Section (I)

1- Isolation of dermatophytes isolates:

In the present study, 220 specimens were collected from patients with confirmed dermatophytes from dermatology out patient clinic of Zagazig University Hospital in Sharkia Governorate during one year (4 seasons). There were different types of Tinea diseases, which included Tinea capitis; 49 cases (22.3%), Tinea corporis; 70 cases (31.8%), Tinea barbae; 9 cases (4.0%), Tinea pedis; 56 cases (25.5%), Tinea cruris; 30 cases (13.6%) and Tinea unguium; 6 cases (2.7%).

2- Total count of the isolated dermatophytes:

In this investigation, a total of 220 isolates including (2) genera and (9) species of dermatophytes resulted (Table 1).

The data presented in table (2) showed that *Trichophyton* was the most prevalent genus represented by 7 species, the most common of which was *Trichophyton rubrum* followed by *Trichophyton violaceum* then *Trichophyton mentagrophytes*. *Microsporum* occupied the second rank represented by two species which were *Microsporum canis* then *Microsporum audouinii*.

Table (1): Total count of the isolated dermatophytes.

Dermatophytes species	T.C of species	% related to genus	% related to total isolates
Trichophyton			
T. mentagrophytes (growth)	28	15.5	12.7
T. mentagrophytes (downy type).	38	21.1	9.6
T. rubrum (granular type).	25	13.8	6.3
T. rubrum (downy type).	43	23.8	10.8
T. violaceum	35	19.4	8.8
T. erinacei	6	3.3	2.7
T. Simi	5	2.7	2.3
Trichophyton	**180**	**100**	**81.8**
Microsporum			
M. canis	38	95	17.3
M. audouinii	2	5	0.9
Microsporum	**40**	**100**	**18.1**

T.C = Total count

Table (2) Dermatomycosis and their Aetiological agents:

Dermatomycosis / Isolated species	Tinea Capitis	%	Tinea Corporis	%	Tinea Cruris	%	Tinea Pedis	%	Tinea unguium	%	Total	%
***Trichophyton*:-**												
***T. mentagrophytes* (granules)**	**0**	**0**	**4**	**11.8**	**4**	**22.2**	**18**	**81.8**	**2**	**25**	**28**	**12.7**
***T. mentagrophytes* (downy)**	**20**	**55.6**	**8**	**23.5**	**5**	**27.8**	**5**	**22.7**	**-**	**-**	**38**	**17.3**
***T. rubrum* (granule).**	**4**	**11.1**	**10**	**29.4**	**3**	**16.7**	**5**	**22.7**	**3**	**37.5**	**25**	**11.4**
***T. rubrum* (downy).**	**4**	**11.1**	**15**	**44.1**	**10**	**72.2**	**10**	**45.5**	**1**	**12.5**	**43**	**19.5**
T. violaceum	**0**	**0**	**18**	**52.9**	**8**	**44.4**	**9**	**40.9**	**-**	**-**	**35**	**15.9**
T. erinacei	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**6**	**2.7**
T. Simi ii	**-**	**-**	**5**	**14.7**	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**2.3**
***Microsporum*:-**												
M. canis	**20**	**55-6**	**10**	**27.8**	**0**	**-**	**8**	**22.2**	**0**	**-**	**38**	**17.2**
M. audouinii	**1**	**2.8**	**-**	**-**	**-**	**-**	**1**	**4.5**	**-**	**-**	**2**	**0.99**
Total	**49** **22.3**	**-**	**70** **31.8**	**-**	**30** **13.6**	**-**	**56** **25.5**	**-**	**6** **2.7**	**-**	**220** **100**	**100**

3- Aetiological agents of different studied Dermatomycosis:

The main causes of tinea capitis, tinea corporis, tinea cruris, tinea pedis and tinea unguium were *Microsporum canis*, *Trichophyton rubrum* (granule), *Trichophyton rubrum* (downy), *Trichophyton violaceum* (Table 2 & Figures 1-12).

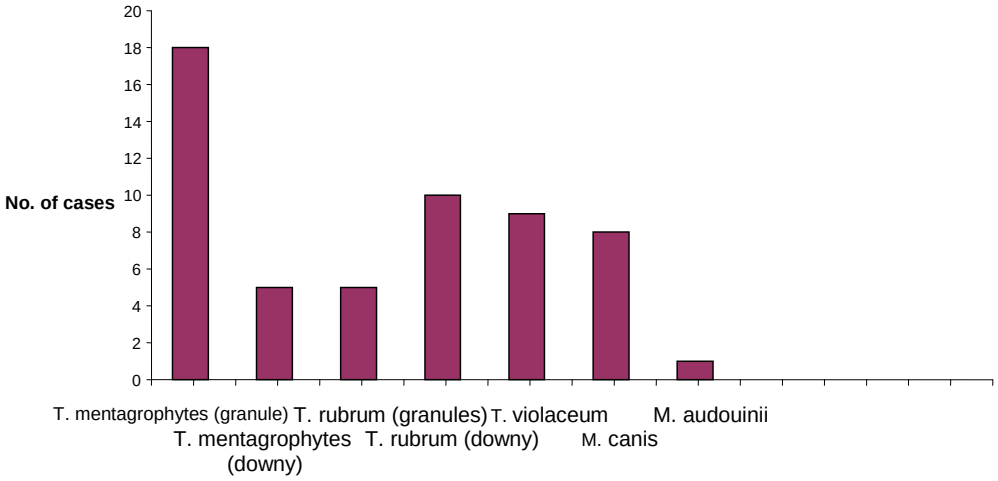


Fig (1): Causal agents of Tinea pedis.

