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FACTORS AFFECTING THE EFFICIENCY OF ACTINOMYCETES IN THE PRODUCTION OF ANTIMICROBIAL

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ABSTRACT

This work aims to study the factors that affect the activity of actinomycetes in the production of antimicrobial whether they are anti-fungal or anti-bacterial. These factors are temperature, pH, concentration of sodium chloride salt (NaCl), carbon sources, nitrogen sources and the best media for the growth of actinomycetes. This was done with three isolates of actinomycetes AC2, AC9 and AC12). The results showed that the best temperature for the growth of the three isolates of actinomycetes was from 35-40oC, and the best pH was between 7 -7.2. The three isolates tolerated salinity with a concentration ranging from 2-5%. The best carbon source for the three isolates was starch, but with different concentrations. The best carbon source for AC2, AC9 and AC12 was starch at concentrations of 20 g/l, 10g\l and 25g\l, which gave inhibition zones 24, 45 and 34mm, respectively. The results indicate that the best nitrogen source for AC2, AC9 and AC12 was ammonium sulfate at concentration 2.5 g/l, sodium nitrate at concentration 1.5g\l, and sodium nitrate at concentration 1.5g\l which gave inhibition zones 20, 27 and 38 mm, respectively.

INTRODUCTION

Antibiotics play a vital role in our daily life because they are used in treatment of so many diseases, and also used in biological control of pathogens in plants. Antibiotics are biological, synthetic or semi synthetic natural substances **(CH *et al.*, 2020)**. Antibiotic overuse and misuse, according to the World Health Organization, has resulted in the resistance of numerous diseases. Antibiotic resistance is spreading among clinically relevant microorganisms like *Staphylococcus aureus*. New resistant strains have been evolving at a faster rate recently, but the rate of discovery of new antibiotics has slowed. As a result, many

scientists have concentrated on microorganism screening programs, particularly for the synthesis of antibiotics by actinomycetes **(Oskay *et al.*, 2004).**

Soil is a complex ecosystem that produces a diverse range of antimicrobial-producing microbes. Actinomycetes produce a vast range of secondary metabolites with a wide range of biological functions, including agriculturally and therapeutically significant substances. **(Saleh et al., 2011).** Because of their ability to create antibiotics and other secondary metabolites, actinomycetes play an important role in maintaining a healthy biological balance in the soil. **Strohl, (2004).**

Producing of antibiotic by actinomycetes has not been well-investigated (**Zulaybar *et al.,* 2006).** The production of antibiotics by different micro-organisms is influenced by the type and concentration of some nutritional requirements like nitrogen and carbon. In addition, the growth conditions such as pH level and temperature play an essential role in antibiotic production by different micro-organisms (**Thakur *et al.,* 2009).**

This study was carried out to isolate beneficial local actinomycete isolates and, improve their efficiency to produce antimicrobial and studying the factors affecting the antimicrobial production.

MATERIALS AND METHODS

This study was carried out at the Microbiological laboratory, department of agricultural microbiology, Faculty of agriculture, Minia University, Minia, Egypt.

1 -Isolation, purification and screening of actinomycetes isolates.

Soil samples were taken from the Farm of the Faculty of Agriculture at Minia University, ranging in depth from 10 to 20 cm. 100 mL sterile distilled water was used to suspend ten grams of incubated soil. One ml of soil suspension was shaken vigorously for one hour before being diluted to 10^{-7} using sterile distilled water. One of the final diluted soil suspension was put on the surface of a sterile nutrient agar plate and cultured for 7 days at 28±2°C. **(Aghighi *et al*., 2004).** After incubation, actinomycetes colonies were picked up and re-cultivated numerous times (by streaking technique) under the same previous isolation conditions to ensure purity. According to their cultural and physical properties, the pure actinomycete isolates were recognized up to the genus level **(Bergeys Manual, 1974).** Following that, the purified actinomycete isolates were divided into groups based on the color of their aerial mycelium **(Shirling and Gottlied, 1966)** and kept on sterile slants of nutritional agar medium in a refrigerator at 4 °C until utilized **(Kutzner, 1972).** Using nutrient agar medium, sub-culturing was normally done every two months.

2- Antagonistic activities of actinomycetes isolates against pathogenic fungi.

The purified actinomycete isolates were streaked on nutrient ager sterile plate media **(Dowson, 1957).** The inoculation plates were incubated for 15 days at 28±2°C.

The antagonistic activity of each culture was tested against different tested fungi using the diffusion plate method, as follows: purified actinomycetes isolates' cultural agar discs were cut with a sterile cork borer 6 mm and placed on nutrient agar plates medium separately seeded by tested fungi: *Rhizctonia solani*, *Macrophomina phaseolina*, and *Fusarium oxysporium*. The inoculation plates were incubated for 3 days at 28±2 °C. After incubation, the antagonistic activities of actinomycetes isolates were measured by measuring the inhibition zone (mm) on the cultural discs. **(Saleh *et al.*, 2011).**

3- Antagonistic activities of actinomycetes isolates against pathogenic bacteria.

The antibacterial activity of the examined actinomycetes was assessed against pathogenic bacteria (*Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus*)was done according to **(Bauer, 1966).** The actinomycete isolates were lawn-cultured on nutritional agar medium plates using intense streaking and incubated at 30 °C for seven days. Using a sterile cork borer, 6 mm agar discs were made from well-grown culture and placed on fresh lawn cultures of test microorganisms, which were subsequently incubated at their respective optimum temperature (37 °C). After 18 to 24 hours, the zones of inhibition for bacteria were measured.

