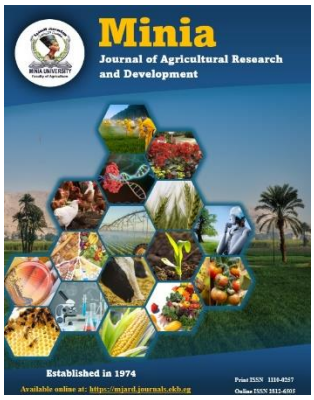
Minia J. of Agric. Res. & Develop., Minia Univ., Vol. 45 (4): 645 - 654, 2025

Minia Journal of Agricultural Research and Development

Journal homepage & Available online at:

https://mjard.journals.ekb.eg



Effect of actinophages on growth of actinomycete isolate and production of bioactive metabolites

Adel M. M. Hammad; Mohamed Z. H. Ali & Enaam M. M. F. Omar

Department of Agricultural Microbiology, Faculty of Agriculture, Minia University

Received: 12 August 2025 Accepted: 24 August 2025

ABSTRACT

The depressive effect of actinophages on growth of actinomycete and its production of bioactive metabolites was investigated in the present study. Moreover, to protect actinomycete against actinophage attack immobilization in alginate beads was used. Presence of actinophages in the liquid culture of actinomcete (AC2) free cells, markedly reduced the fresh and dry weight of its mycelium as compared to the free cells culture in absence of actinophages. Whereas, the highest fresh and dry weight of mycelium were recorded in alginate immobilized culture. In case of actinomycete immobilized cells, presence of actinophages had no marked effect on the fresh and dry weight of mycelium. The highest amount of metabolites were produced by immobilized actinomycete as compared to that produced by the free cells. Presence of actinophages had no marked effect on the amount of metabolites produced by immobilized cells. Using gas chromatography–mass spectrometry (GC-MS) analysis, five chemical compounds were detected in metabolites of actinomycete isolate (AC2).

Keywords: Actinomycetes, actinophages, immobilization, metabolites

INTRODUCTION

Several actinomycetes are able to produce many antibiotics as secondary metabolites**.** Actinomycetes, especially *Streptomyces* species (spp.) are very important since they have long been used against bacterial infections as biocontrol agents **(Cox *et al.*, 2017).**

Quach *et al.* (2016) reported that *Streptomyces* spp. are Gram-positive bacteria localized in the soil and they have a part in the medical industry. More than two-thirds of antibiotics that clinically used are produced by *Streptomyces* spp.

Zhu *et al*. (2014) determined that secondary metabolites are mostly produced by filamentous microorganisms (fungi and bacteria of the order Actinomycetales), which account for 90% of all antibiotics known. Actinomycetes, the majority of which belong to the genus *Streptomyces*, produce almost two-thirds of all antibiotics. Until recently, tens of thousands of natural antimicrobial agents have been extracted

* Corresponding author: Enaam M. M. F. Omar
E-mail address: mnn287212@gmial.com

from microbial sources, but this number likely represents just a small part of the total number of bioactive chemicals that could be created. **Lamsa *et al.* (2016)** reported that *Streptomyces* are productive sources of antibacterial, antifungal, and anticancer compounds which were widely used for treating various human infections.. In addition, **Peters *et al.* (2018)** reported that actinophage infection and lysis of cells have been recorded with a few actinomycetes which used in the pharmaceutical industries leading to low-quality of the end-products, failure of fermentation, and consequently substantial economic losses. Interestingly, infection of *Streptomyces* with actinophages was detected in the streptomycin commercial production resulting in lower yields of streptomycin.

The present study aimed to investigate the depressive effect of actinophages on growth of actinomycete and its production of bioactive metabolites. Moreover, attempt will be made to protect actinomycete against actinophage attack.

MATERIALS AND METHODS

Actinomycetes isolates

An actinomycete isolate (AC2) of high antagonistic activity against *Staphylococcus aureus, Escherichia coli, Klebselia* sp. and *Bacillus cereus* which was isolated previously by **Hammad *et al.* (2025)** from soil and compost samples was used in this study.