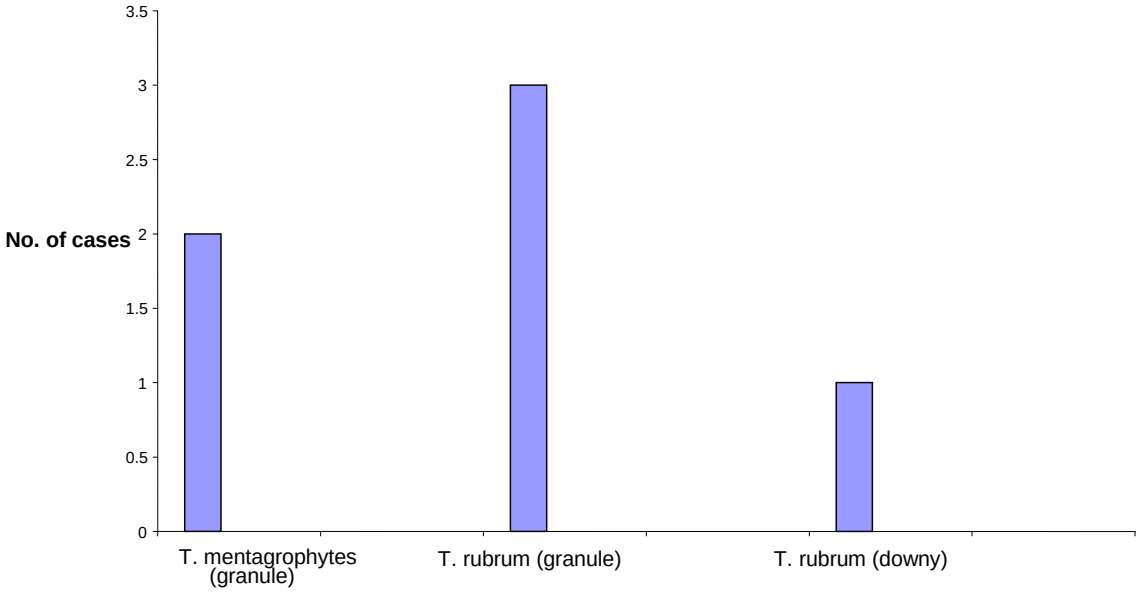


Fig (2): Causal agents of Tinea unguium.

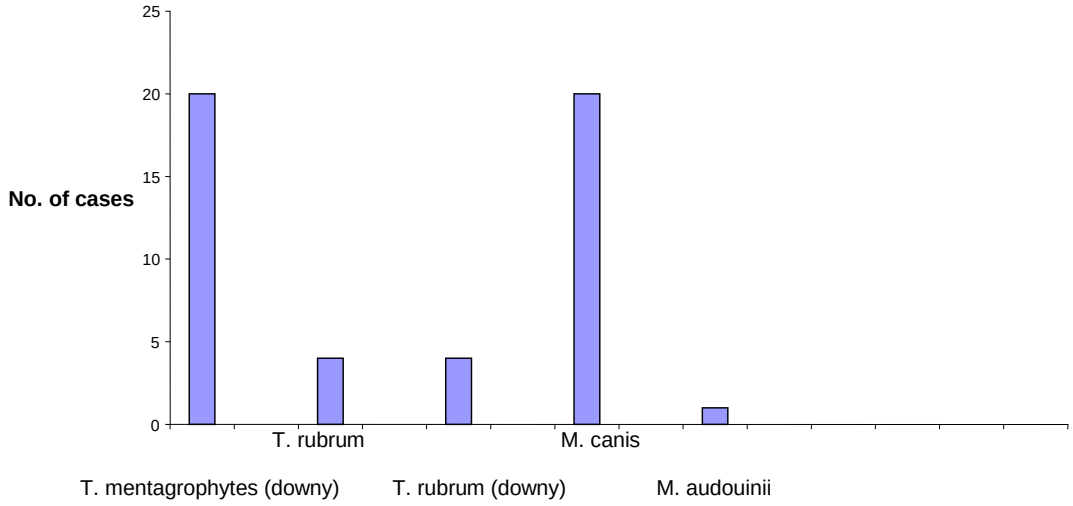


Fig (3): Causal agents of Tinea capitis.