4- Factors affecting production of antimicrobial by actinomycete isolates:

4-1:The optimum pH for actinomycetes isolates.

A loop full of the tested isolate from a 7day old culture was obtained and serially diluted in sterile distilled water from 10^{-1} to 10^{-7}. It was agitated with a vortex, and about 0.1 ml of the suspension was removed and inoculated on starch nitrate agar media **(Waksman, 1961)** at pH values of 6.5, 6.8, 7, 7.2, and 7.4 using the spread plate technique. The experiment was repeated twice, and the colonies were counted using a log colony forming unit (CFU). After a 7day incubation period at 30°C, the isolates were tested. The outcome was recorded **(Laidi et al., 2006).**

4-2:The optimum temperature for actinomycete isolates.

A loop full of the tested isolate from a 7day old culture was obtained and serially diluted in sterile distilled water from 10^{-1} to 10^{-7}. It was vortexed, then 0.1 ml of the suspension was removed and infected using the spread plate procedure. After incubation the isolates at 30, 35, 40, and 45°C, colonies were counted using log colony forming unit (CFU). The outcomes were documented **(Laidi *et al.*, 2006).**

4-3: Effect sodium chloride concentration on actinomycete isolates.

On starch nitrate agar media supplemented with 2, 5, 7, and 10% sodium chloride, the isolates were evaluated for their sodium chloride tolerance. Spread plate technique was used to inoculate agar plates with tested isolates. The experiment was carried out twice. After incubation the isolates at 30°C for 7 days, the colony was counted with a log colony forming unit (CUF), and observations were made to record the greatest salt concentration that supports growth (**Santhi *et al.*, 2010).**

4-4: Influence of different carbon and nitrogen sources on antimicrobial activity.

Using the agar diffusion method, the effect of various carbon and nitrogen sources on antifungal activity was investigated. Two ml of the preceding spore suspension were put into a 250 ml conical flask containing 50 ml of starch nitrate broth medium and incubated for seven days on a rotating shaker at 28±2 °C . (200 rpm). Following growth, the appropriate volume of cell-free filtrate was transferred to the wells pored in nutritional agar plates that had previously been inoculated with *Macrophomina phaseolina* (most sensitive test organism). In the starch nitrate medium, different carbon sources were tested by replacing 20 g L-1 starch (control) with 15.0, 20.0, 25.0 g L^{-1} corn yellow, glucose, dextrin, and mannitol, respectively. In the starch nitrate medium, different nitrogen sources were tested by replacing 2.0 g L^{-1} KNO3 (control) with 1.5, 2.0, 2.5, and 2.5 g L^{-1} $NaNO_3$, $(NH_4)_2SO_4$, and urea, respectively. In the basal medium (starch nitrate broth), each carbon and nitrogen source was supplied at the same C|N ratio and in place of starch and KNO_3, respectively. The

inhibition zone (mm) around the isolates was measured to get the results**. (Saleh *et al*., 2011).**

RESULTS AND DISCUSSION

1- Isolation and purification of actinomycetes isolates.

The Faculty of Agriculture, Minia University, twelve actinomycete isolates were isolated from Egyptian soil samples for this study. They came from well-developed branching, non-septate, non-fragmented aerial mycelia bearing long spore chains and non-motile spore that did not end in verticillate sporophores, indicating that they belonged to the genus s*treptomyctes* **(Bergey,s Manual, 1974).**

Table (1) indicates that actinomycete isolates were divided into three groups based on the color of their aerial mycelium. The majority of actinomycete isolates were greyish in color, accounting for 7 isolates (58.33%) of all actinomycete isolates, followed by whitish and creamy, accounting for 3 (25%) and 2 (16.66) isolates, respectively. As a result, the actinomycete isolates AC 2, AC 9, and AC12 will be employed in future investigations to determine which isolate is the most effective in terms of antifungal and antibacterial activity. According to (**Rizk *et al*., 2007)** noted that in the soil, actinomycetes of the grey and white color series predominate over those of the yellow, red, violet, and green color series.

2- Antagonistic activities of different actinomycete isolates against different plant pathogenic fungi.

Data presented in Table (2) and Figure (1) indicated that actinomycetes isolates had varying degrees of antagonistic activity (mm of inhibition zone). (AC1,AC2, AC3, AC4, AC5, AC6,AC7,AC8, AC9, AC10, AC11and AC12)organism tested (*Macrophomina phaseolina* , *Fusarium oxysporium* and *Rhizctonia solani*).

All isolates gave antagonistic activity against *Macrophomina phaseolina* . The inhibition zone reached 32,28, 29.5, 33, 30.5, 35, 21, 31, 37.5, 29.5, 31 and 34. 5 respectively. while 7 isolates of actinomycetes (AC2, AC7, AC8, AC9, AC10, AC11 andAC12) had an anti-fungal effect (*Rhizctonia solani*) and the inhibition zone reached 35, 25, 22, 21.5, 25, 24.5 and 22 respectively, while none of the isolates had any inhibitory effect against Fusarium oxysporium. On the other hand, actinomycetes isolates AC9 showed more inhibition effect than the other isolates and control. These results in agree with those of (**Singh *et al.*, 2017**) who reported that Six isolates were discovered to have antifungal activity against various fungi among 80 identified actinomycetes. ACITM-1 was chosen based on screening since it inhibited all of the fungus tested, including *Macrophomina phaseolina*, *Fusarium oxysporium*, *Colletotrichum truncatum*, and *Rhizoctonia solani*. **Tlemsani *et al.* (2020)** indicated that among 61 actinomycetes isolates, only 18 or 29.50% are active on at least one FOC isolate and 70.5% did not show any antifungal activity against the three FOC isolates. **Rostami Mehrouyie *et al.* (2021)** showed that three actinomycetes isolates (R1.6, R5.52, and R5.56) were chosen for further study because they had the highest inhibition zone size against *Macrophomina phaseolina*.