Actinophages

Two actinophage isolates specific to actinomycete isolate (AC2) were isolated previously by **Hammad *et al.* (2025)** from soil and compost samples and designated **ØAC2a** and **ØAC2b** were used in this study.

Preparation of actinomycete cultures

The actinomycete isolate was intense streaked on plates containing starch nitrate agar medium and incubated at 30°C for seven days. Using a sterile cork borer, 6 mm agar discs were taken from well-grown actinomycete culture plates. Five discs were placed in a 100ml conical flask containing 50 ml of starch nitrate broth medium and incubated for 7 days at 30°C (giving 10^8cfu/ml). This liquid culture was used to prepare free cells culture and immobilized cells.

a- Alginate-immobilized cells inoculum:

Alginate immobilized beads of 2mm in diameter containing cells of actinomycete isolate (AC2) were prepared as described by **Hammad (1998)**

b- Free cells culture

One liter conical flasks each containing 500 ml of starch nitrate broth medium were prepare and subjected to the following treatments:

1- Inoculation with free cells.
2- Inoculation with free cells and phage suspension.
3- Inoculation with immobilized cells.
4- Inoculation with immobilized cells and phage suspension.

Three replicates were involved for each treatment.

In case of inoculation with free cells, 5 ml of the prepared actinomycete liquid culture (10^8cfu/ml) were added to each flask.

In case of inoculation with the immobilized cells, a calculated weight of beads containing the same number of actinomycete cells (in the 5 ml of free cells inoculum) was added to each flask.

In treatments, which received phages, 5 ml of mixed phage suspensions were added to each flask. This phage mixture was prepared by mixing equal amounts of the

high titer suspensions of the two phage isolates.

The flasks were incubated at 30ºC for 10 days, and then the contents of every flask were centrifuged at 10 rpm for 15 min. The supernatant of every treatment was collected and stored at 4ºC.

The fresh and dry weight of biomass of every treatment was determined. The biomass was dried at 70ºC until constant weight.

Extraction of bioactive metabolites:

Antimicrobial substances were extracted from the supernatant of every treatment using three organic solvents (ethyl acetate, ethanol, and diethy either) at rate of 1:10 as described by **Raja *and* Prabakaran (2011)**. Each solvent was mixed with the supernatant and subjected to vigorously stirring for overnight. The extracts were then separated, dried and stored at 4°C.

The extract was subjected to GC/MS Analysis.

Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of the samples were performed according to **Mokhtar *et al*. (2023)** using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University.

RESULTS AND DISCUSSION

Effect of actinophages on growth of actinomycete (AC2)

Data presented in **Table (1)** indicated that presence of actinophages in the liquid culture of actinomcete (AC2) free cells, markedly reduced the fresh and dry weight of its mycelium as compared to the free cells culture in absence of actinophages.

Moreover, the highest fresh and dry weight of mycelium were recorded in alginate immobilized culture. These data may demonstrate that the immobilization system gives reasonable condition for development and increase of the immobilized cells interior the beads. Similar observations were found by **Fathy (2004).**

In case of immobilized cells of actinomycete AC2 the presence of phages had no pronounced effect on the fresh and dry weight of actinomycete mycelium. Such results indicate that the immobilization process protect the immobilized cells against phage infection. Such protection is due to the presence of the actinomycete cells interior beads of alginate that may prevent adsorption of phage particles on the surface of actinomycte cells, and hence no infection can be happened.

Therefore, the lytic action of phages in case of immobilized cells is restricted only on the cells released from alginate beads **(El-Balkhi *et al.*, 2006).**

Table (1): Fresh and dry weight of actinomycete (AC2) grown for 10 days in form of free and immobilized cells in presence and absence of actinophages.

Actinomycte Isolates	**Fresh weight of mycelium(g.)**			
	F	**F+P**	**M**	**M+P**
AC2	15.80	10.00	16.40	15.94
	Dry weight of mycelium(.g)			
	1.00	0.60	1.24	1.16

F= free cells; F+P= Free cells + phage; M= Immobilized cells; M+P= Immobilized cells +phage

Effect of actinophages on production of bioactive compounds by actinomycte (AC2):

Data presented in **Table (2)** indicated that in case of using any solvent (ethanol, diethyl either or ethyl acetate) the highest amount of bioactive compounds was produced by immobilized actinomycete isolate (AC2) as compared to that produced by the free cells. These data may indicate that the immobilization process provides convenient condition for multiplication and growth of immobilized cells interior the beads.