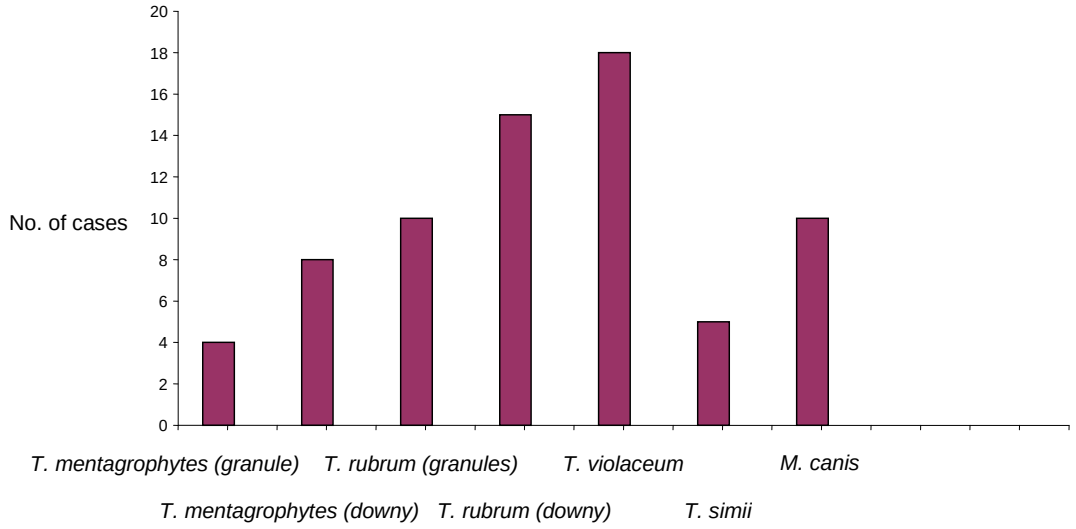


Fig (4): Causal agents of Tinea corporis.

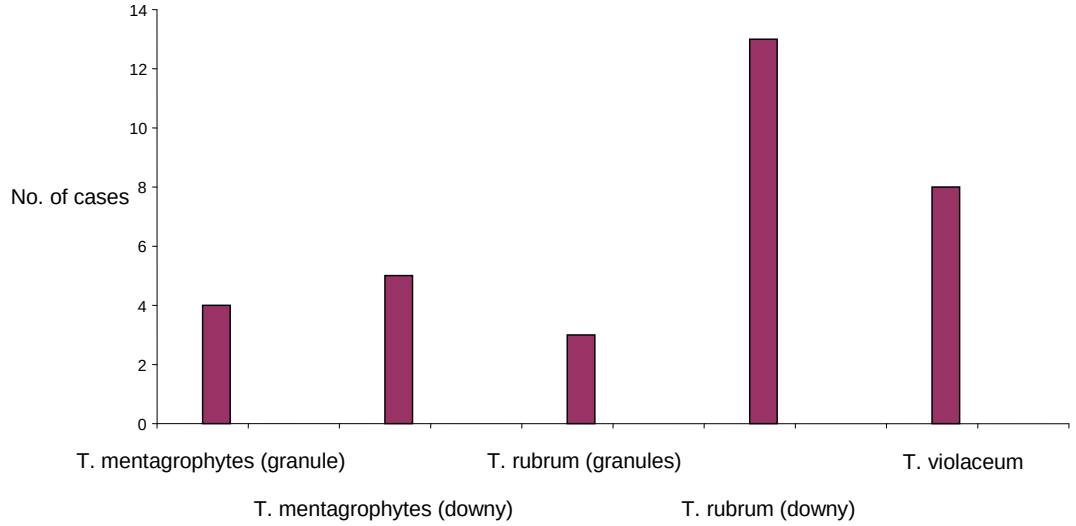


Fig (5): Causal agents of Tinea cruris.

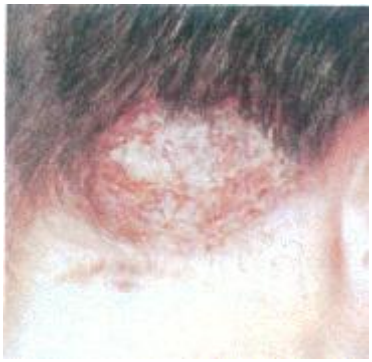


Fig (6): Tinea capitis (Scaly type).

Fig (7): Tinea capitis (black – dot type).

Fig (8): Consecutive scars in scalp ringworm Produced by *Trichophyton violaceum.*

Fig (9): Tinea corporis caused by *T. rubrum.*

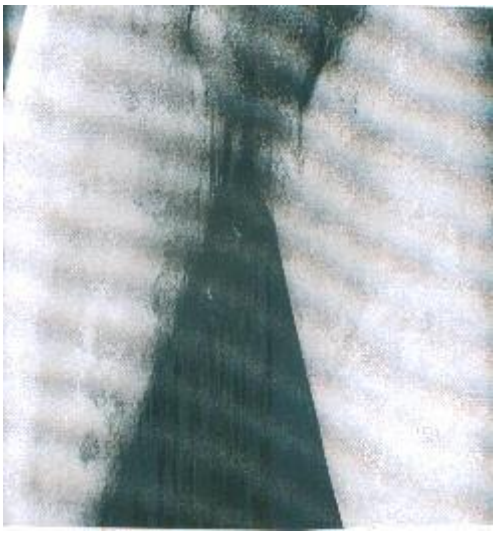


Fig (10): Tinea cruris.

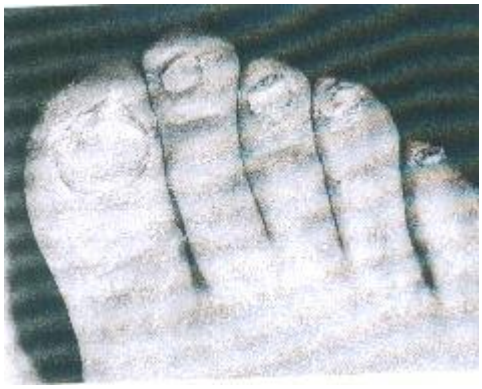


Fig (11): Onychomycosis caused by *T. rubrum*.