3- Antagonistic activities of different actinomycete isolates against different pathogenic bacterial strains.

Data in Table (3) and Figure (2) showed that among all 12 tested actinomycete isolates only AC2 showed that antibacterial effect against the two pathogenic bacteria *(Escherichia coli* and *Staphylococcus aureus*) with inhibition zones 25 and18mm, respectively. While they had not any effect against *Pseudomonas aeruginosa* and *Bacillus*

cereus. This completely matches with the results found by **Kumar *et al.* (2010)** indicated six isolates of actinomycetes were highly active against *Staphylococcus aureus* strains. Seven isolates were highly active with an inhibition zone more than 20 mm in diameter. Most of the isolates inhibited growth of the gram-negative bacteria tested. **Sah *et al.* (2021)** discovered that antibacterial activity was observed in the isolated actinomycetes (T18) against both gram-positive and Gram-negative test microorganisms. The prospective isolate showed inhibitory zones of 9 mm,26 mm , 26 mm, and 14 mm against *P. aeruginosa*, *B. subtilis, S. aureus,* and *E. coli*, respectively.

4- The effect of varying pH levels on actinomycetes' growth.

To study the effect of pH value of the growth medium on the three actinomycete isolates, different values (i.e. 6.5, 6.8, 7, 7.2 and 7.4) were tested using starch nitrate agar medium. Figure (3) showed that the selected three actinomycete isolates can grow and produce antimicrobial agents (antifungal and antibacterial) at different initial pH levels (6.5, 6.8, 7, 7.2 and 7.4). The best pH for isolates AC2, AC9 was 7.2, while the best pH for isolate AC12 was pH 7. According to **Charousová *et al.* (2021)** *Streptomycetes* are known to favor neutral to alkaline environments, with a pH range of 6.5-8 being ideal for growth. The researchers discovered that the strain VY31 grew best in the pH range of 6 to 8, with pH 7 being the best. **Salaria and Furhan, (2021)** indicated that the initial pH of the production medium had a direct impact on actinomycetes' bioactive metabolite synthesis. At pH 7, the highest inhibitory action was seen. In *Streptomyces* strains, neutral pH was found to be the most suitable for bioactive metabolite production. Antibiotic production improved as the pH was raised from 5.0 to 7.0, but as the pH was elevated higher, antibiotic production declined.

5- The effect of varying temperatures on actinomycetes' growth.

Three actinomycete isolates, 7 days old after culturing in starch nitrate agar medium were used to study effect of various temperature degrees on actinomycetes' growth and activity. The results indicated that the selected three actinomycetes isolates can grow and produce antifungal and antibacterial agents at different degrees of temperature (30, 35, 40, and 45°C). the results showed that the best temperature for isolates (AC12, AC9) was 40°C, while the best temperature for isolate AC2 was 35°C. (Figure 4). These results are in agreement with those of **Muleta and Assefa, (2018)** noted that the isolates grew at temperatures ranging from 15 to 37°C, with a higher number of colonies and colony diameter than the other temperature ranges. The findings also revealed that at 45°C, none of the isolates grew. **Al-agamy *et al.* (2021)** indicated that the stability and activity of some actinomycete strains were shown to be best at 35°C.**Charousová *et al.* (2021)** reported that the strain VY31 has optimal temperature of 30°C. They also discovered that the optimum growth temperature for most actinomycetes is 23-37°C, and that most actinomycetes are mesophiles with an optimum growth temperature of 30°C.

6-Effect of sodium chloride concentration on growth and activity of actinomycetes.

Four concentrations of sodium chloride (2, 5, 7 and 10%) were used to study their effect on the growth of three actinomycetes isolates. The data in Table (4) summarize the salt tolerance of isolates(AC2,AC9 and AC12), which indicated that the isolate tolerated salt concentration up to 5%, while above this concentration from 7% to 10% no growth was seen detected. The best concentration

of NaCl was (2%) for the three studied isolates. Generally 2% NaCl concentration was optimum for production of antibiotic by the three studied actinomycete isolates. Our results are in agreement with those of **Niemhom and Thawai, (2020)** who noted that NaCl tolerance was found between 0-5% concentrations. **Salaria and Furhan, (2021)** indicated that NaCl has been shown to have a considerable impact on actinomycetes' proliferation and generation of bioactive metabolites. The optimal concentration of NaCl for the synthesis of antibiotics in isolate A41 was 1.5 percent. At a salinity of 2%, a *Streptomyces sp*. SRDP-TK-07 was previously shown to produce more bioactive metabolites (**Rakesh *et al.*, 2013**).

7-Effect of different carbon sources on antifungal activities of actinomycete isolates.

