
Salt affected soil	11%
Total	65%

Table 5: Distribution of antimicrobial spectrum of 65% active bacterial

% Active bacterial	Activity			
	Anti-bacterial	Plant pathogenic bacteria	Anti-yeast	Anti-filamentous fungi
10	←			
10		←		
7			←	
14				→
9			←	→
15	←			→

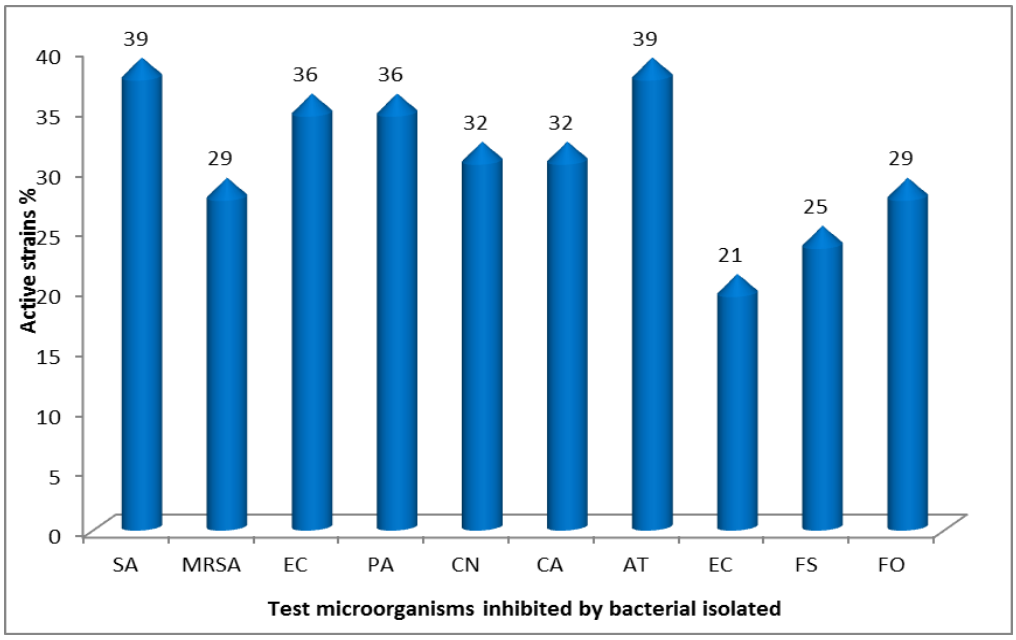

Figure 1: Percentages of active bacterial isolated against each test microorganism

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Table 6: Top 28 bacterial isolated having antimicrobial activity against ten tested microorganisms by disc diffusion method and hyphal growth inhibition test

Active strain code	Inhibition zone(mm)								% inhibition	
	SA	MRSA	EC	PA	AT	EC	CN	CA	FS	FO
CBF3	-----	-----	----	----	----	------	----	----	----	----
CBF4	-----	-----	----	---	-----	----	----	----	------	-----
BF8	---	---	---	---	----	----	---	----	----	----
BF11	---	7.80	13.42	17.20	24.70	21.00	----	---	----	-----
SMF4	30.95	23.54	22.50	34.85	31.45	21.54	18.90	32.45	90	100
SMF7	---	---	----	-----	------	------	-----	-----	---	-----
SMF9	14.00	15.00	----	----	-----	24.10	-----	-----	-----	----
SMF12	-----	14.25	21.00	-----	-----	18.55	13.20	-----	86	-----
SWF1	25.77	-----	-----	31.00	40.33	25.26	-----	25.60	-----	-----
SWF7	20.25	22.25	12.55	-----	18.45	-----	7.80	-----	87	95
SWF9	-----	----	-----	-----	------	-----	-----	-----	-----	----
SOF2	-----	----	-----	-----	------	-----	-----	-----	----	----
SOF4	-----	----	-----	-----	-----	-----	-----	-----	----	----
SOF5	-----	-----	-----	-----	32.20	35.56	-----	-----	-----	-----
SOF9	11.58	-----		11.80	-----	12.44	-----	----	-----	-----
SOF12	12.90	18.65	23.25	33.62	11.25	15.95	25.85	8.95	100	95
ACF4	------	-----	-----	-----	-----	------	-----	------	-----	-----
ACF8	-----	-----	-----	11.20	-----	-----	15.75	-----	86	90
AMF5	23.65	-----	10.51	-----	-----	-----	14.85	-----	-----	95
AMF11	22.32	28.25	14.70	20.20	9.00	18.20	34.37	14.15	97	100
PF4	-----	-----	-----	-------	-----	-----	33.62	17.52	-----	-----
RF8	------	-----	-----	-----	-----	-----	-----	-----	------	-----
BF5	7.72	-----	32.87	-----	-----	-----	13.88	20.65	85	100
MF1	-----	------	------	-----	-----	-----	-----	-----	-----	-----
CF3	11.72	-----	12.80	8.95	-----	-----	28.87	-----	-----	-----
OF2	19.33	7.75	-----	11.24	-----	-----	-----	-----	-----	-----
OF7	-----	-----	11.55	-----	22.15	-----	-----	-----	-----	92
OF9	-----	------	-----	18.95	18.22	-----	11.75	----	-----	-----

Top 18 bacterial isolated that can inhibit each group of the tested bacteria and yeast from disc diffusion method with inhibition zone over 25 mm and hyphal growth inhibition over 80% were selected for further study (Table 6). Among the 18 new active isolates from soil, the highest number was from Tanta, Sheben El-Kom and El-Mansora (33.33%), El-Tahrir and Ismailia (27.78%) followed by Sedi-Salem and Kafr-El-Sheikh (16.67%), EL-Tall El Kebeer (16.67%) and the least active isolates from Borg El-Arab and El-America (5.56). According to the spectrum of 18 active bacteria, it was found that most of bacterial inhibited three tested microorganisms (5 isolates) followed by 7, 6, 5, 3 and 2 isolates that inhibited 1, 1, 3, 4 and 2 tested microorganisms, respectively. Only three bacterial isolates SOF12, SMF4 and AMF11 inhibited all the tested microorganisms with high potential inhibitory activity against both filamentous fungi (Table 6).