Fig (12): Tinea pedis.

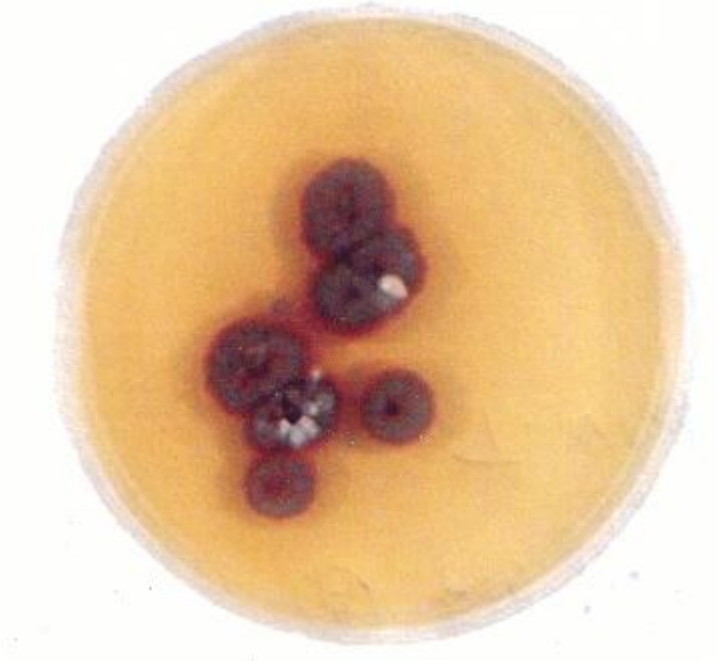
4- Identification of dermatophytes isolates:

Fig. 13 (A, B) indicated that the isolate produced a purplish-red thallus with white sectors and a glabrous-wrinkled texture. The reverse color was lavender to purple. The microscopic examination indicates that the isolate produced pyriform microconidia and rare or irregularly shaped macroconidia and chains of asymmetrical chlamydospores. From the above results this isolate was named ***Trichophyton violaceum.***

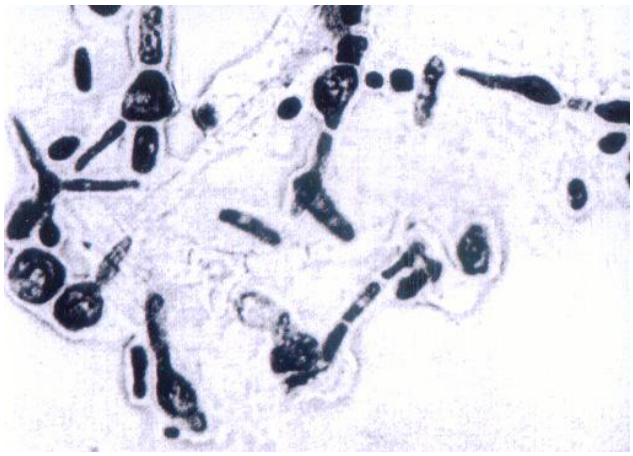
Fig. 14 (A, B, C) indicated that the isolate produced white to pale pink thallus with blood red to reddish-brown reverse color and the microscopic examination indicated that few pyriform, lateral microconidia and pencil-shaped macroconidia, the arthroconidia were produced from hyphae and macroconidia . From these above results the isolate was named ***Trichophyton rubrum.***

Fig. 15 (A, B) indicated that the organism produced on Sabouraud`s dextrose agar, with flat colonies, white to cream-coloured with a dense cottony surface. Colonies usually have a bright golden-yellow to brownish-yellow reverse pigment. Macroconidia were typically

spindle-shaped. A few pyriform to clavate microconidia were also present. Macroconidia and/or microconidia did not produce on primary isolation media and it was recommended that sub-cultures be made onto boiled polished rice grains to stimulate sporulation. From all these results the isolate was named ***Microsporum canis.***



(A)



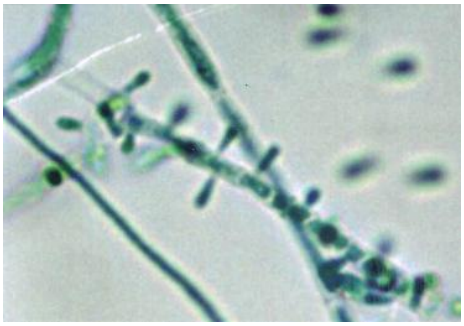
(B)