The present study showed that the three selected actinomycetes isolates (AC2, AC9, and AC12) were able to exploit varied carbon sources for growth and antifungal production. The antifungal activities of actinomycetes isolates against *Macrophomina phaseolina* were influenced by the carbon source. The most optimal carbon source for isolate AC2 was starch at a concentration of 20 g/l which resulted in a 34 mm inhibition zone after 7 days of incubation at 28±2°C on a rotary shaker. This was followed by dextrin at a concentration of 25 g/l and mannitol at a concentration of 20 g/l which resulted in 29 mm and 20 mm inhibition zone respectively after 7 days of incubation under the same conditions, however, the results showed that corn yellow did not have any effect on antifungal activities. (Table 5) and(Figure5). The most suitable carbon source for isolate AC12 was starch a concentration of 25g/l which resulted in a 45 mm inhibition zone after 7 days of incubation at 28± 2°C on a rotary shaker, followed by mannitol at a concentration of 20 g/l, corn yellow and dextrin at concentration of 15 g /l which resulted in 30 mm and 29 mm inhibition zone respectively after 7 days of incubation under the same conditions. Also, the results shown in (Table 6) and(figure 5) suggested that the best carbon source for isolate AC9 was starch at a concentration of 10 g/L which resulted in 24 mm inhibition zone after 7 days of incubation at 28± 2°C on a rotary shaker followed by Mannitol at a concentration of 15 g/L and dextrin 10 g/L which resulted in 24 mm and 20 mm inhibition zone respectively after 7 days of incubation under the same conditions, while corn yellow had no effect on the antifungal activities of this isolate. According to **Sudiana *et al.* (2020)** The capacity of actinomycetes to use diverse carbon sources influences their ability to live in the soil. They discovered that the isolate TG01 could use a variety of carbon sources and could endure a wide range of salinity levels (1-5 %). Microbes' ability to live in varied environmental situations is linked to their carbon absorption pattern and ability to exploit a certain carbon source. **Chali *et al.* (2021)** indicated that high antibacterial and antifungal metabolite synthesis was obtained in conditions containing starch, followed by glycerol, D-xylose, D-glucose, D-fructose, sucrose, and maltose as major sources of

carbon for the generation of antibacterial metabolite by LCHAACC13 isolate.

8-Effect of different nitrogen sources on antifungal activities of actinomycete isolates.

The present study showed that the three selected actinomycete isolates (AC2, AC9 and AC12) could use different nitrogen sources for growth and antifungal activities; however the antifungal activities were influenced by the type of nitrogen source. Generally, the suitability of inorganic nitrogen on the antifungal activity was more active as compared to organic nitrogen expect urea. It was found that $NaNO_3$ was the most suitable source of nitrogen for the anti-fungal activity by isolates AC9, AC12 which resulted 38 and 27 mm inhibition zones after 7 days of incubation on a rotary shaker, respectively, followed by urea, which gave 32 and 27 mm of inhibition zones, respectively. Followed by $(NH_4)_2SO_4$ which gave 22 and 22 mm of inhibition zones, respectively after 7 days. On the other hand the most suitable nitrogen source for the antifungal activities of the isolate AC2 was $NaNO_3$, followed by($NH_4)_2SO_4$ which resulted in 20 and 20 mm inhibition zones respectively after 7 days, while urea did not affect the antifungal activities for isolate AC2.(Table 7 and Figure 6). our results are in agreement with those of **Al-Zahrain, (2007)** who indicated that the highest antimicrobial activity was obtained by *Streptomyces* isolate J12 culture, which containing 3 g L^{-1} KNO_3 followed by $NaNO_3$ and $(NH_4)_2SO_4$ at concentration of 2.5 g L^{-1}. **Refaat *et al.* (2017)** showed that organic forms of nitrogen (urea, casein, yeast extract, and peptone) were shown to be less suited than inorganic forms of nitrogen (KNO_3, $NaNO_3$, $(NH_4)_2.SO_4$ and NH_4NO_3), while sodium nitrate in the medium produced the most active metabolites.

Table (1): Numbers and colors of actinomycetes isolates.

Color of aerial mycelium	Isolates	
	Total	**%**
Gray	7	58.33
White	3	25
Creamy	2	16.66
Total	**12**	**100**

*Growing on nutrient agar medium for 15 days at 30 ^{o}C

Table (2): Antagonistic activities of different actinomycete isolates against different plant pathogenic fungi.

Pathogenic fungi	***Macrophomina. phaseolina***	***Rhizctonia solani***	***Fusarium oxysporium***
Actinomycetes isolates	**Inhibition zone(mm)**		
AC1	+ **(32)**	**- (0)**	**- (0)**
AC2	+ **(28)**	+ **(35)**	**- (0)**
AC3	+ **(29.5)**	**- (0)**	**- (0)**
AC4	+ **(33)**	**- (0)**	**- (0)**
AC5	+ **(30.5)**	**- (0)**	**- (0)**
AC6	+ **(35)**	**- (0)**	**- (0)**
AC7	+ **(21)**	+ **(25)**	**- (0)**
AC8	+ **(31)**	+ **(22)**	**- (0)**
AC9	+ **(37.5)**	+ **(24.5)**	**- (0)**
AC10	+ **(29.5)**	+ **(22)**	**- (0)**
AC11	+ **(31)**	+ **(21.5)**	**- (0)**
AC12	+ **(34.5)**	+ **(25)**	**- (0)**

- (Negative inhibition zone) + (positive inhibition zone)

Table (3): Antagonistic activities of different actinomycete isolates against different pathogenic bacterial strains.

Bacteria strains	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Bacillus cereus***
Actinomycetes Strains	**Inhibition zone (mm)**			
AC1	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC2	+ **(18)**	+ **(25)**	- **(0)**	- **(0)**
AC3	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC4	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC5	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC6	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC7	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC8	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC9	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC10	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC11	- **(0)**	- **(0)**	- **(0)**	- **(0)**
AC12	- **(0)**	- **(0)**	- **(0)**	- **(0)**

- (Negative inhibition zone) + (positive inhibition zone)

Table(4):Effect of sodium chloride concentration on growth of actinomycetes.