Moreover, in case of using any solvent (ethanol, diethyl either or ethyl acetate) the lowest amount of bioactive compounds were produced by free cells of actinomycete in presence of actinophages as compared to the other treatments. Such results may indicate that presence of actinophages depressed the growth of actinomycete and reduced its growth and hence reduced the production of metabolites. **Peters *et al*. (2018)** reported that actinophage infection and lysis of cells have been recorded with a few actinomycetes which used in the pharmaceutical industries leading to low-quality of the end-products, failure of fermentation, and consequently substantial economic losses. Interestingly, infection of *Streptomyces* with actinophages was detected in the streptomycin commercial production resulting in lower yields of streptomycin.

On the other hand, presence of actinophages had no marked effect on the amount of metabolites produced by immobilized cells of actinomycete. Such result may indicate that the immobilization process protect immobilized cells against phage infection **(Fathy, 2004)**.

Gas chromatography–mass spectrometry (GC-MS) analysis:

Results in **Table (3)** indicated that five chemical compounds were found in the ethanolic extract of actinomycete isolate (AC2) metabolites.

Table (2): Weight of bioactive compounds (g)produced by actinomycete (AC2) extracted by different solvents

Solvent	Treatments			
	F	**F+P**	**M**	**M+P**
Ethanol	2.92	2.68	3.16	3.04
Diethyl either	1.04	0.82	1.26	1.20
Ethyl acetate	0.84	0.72	2.00	1.96

F= free cells; F+P= Free cells+ phage; M= Immobilized cells; M+P= Immobilized cells +phage

Table (3): The detected compounds in the ethanolic extract of actinomycete isolate (AC2) metabolites.

No	Compound Name	Molecular Formula	Molecular Weight
1	2-Nonadecanone 2,4-dinitrophenylhyda zine	C25H42N4O4	462
2	Carbamic acid, N-[10,11-dihydro-5-(2-methylamino-1-oxoethy l)-3-5H-dibenzo[b,f]azepinyl]-, ethyl ester	C20H23N3O3	353
3	Ethyl 5-[(methylamino)acetyl]-10,11-di hydro-5h-dibenz o [b,f]azepin-3-ylc arbamate #	C20H23N3O3	353
4	Phthalazine, 1,2-Dihydro-1,4-Dipheny	C20H16N2	284
5	Palmitic acid, 2-(tetradecyloxy) ethyl ester	C32H64O3	496

The first compound is **2-Nonadecanone 2,4-dinitrophenylhyda zine**. As shown in **Figure (1)** its formula C25H42N4O4 and MW 462.Thiscompound being a long-chain **aliphatic ketone**, has shown **antimicrobial properties** in some studies. It may have **antibacterial** and **antifungal** effects, likely due to its ability to disrupt microbial cell membranes or interfere with microbial metabolism. This compound is sometimes used in formulations for treating **skin infections** or incorporated into **antimicrobial agents (Jeeva and Krishnamoorthy, 2018).**