Fig (13): Culture of *Trichophyton violaceum.*

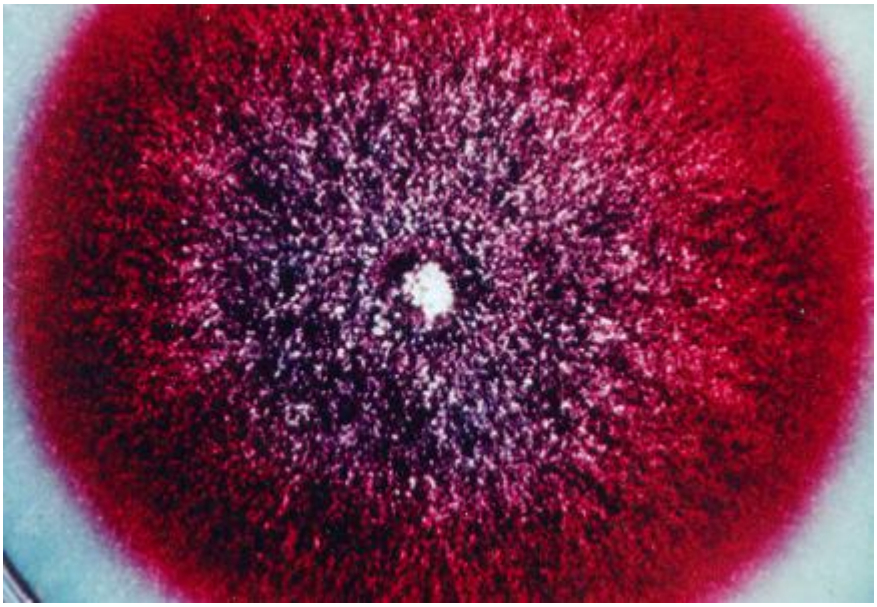
A- Thallus color, purple red with white sector.

B- Chain of asymmetrical chlamydospores.

(A)



(B)



(C)

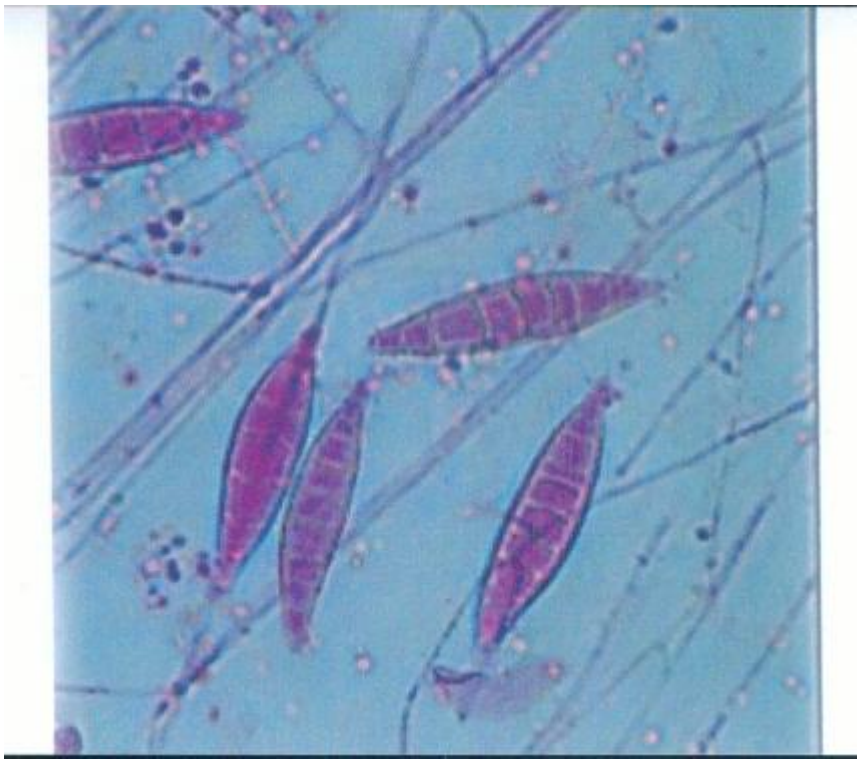
Fig (14): Culture of *Trichophyton Rubrum.*

A- Thallus color, white to pale pink.

B- Arthroconidia produced from hyphae and macroconidia,

C- Blood red to reddish brown reverse color.

(A)



(B)

Fig (15): Culture of *Microsporum Canis.*

A-The colonies were flat with dense cottony surface.

B- Macroconidia were typically spindle-shaped.

Section (II)

1- Isolation and differentiation of actinomycetes isolates:

Ten soil samples were collected as available from cultivated areas of different regions in Egypt, 4 soil samples were collected from El-Qalubia governorate, 3 soil samples from El-Sharkia governorate, 1 soil sample from Kfr EL-Sheik governorate, and 2 soil samples from El-Dakhalia governorate.

The collected soil samples were dried and used for further investigations.

1-1 Soil dilution:

Ten gm of air-dried soil were transferred to 250 ml conical flasks having 100 ml sterile water, flasks were gently shaken for 20 minutes and then a serial dilution was prepared.

One drop of each soil dilution was placed over the surface of inorganic salts starch agar, then carefully spreaded over the whole agar surface using a sterile glass spreader. Seeded plates were kept at 28°c for 10 days. Developing actinomycete colonies were transferred to slants of inorganic salts starch agar.

One hundred ninety two were isolated of actinomycetes culture by the described previous method. The collected isolates were screened for antifungal activity. The collected isolates of actinomycetes with white and grey aerial mycelium were recorded and selected from 10 soil samples collected from different governorates of Egypt (Table 1).

Out of these 192 isolates, 80 isolates produced white aerial mycelium with brown pigmentation and 92 isolates produced grey aerial

mycelium with pale yellow pigment and 20 isolates produced grey aerial mycelium with pink pigments.