	Growth of different actinomycetes isolates		
Concentration of NaCl%	Actinomycet isolate 2	Actinomycet isolate12	Actinomycet isolate 9
0%	++	++	++
2%	++	++	++
5%	+	+	++
7%	-	-	-
0%	-	-	-

++ (well growth) + (modernity growth) - (no growth)

Table (5): The effect of carbon sources (starch, dextrin, mannitol and corn yellow) on antifungal activity by actinomycetes isolates(AC2 and AC12).

	Starch			**Dextrin**			**Mannitol**			**Corn yellow**		
Concentration g\l	**15**	**20**	**25**	**15**	**20**	**25**	**15**	**20**	**25**	**15**	**20**	**25**
	Inhibition zone (mm)											
Actinomycetes isolate 2	+ 20	+ 34	- 0	+ 26	+ 21	+ 29	- 0	+ 20	- 0	- 0	- 0	- 0
Actinomycetes isolate 12	+ 28	+ 37	+ 45	+ 29	+ 24	+ 27	+ 25	+ 31	+ 20	+ 30	+ 30	+ 30

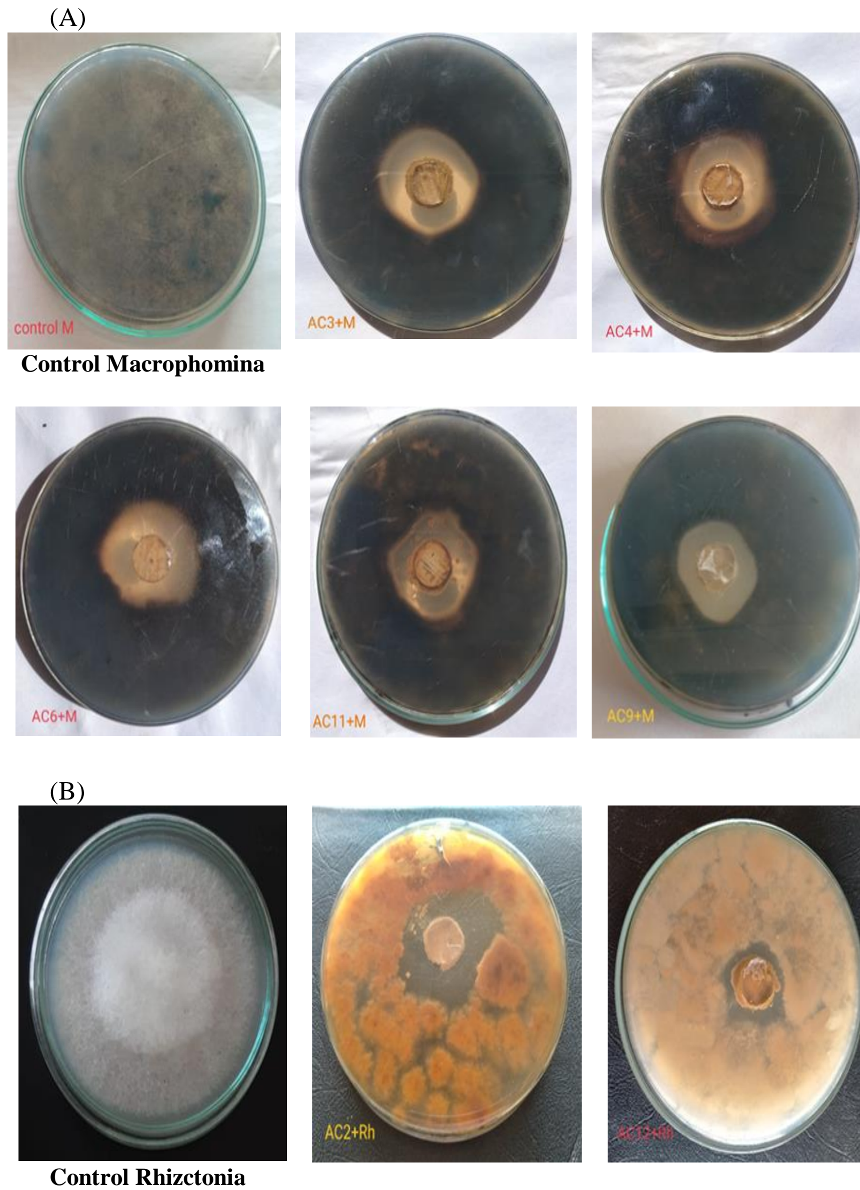
Table (6): The effect of carbon sources (starch, dextrin, mannitol and corn yellow) on antifungal activity by actinomycetes isolates(AC9).

	Starch			**Dextrin**			**Mannitol**			**Corny yellow**		
Concentration g\L	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**	**5**	**10**	**15**
	Inhibition zone (mm)											
Actinomycete isolate 9	- 0	+ 24	- 0	+ 20	**-** 0	+ 15	+ 15	+ 14	+ 24	- 0	- 0	- 0

Table (7)-The effect of nitrogen sources (NaNO3, (NH4)2SO4, Urea) on antifungal activity.

	$NaNO_3$			$(NH_4)_2SO_4$			Urea		
Concentration g\l	**1.5**	**2.0**	**2.5**	**1.5**	**2.0**	**2.5**	**1.5**	**2.0**	**2.5**
Inhibition zone(mm)									
Actinomycete isolate 2	- (0)	- (20)	- (0)	- (0)	- (0)	+ (20)	- (0)	- (0)	- (0)
Actinomycete isolate 12	+ (27)	- (0)	- (0)	- (0)	+ (22)	+ (22)	+ (15)	+ (20)	+ (25)
Actinomycete isolate 9	+ (38)	+ (28)	+ (35)	- (0)	+ (22)	- (0)	+ (25)	+ (27)	+ (32)

- (Negative inhibition zone) + (positive inhibition zone)



Figure(1): Antagonistic activities of actinomycete isolates against *Macrophomina phaseolina* and *Rhizctonia solani* compared with control.