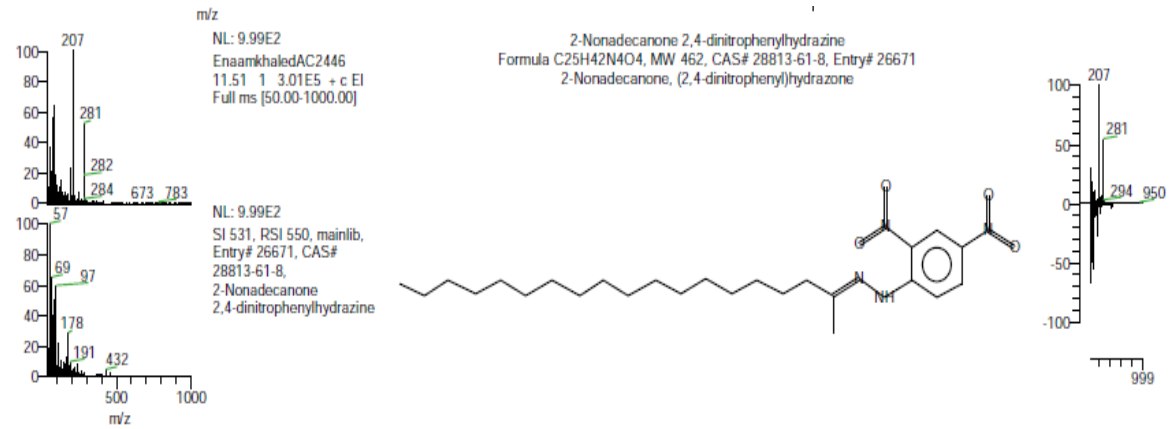


Figure (1): GC/MS chromatogram and chemical structure of ethanolic extract of actinomycete isolate (AC2) metabolites.

The second compound is **Carbamic acid, N-[10,11-dihydro-5-(2-methylamino-1-oxoethy l)-3-5H- dibenzo[b,f]azepinyl]-, ethyl ester.** The formula of this compound is C20H23N3O3 and MW 353as indicated in **Figure (2).**There's no direct evidence to suggest this compound has **strong antibacterial** or **antifungal** activity. It is more likely a **pharmacologically active compound** used for treating **neurological conditions** rather than infections. The structure suggests it might be used in psychotropic or **neuropharmacological treatments (Desbois and Smith, 2010).**

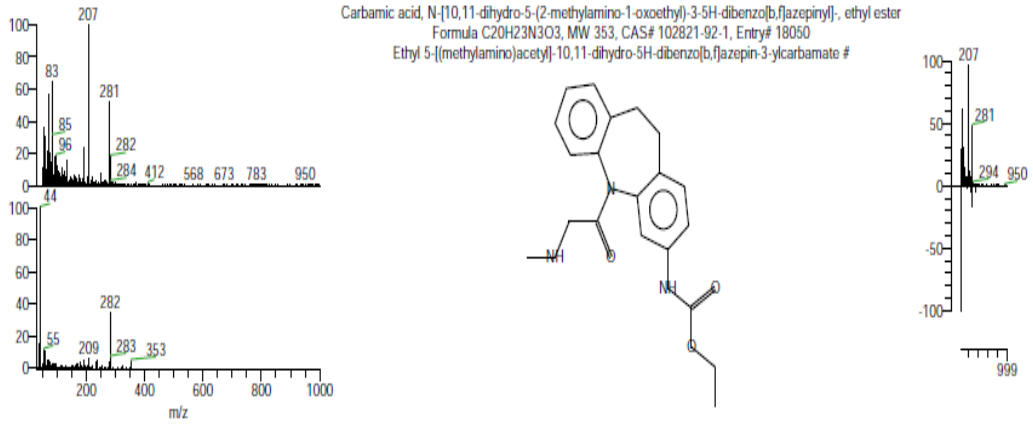


Figure (2): GC/MS chromatogram and chemical structure of ethanolic extract of actinomycete isolate (AC2) metabolites.

Moreover, **Ethyl 5-[(methylamino)acetyl]-10,11-di hydro-5h-dibenz o [b,f]azepin-3-ylc arbamate** was detected in the metabolite ethanolic extract of actinomycete AC2 which has MW 353 and formula C20H23N3O3 as shown in **Figure (3).**This compound seems to be more related to **psychiatric or neurological** drugs, with no clear connection to **antibacterial** or **antifungal** properties, while some research indicates potential anticancer activity. It likely works on **neurotransmitter systems** rather than having antimicrobial effects. **(Sharma and Sharma, 2011)**.