The collected isolates were differentiated into 3 groups according to the type of spore chain morphology as follows:

Group one:-

Eighty isolates that produced long, straight chain of spores with brown pigments.

Group two:-

Ninety two isolates that produced spiral chain of spores consisting of 2-3 turns. The spiral was loose.

Group three:-

Twenty isolates that produced primitive spiral chain of spores with pink pigments.

Table (3): Total count of actinomycetes in some soil samples collected from some regions of Egypt.

No. of Sample	Governorate	Location of collected soil	Soil Type	Plant Cover	Total Count of actinomycetes
1	Kalyobia	Benha	Clayoam	Zea	18
2	Kalyobia	Kafr-Shoker	Clayoam	Vegetables	10
3	Kalyobia	Gamgara	Clayoam	Weeds	8
4	Kalyobia	Qaliob	Clayoam	Vegetables	20
5	El – Sharkia	Zagazig	Clayoam	Wheat	10
6	El – Sharkia	San El-hager	Sandy soil	Triticum Vulgare	30
7	El – Sharkia	Bilbiaus	Clayoam	Wheat	35
8	Kafr El-sheikh	Baltiim	Clayoam	Wheat	18
9	El-Dakahlia	Mit Gahemer	Clayoam	Zea	12
10	El-Dakahlia	Kom El-noor	Clayoam	Gossypium barbadense	31

Details of biological characterization will be restricted to the isolates of group one, two, and three that were proved to be antagonistic to dermatophytes.

1-2 Chemical composition of actinomycetes cell wall:-

The chemical analysis of the whole cell wall hydrolysates of isolate from group two and group three revealed that they contain LL-diaminopimilic acid, with no sugar characteristic but chemical analysis of group one showed that it contains meso-diaminopimilic acid with arabinose and galactose as characteristic sugars.

Accordingly, the isolate of group one was belonging to cell wall chemo-type IV, however, the isolates of group two and three were considered belonging to cell wall chemo-type I.

2- Biological characterization and identification of group one:-

Group one: (Long straight)

This group included 20 isolates, and was represented by isolate No. B – 8.

2-1 Morphological characteristics:-

a- Spore chain morphology:

The isolates of this group produced on solid media, developed colonies, which consisted of extensively branched, unfragmenting substrate mycelium and very long, straight, rarely branching aerial hyphae. The aerial hyphae fragment into spores (Fig. 16).

b- Spore morphology and ornamentation:

Electron microscope examination revealed that the isolate produced long cylindrical to oblong spores of unequal length. Spore surface were smooth. Spore chains may contain more than 50 spores in one chain (Fig. 17).

2-2 Cultural characteristics:

Cultural characteristics of the isolates of group I on different agar media indicated the close similarity between them. Isolate no. B-8 produced a white to pale yellow aerial mycelium on starch nitrate, glycerol asparagines and soybean meal. It produced a pale brown substrate mycelium on starch nitrate and glycerol asparagines medium, pale brownish yellow substrate mycelium on fish extract medium and grayish brown substrate mycelium on soybean meal (Table 4).

The isolate produced brown pigments on starch nitrate and glycerol asparagines medium .The diffusible white pigments produced were not sensitive to pH. The isolate did not produce pigments when cultivated on either peptone – yeast iron agar or tyrosine agar.

The optimum growth temperature of the isolate was 28ºC, heavy growth at 37ºC, weak growth at 10ºC, and no growth at 4ºC, 45ºC or 52ºC., The isolate produced heavy growth at 10% NaCl, and was not inhibited by 0.01% sodium azide, 0.1% phenol.

2-3 Growth at different carbon and nitrogen sources:

Isolate No. B-8 could assimilate, L-arabinose, sucrose, meso-imsitol, mannitol, maltose, D-Fructose, L-rhamnose, D-mannose, D-lactose, salicin, trehalose, dextran. It could not assimilate D- xylose.

The isolate was able to utilize Dl- amino – n- butyric acid, L-cysteine, L-valine, L-threonine, L-serine, L-phenylalanine, L-methionine, L-arginine, and hydroxyproline.

2-4 Antimicrobial potentialities to different antibiotics:

The isolate No. did not produce any antibiotics. The isolate was not sensitive to rifampicin, cephaloridine, oleandomycin and penicillin G. It was sensitive to gentamycin, neomycin, streptomycin and dimethyle chlorotetracycline.

2-5 Production of enzymes:

The tested isolate showed the following positive enzymatic activities**:** proteolytic, lipolytic, pectinolytic, and celluolytic. They reduced nitrates to nitrites, hydrolyzed sodium hippurate, showed positive catalase reaction, succeeded to coagulate and peptonized milk, but failed to produce hydrogen sulphide. It degraded L-tyrosine, tween 80, starch, casein, gelatin, cellulose, asculine, urea and testosterone, but it could not degrade adenine, arbutin, DNA and RNA.

2-6 Chemical analysis of cell wall:

a- Type of diaminopimilic acid:

The cell wall of the isolate No. B-8 was found to contain meso-diaminopimilic acid.

b- Type of characteristic sugars of cell wall:

The chemical analysis of the cell wall of the isolate showed that they contained galactose and arabinose as characteristic sugar components of cell wall.