(A)- Antagonistic activities of actinomycete isolates against *Macrophomina phaseolina.*

(B)- Antagonistic activities of actinomycete isolates against *Rhizctonia solani.*

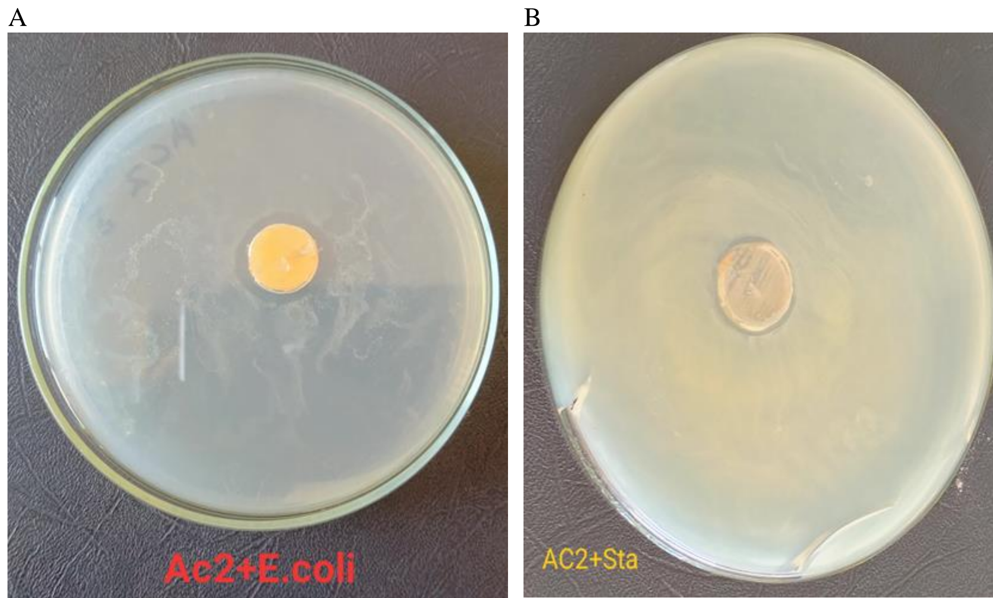


Figure (2): Antagonistic activities of actinomycete isolate AC2 against *Escherichia coli*(A) and (B) *Staphylococcus aureus*

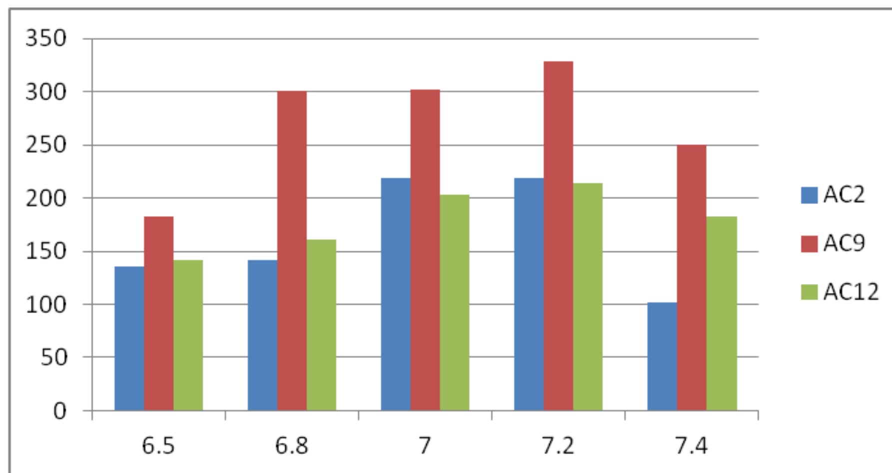


Figure (3): Growth of actinomycetes isolates on different pH degrees.

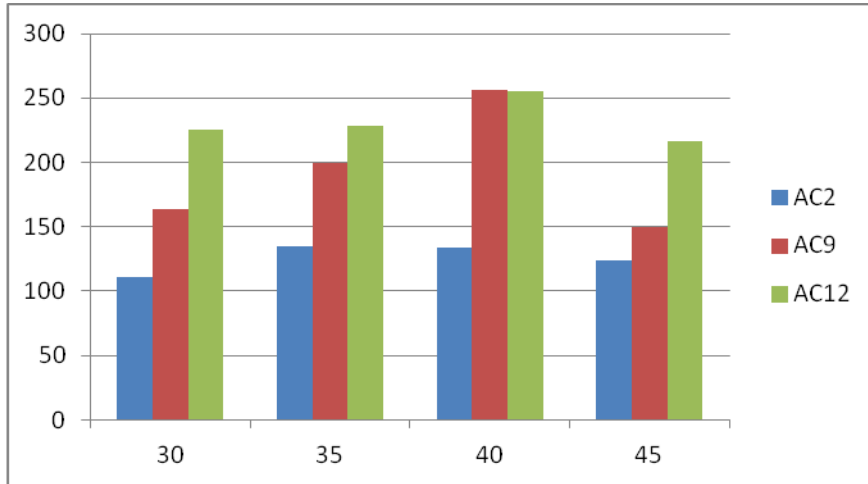


Figure (4): Growth of actinomycete isolates at different temperature degrees.