Phthalazine, 1,2-Dihydro-1,4-Diphenyl:

Phthalazine, 1,2-dihydro-1,4-diphenylhas MW 284 and its formula is C20H16N2 **(Figure 4).** It is known to bean antitumor agent it has also shown potentially targeting certain cancers by inhibiting the growth and spread of blood vessels. Additionally, it has demonstrated antimicrobial and anti-inflammatory properties **(Maghdu and Palaniyappan, 2014).**

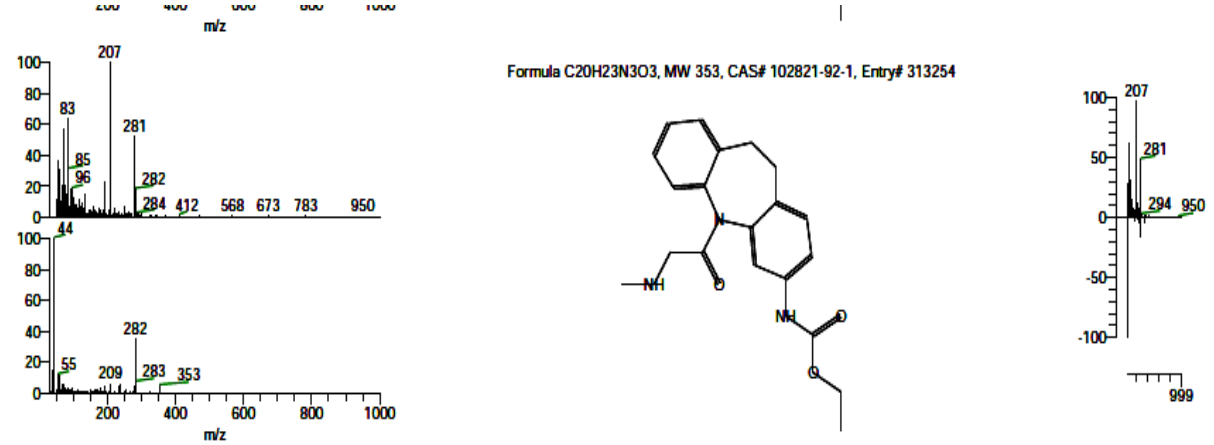


Figure (3): GC/MS chromatogram and chemical structure of ethanolic extract of actinomycete isolate (AC2) metabolites.

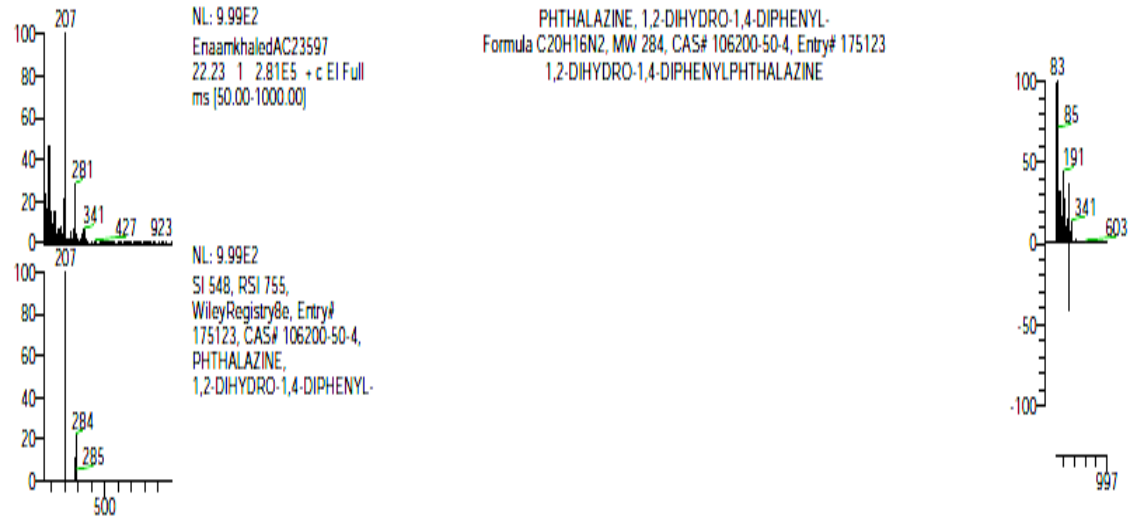


Figure (4): GC/MS chromatogram and chemical structure of ethanolic extract of actinomycete isolate (AC2) metabolites.