2-7 Identification of the isolate No. B-8:

Consulting the references of the taxonomy of actinomycetes and on the basis of the obtained biological characteristics, isolate No. B-8 was identified as ***Nocardiopsis halotolerans*** (Al-zarban *et al*., 2002).

Table (4): Cultural characteristics of 14-days old culture of isolate No. B-8 on different agar media.

Color / Media			
	Aerial mycelium	**Substrate mycelium**	**Medium**
Starch nitrate	Pale yellow	Pale brown	Brown
Soybean meal	Pale yellow	Grayish brown	Non pigmented
Glycerol asparagine	White	Pale brown	Brown
Fish meal extract	Pale yellow	Pale brownish	Non pigmented

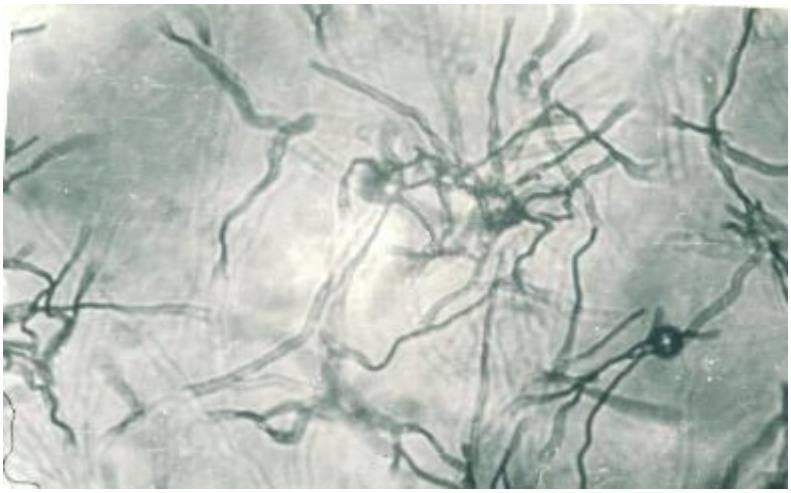


Fig (16): Microphotograph of spore chain of isolate No. B-8 (400 X).

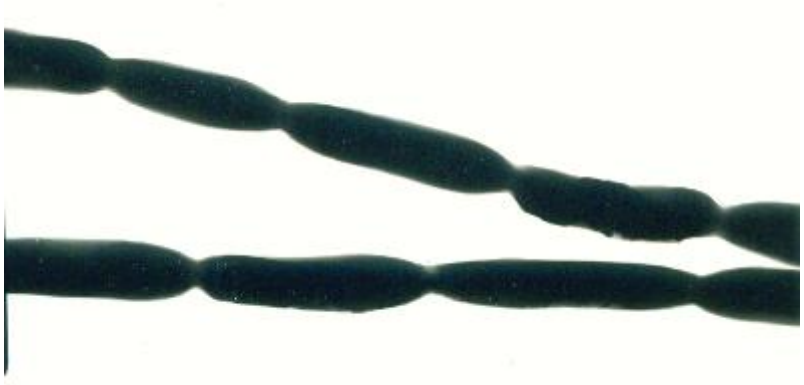


Fig (17): Electron micrograph of isolated spores No. B-8 (25,000 X)

3- Biological characterization and identification of group two:-

Group two included 92 isolates, and was represented by isolate No. A-10

3-1 Morphological characteristics:-

a- Spore chain morphology:

The direct microscopic examination of culture streaks of the isolate revealed that it produced spiral chain of spores consisting of 2 – 7 turns. The spirals were loose (Fig. 18).

b- Spore morphology and ornamentation:

Electron microscope examination of spores revealed that the isolate produced long, cylindrical spores with smooth surface (Fig.19).

3-2 Cultural characteristics:

Cultural characteristics of the isolates on different agar media indicated the close similarity between them. Isolate No. A-10 produced medium grey aerial mycelium on starch nitrate agar and soybean agar, pale medium grey aerial mycelium on glycerol asparagine agar, and a white aerial mycelium on fish meal extract agar. The isolate produced non-pigmented substrate mycelium on starch nitrate agar and glycerol asparagine agar, but it produced cream pigment on soybean meal agar and fish meal extract agar. The isolate produced non-pigmented substrate mycelium on starch nitrate agar and glycerol asparagine agar, but it produced cream pigment on soybean meal agar and fish meal extract agar. Diffusible pale yellow pigments were produced on soybean meal agar. The yellow substrate and diffusible pigments produced were not

sensitive to pH. The isolate did not produce melanin pigments, when cultivated on either peptone yeast iron agar or tyrosine agar (Table 5).

The optimum growth temperature of the isolate was 28ºC, moderate growth at 37ºC, weak growth at 10ºC and no growth at 4ºC, 45ºC or 52ºC. The isolate was sensitive to sodium chloride concentration higher than 5%. It was not inhibited 0.01 or 0.02% sodium azide, 0.1% phenol.

3-3 Growth on different carbon and nitrogen sources:

Isolate No. A-10 could assimilate L-arabinose, sucrose, D-xylose, meso-inositol, mannitol, maltose, D-practose, L-rhamnose, D-mannose, D-lactose, inulin, trehalose, D-galactose, cellobiose, sodium acetate, sodium citrate and sodium pyruvate.