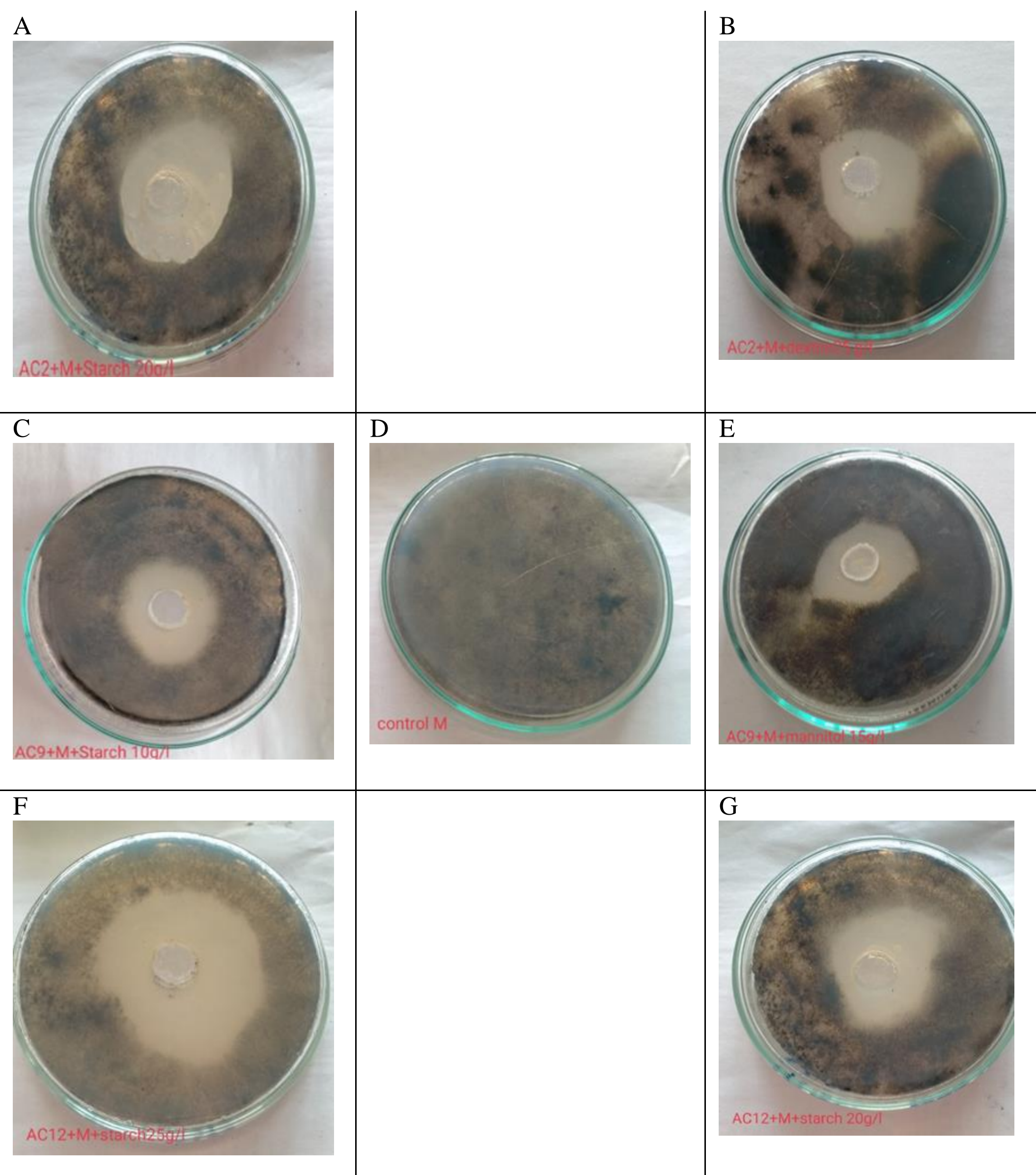


Figure (5): The effect of carbon sources on the efficiency of antifungal production against *Macrophomina phaseolina* by three actinomycetes isolates (AC2,AC9 and AC12) as compared with control.

A-actinomycetes isolates 2 (starch 20g\l).
B- actinomycetes isolates 2 (dextrin 25g\l).
C-actinomycetes isolates 9 (starch 10g\l).
D-control *Macrophomina phaseolina*.
E-actinomycetes isolates 9 (mannitol 15g\l)
F- actinomycetes isolates 12 (starch 25g\l)
G- actinomycetes isolates 12 (starch 20g\l)