Palmitic acid, 2-(tetradecyloxy) ethyl ester

The molecular weight of **palmitic acid** is 496 and its formula is C32H64O3 as indicated in **Figure (5). Palmitic acid** itself has shown some **antibacterial** properties, though it's not typically classified as a primary antimicrobial agent. The **ester form** may improve its penetration and effectiveness in topical formulations. It exhibits both antifungal and antibacterial properties, though its effectiveness can vary depending on the specific microorganism and concentration. While it can inhibit the growth of certain bacteria and fungi, but there is limited evidence supporting it as a strong **antifungal** or **antibacterial** agent (**Saravanan *et al.*, 2016).**

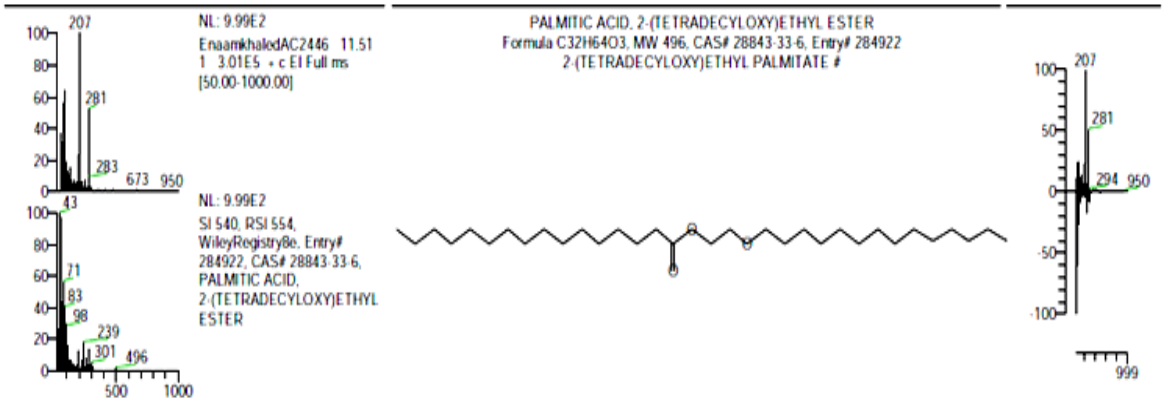


Figure (5): GC/MS chromatogram and chemical structure of ethanolic extract of actinomycete isolate (AC2) metabolites.

REFERENCES

Cox G, Sieron A, King AM, De Pascale G, Pawlowski AC and Koteva K, (2017). A Common Platform for AntibioticDereplication and Adjuvant Discovery. Cell Chem Biol. 24(1):98–109.

Desbois, A. P., Smith, V. J., (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl. Microbiol. Biotechnol, (85): 1629–1642.

El-Balkhi, M. A., Saad, O. A. O., Hammad, A. M. M. (2006). Protection of phosphate dissolving bacteria against bacteriophage attack. Damascus University J. Basic Science Vol. 22 No. 1: 43-57.

Fathy, shimaa, H. (2004). Protection of certain nitrogen fixing and phosphate dissolving bacteria against bacteriophage attack. M. Sc. Thesis, Dept. Agric. Microbiology, Fac. Agric. Minia Univ. Egypt.

Hammad, A. M. M. (1998). Evaluation of alginate-encapsulated *Azotobacter chroococcm* as a phage-resistant and an effective inoculum. J. Basic Microbiol., 38 (1): 9-16.

Hammad, A. M. M.; Ali, M. Z. H. and Omar, Enaam M. M. F. (2025). Actinophages of an actinomycete isolate of high antagonistic activity against certain pathogenic bacteria. (Isolation and characterization). Minia J. Agric. Res &Dev., 45 (4): 501-514.

Jeeva, S. and Krishnamoorthy, A. S. (2018). Antifungal Potential of Myco-molecules of Coprinopsiscinerea (Schaeff) S. Gray s.lat. against Fusarium spp. Madras Agric. J., 105 (1-3): 56-60.