DISCUSSION

Soil is a primary source of microorganisms and antibiotics producing microorganisms [25,26]. The numbers and species of microbes in soil is depend on environmental conditions like nutrient availability, soil texture, presence of moisture in soil and type of vegetation cover, and their number varies according to the type of environmental condition [27]. From ancient times is well understood that, natural products have a key role in the discovery and development of many antibiotics [28]. Antibiotics are one of the important pillars of modern medicines [29], but old antibiotics lose their efficacy and they are necessarily replaced with new ones

for many species of pathogenic bacteria [30]. Considerable research is being done in order to find new antimicrobial producing bacteria isolated from soil [31-33]. The present study focus on the isolation of a bacterial strain having antimicrobial activity from soil samples collected from different locations and different types of soils including, calcareous soil from Borg El-Arab and El-Ameria, sandy loam soil from South El-Tahrir and Ismailia, salt affected soil from Sedi Salem and Kafr El-Sheikh, alkaline soil from El-Tal El-Kebeer and silty clay loam soil from Tanta, Sheben El-Kom and El-Mansora.

Research in finding newer antibiotics and increasing productivity of such agents has been a very important activity [34, 35]. This is because some important drugs are expensive and/or have side effect to the host, some microbes have no successful antibiotics and others are developing multidrug resistance. From results, A total of 28 isolates of bioactive bacterial from 112 isolates were tested for antimicrobial activity against bacteria and yeasts by disc diffusion method against human pathogenic bacteria: *S. aureus, methicillin-resistant S. aureus* (MRSA)*, E. coli, P. aeruginosa*, two species of plant pathogenic bacteria *Agrobacterium tumefaciens* and *Erwinia carotovora,* two species of yeasts: *C. neoformans* and *C. albicans*, hyphal growth inhibition against two species of plant pathogenic filamentous fungi: fungi *Fusarium solani* and *Fusarium oxysporum*. Sixty five percents of the isolates showed antimicrobial activity against at least one tested microorganism, 4% isolates were from the calcareous soil, 18% from sandy loam soil, 11% from the alkaline soil, 21% from the alluvial soil and 11% from the salt affected soil (Table 4). The effect of soil pH on the biocontrol efficacy of *P. fluorescens* has been described [36]. These differences in results have been attributed to abiotic or biotic soil environment. Abiotic activity has been shown to be responsible for the antimicrobial activities of clay minerals used in the treatment of a mycobacterial skin infection, “Buruli Ulcer” [37]. Soil texture was found to influence survival of *Pseudomonas fluorescens* and *Bacillus subtilis* in soil [38], while soil temperature and pH and the presence of roots affected the leaching of a genetically modified strain of *P. fluorescens* in soil [39]. Biotic factors, including predation and antimicrobial-producing or lytic microorganisms were suggested as mechanisms of killing microorganisms introduced into soils [40-43]. Prior inoculation of soil with one strain of *P. fluorescens* reduced the ability of a second *P. fluorescens* strain to colonize [44]. A phenazine pigment produced by a *P. fluorescens* strain was shown to be responsible for biological control of a root disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* [45], and it has been shown that filaments of the biocontrol fungus *Trichoderma* grow towards fungal pathogens and release antibiotics and lytic enzymes [46].

The diversity of plants in an ecosystem and the composition of root exudates influence the functioning and structure of microbial communities [47-50]. The rhizospheric microbial community structure is influenced by plant litter and root exudates [51-53]. Both the composition of litter and the quality and quantity of root exudates are influenced by plant species and genotype, growth stage, and environmental conditions (light, pH, temperature, and nutrients, among others). The composition of root exudates is also affected by the microbial community as the result of plant–microbe chemical communication. Top 18 bacterial isolated that can inhibit each group of the tested bacteria and yeast from disc diffusion method with inhibition zone over 25 mm and hyphal growth inhibition over 80% were selected (Table 6). Among the 18 new active isolates from soil, the highest number was from El-Tahrir and Ismailia (16.67%) followed by Tanta, Sheben El-Kom and El-Mansora (11.11%), Sedi-Salem and Kafr-El-Sheikh (11.11%), EL-Tall El Kebeer (5.56%) and no active isolates from Borg El-Arab and El-Ameria. These soils are different in texture and other chemical properties (Table 1). Therefore, some of them are nutrient poor (Borg El-Arab and El-America) with a few dominant microbial species and antibiotic producing bacteria occupy niches with similar biotic characteristics. The low levels of antibiotic producing bacteria isolated in the current study may have several contributing factors [54]. The sandiness of the soil allowed easy drying and several species could have been lost during transport. In addition, sampling of sandy loam temperate soils during late spring (May) has been previously shown to recover a bacterial biomass only around half that of late summer (August).

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