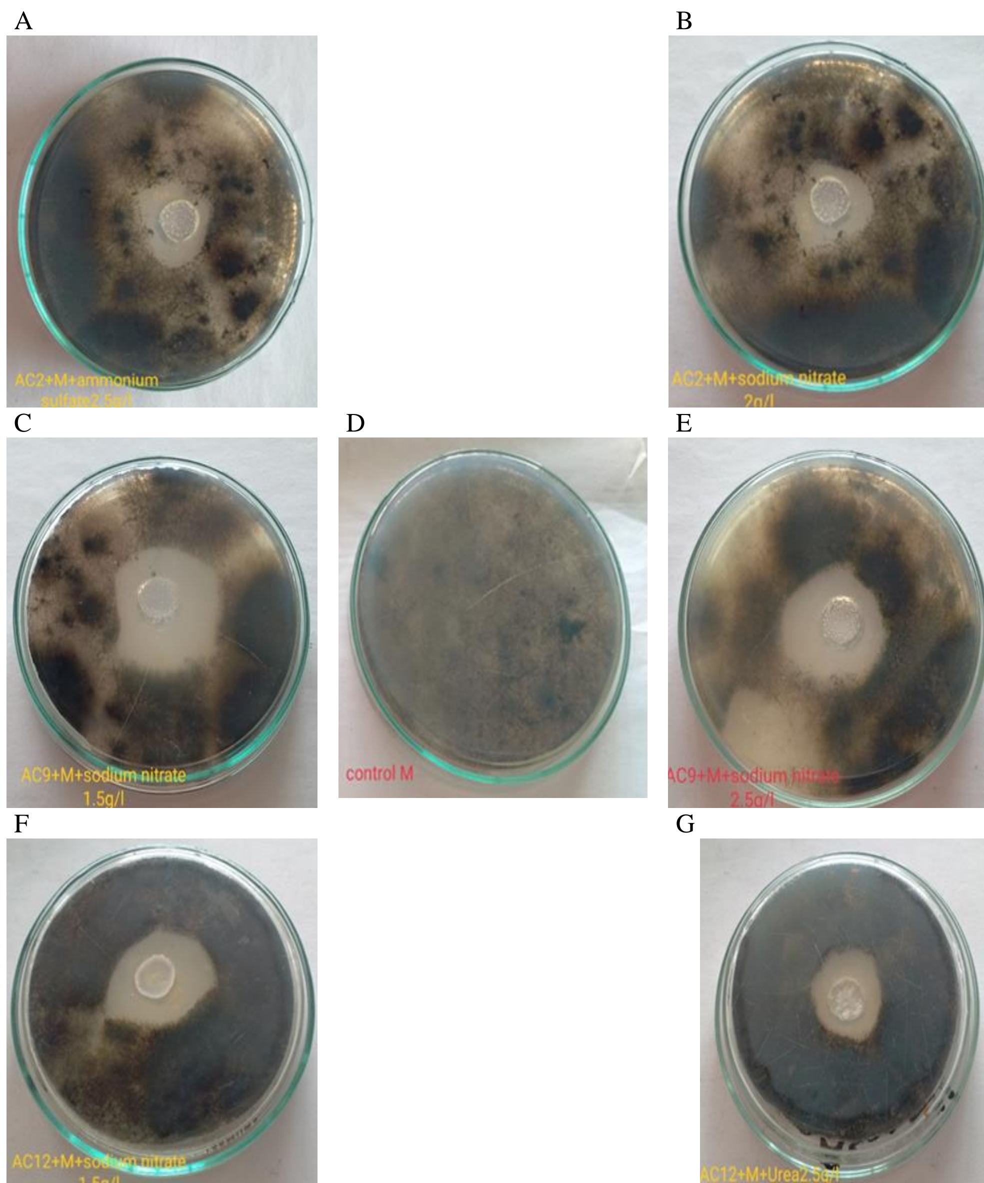


Figure (6): Effect of different nitrogen sources on antifungal activities of three isolates of actinomycetes on production of the antifungal against *Macrophomina phaseolina*)as compared with control.

A-actinomycetes isolates 2 ($(NH_4)_2SO_4$ 2.5g\l).
B-actinomycetes isolates 2 ($NaNO_3$ 2 g\l).
C- actinomycetes isolates 9 ($NaNO_3$ 1.5g\l).
D-control *Macrophomina phaseolina*.
E- actinomycetes isolates 9 ($NaNO_3$ 2.5g\l).
F- actinomycetes isolates 12 ($NaNO_3$ 1.5g\l).
G- actinomycetes isolates 12 (Urea 2.5g\l).

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الملخص العربي

العوامل المؤثرة على كفاءة الاكتينوميستات في إنتاج مضادات الميكروبات

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يهدف هذا البحث إلى دراسة العوامل التي تؤثر على كفاءة الاكتينوميستات في إنتاج مضادات الميكروبات ، سواء كانت مضادة للفطريات أو للبكتيريا وهذه العوامل هي درجة الحرارة – pH – تركيز ملح كلوريد الصوديوم (NaCl) – الكربون مصادر – مصادر النيتروجين و أفضل بيئة لنمو الاكتينوميستات وقد تم ذلك على ثلاث عزلات من الاكتينوميستات (AC2 ، AC9و AC12)وأظهرت النتائج ان أفضل درجة حرارة لنمو العزلات الثلاثة من الاكتينوميستات كانت تتراوح من ٣٥ – ٤٠ درجة مئوية وان أفضل pH للثلاثة عزلات كانت تتراوح من 7 –7.2 وان العزلات الثلاثة تحملت الملوحة بتركيز يتراوح من ٢–٥ %. وكان أفضل مصدر كربوني للعزلات الثلاثة هو النشا ولكن بتركيزات مختلفة حيث كان افضل مصدر كربوني بالنسبة (AC2) هو نشا ٢٠جرام/لتر وبالنسبة(AC9) ١٠جرام/لتر وبالنسبة(AC12) نشا ٢٥ جرام/لتر والتي اعطت هالة رائقة بقطر(٣٤و٤٥و ٢٤)ملليمتر على التوالي .وكان أفضل مصدر نيتروجيني للسلالة AC2 نترات الصوديوم ٢ جرام/لتر وكبريتات الامونيو 2.5 جرام/لتر وللسلالة (AC9) نترات الصوديوم 1.5جرام/لتر بينما كان(AC12)هو نترات الصوديوم 1.5 جرام/لتر والتي اعطت هالة رائقة بقطر (٢٠،٢٧،٣٨) ملليمتر على التوالي