Lamsa A, Lopez-Garrido J, Quach D, Riley EP, Pogliano J, Pogliano K. (2016). Rapid Inhibition Profiling in Bacillus subtilis to Identify the Mechanism of Action of New Antimicrobials. ACS Chem Biol. 11(8):2222–2231.

Maghdu, N. A. and Palaniyappan K. D. (2014). *In vitro* antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *Lepidagathis cristata* Willd. (Acanthaceae) inflorescence.Asian Pacific Journal of Tropical Biomedicine 4 (2): 661-664.

Mokhtar, F.Y., Nasr, A.E., Elaasser, M.M. &Elsaba, Y.M. (2023). Bioactive secondary metabolites from *Aspergillus fumigatus* ON428521 Isolated from Wadi El Rayan, El Fayum Governorate. *Egyptian Journal of Botany*, *63*(1), 233-250.

Peters CE, Lamsa A, Liu RB, Quach D, Sugie J, Brumage L, (2018). Rapid Inhibition Profiling Identifies aKeystone Target in the Nucleotide Biosynthesis Pathway. ACS Chem Biol; 13(12):3251–8.

Quach D.T, Sakoulas G, Nizet V, Pogliano J, Pogliano K. (2016). Bacterial Cytological Profiling (BCP) as aRapid and Accurate Antimicrobial Susceptibility Testing Method for Staphylococcus aureus. EBioMedicine.2016; 4:95–103.

Raja, A., & Prabakaran, P. (2011). Preliminary screening of antimycobacterial effect of psychrophillic Actinomycetes isolated from Manali ice point: Himachal predesh. Journal of Microbiology and Antimicrobials, 3(2), 41-46.

Saravanan K.; Pushpesh K. M. and Girendra K. G. (2016). isolation and characterization of palmitic acid, 2- (tetradecyloxy) ethyl ester and beta sitosterol from methanolic extract of bauhinia purpurea leaves. European Journal of Biomedical and Pharmaceutical Sciences, 3, (12): 264-268.

Sharma, P. and Sharma, A. (2011). Antibacterial and antifungal activities of fatty acids from plant sources. Pharmaceutical Biology, 49*(*7), 672-676.

Zhu, H., Sandiford, S. K., & van Wezel, G. P. (2014). Triggers and cues that activate antibiotic production by actinomycetes. Journal of Industrial Microbiology and Biotechnology, 41(2), 371-386

الملخص العربى

تأثير الاكتينوفاجات على نمو عزلة اكتينوميسيتات وانتاجها لمواد ذات نشاط بيولوجى

عادل محمود محمد حماد ، محمد زكى حسن على ، إنعام محمد محسن فكرى عمر
قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا

تم دراسة التأثير المثبط للأكتينوفاج على نمو عزلة أكتينوميسيتات وإنتاجها لمواد نشطة بيولوجيا. ، لحماية عزلة الأكتينوميسيتات من الاصابة بالأكتينوفاج، تم استخدام تثبيت خلايا الاكتينوميستات في حبيبات الألجينات. أدى وجود الأكتينوفاجات في المزرعة السائلة لخلايا الأكتينومسيتات الحرة إلى انخفاض ملحوظ في الوزن الطازج والجاف للميسيليوم مقارنةً بمزراعة الخلايا الحرة في غياب الأكتينوفاجات. في حين تم تسجيل أعلى قيم للوزن الطازج والجاف للميسيليوم في المزرعة المثبتة بالألجينات. في حالة الخلايا المثبتة ، لم يكن لوجود الأكتينوفاج أي تأثير واضح على الوزن الطازج والجاف للميسيليوم. تم إنتاج أعلى كمية من المواد النشطة بيولوجيا بواسطة الأكتينوميسيت المثبتة مقارنةً بتلك التي تنتجها الخلايا الحرة. لم يكن لوجود الأكتينوفاجات أي تأثير ملحوظ على كمية المواد النشطة بيولوجيا التي تنتجها الخلايا المثبتة. وباستخدام تحليل الغاز الكروماتوجرافي (GC-MS)، تم الكشف عن خمسة مركبات كيميائية في المواد النشطة بيولوجيا المنتجة بواسطة عزلة الأكتينوميسيتات (AC2).