It could utilize, DL-amino- n-butyric acid, L-cystine, L-valine, L-threonine, L-Serine and L-merhionine.

3-4 Antimicrobial potentialities and their sensitivity to different antibiotics:

Twenty isolates of group two were assayed for the production of antimicrobial substances. They failed to produce any antibiotics. The isolate was not sensitive to rifampicin, cephaloridine and penicillin G, but were sensitive to gentamycin, neomycin, streptomycin and oleandomycin.

3-5 Production of enzymes:

The isolate could produce lipolytic, keratinolytic, cellulolytic, pectinolytic and chitinolytic enzymes. It reduced nitrate, produced also hydrogen sulfide, coagulated and peptonized milk. The isolate degraded

guanine, elastin, L-tyrosine, urea, gelatin, tween 80, and glycogen, but could not degrade testosterone.

3-6 Identification of isolate No. A – 10:

Consulting the references of the taxonomy of actinomycetes and on the basis of the obtained biological characteristics, isolate No.A-10 was identified as *Streptomyces diastaticus* (Pridham *et al*., 1958).

Table (5):- Cultural characteristics of 14-day-old culture of isolate No. A-10 on different agar media.

Color / Media	Aerial mycelium	Substrate mycelium	Medium
Starch nitrate	Pale grey	Non pigmented	Non pigmented
Soybean meal	Pale grey	Cream	Pale yellow
Glycerol asparagine	Medium grey	Non pigmented	Non pigmented
Fish meal extract	White	Cream	Non pigmented

Fig (18): Microphotograph of spore chains of isolate No. A – 10 (400 X).

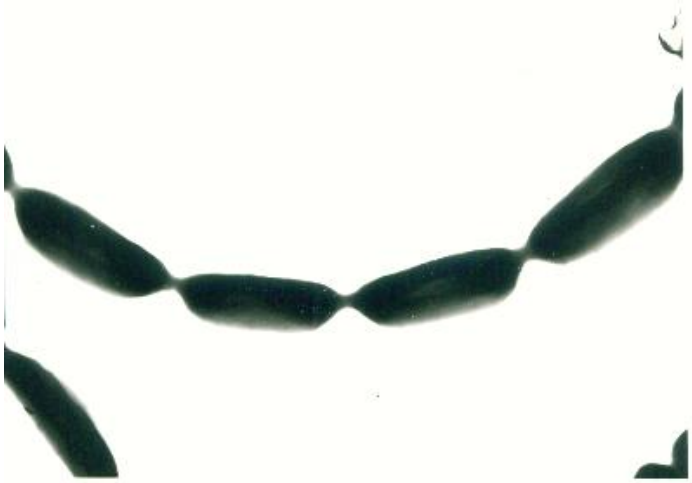


Fig (19): Electron micrograph of spore of isolate No. A – 10 (25,000 X).

4- Biological characterization and identification of group three:-

Group three included 80 isolates, and was represented by isolate No. R – 4.

4-1 Morphological Characteristics:

a- Spore chain morphology:

The direct microscopic examination of culture streaks of the isolate revealed that it produced primitive spiral chains of spores that arise on a long main sporophore. Spore chains were stalked, and sometimes straight, hooked, looped or in the form of primitive spirals of 1-2 turns. Spore chain morphology of the isolate indicated that it belonged to the retinaculiaperti group (Fig. 20).

b- Spore morphology and ornamentation:

Electron microscope examination of spores revealed that the isolate produced short cylindrical spores with more or less rounded ends. Spores were smooth-surfaced (Fig. 21).

4-2 Cultural characteristics:

Cultural characteristics of the isolates on different agar media indicated the close similarity between them. Isolate No. R-4 produced a medium grey, aerial mycelium on starch nitrate agar and soybean meal agar, a pale grey to grey aerial mycelium on glycerol asparagine agar and a pale grey to red grey, aerial mycelium on fish meal extract agar. It produced a reddish-pink substrate mycelium on soybean meal agar and fish meal extract agar. The substrate mycelium was non-pigmented on starch nitrate agar and glycerol asparagine agar. Diffusible pink pigments

were produced on soybean meal agar and fish extract agar. The pink substrate and diffusible pigments produced were sensitive to pH.

When cultivated on peptone – yeast iron agar and tyrosine agar, the isolate did not produce melanin pigments (Table 4).

The optimum growth temperature of the isolate was 28ºC, moderate growth at 37ºC, weak growth at 10ºC and no growth at 4ºC and 45ºC. The isolate was sensitive to sodium chloride concentrations higher than 5%. It was not inhibited by 0.01% sodium azide and 0.1% phenol.

4-3 Growth on different carbon and nitrogen sources:

Isolate No. R-4 assimilated the following: L-arabinose, sucrose, D-xylose, meso-inositol, mannitol, maltose, L-rhamnose, D-lactose, inulin, dextran, D-galactose, cellobiose, sodium acetate, sodium citrate and sodium pyruvate.

It utilized DL-amino-n-butyric acid, L-cysteine, L-valine, L-serine, L-phenylalanine and L-methionine.

4-4 Antimicrobial potentialities and their sensitivity to different antibiotics:

Ten isolates of group three were assayed for the production of antimicrobial substances. These isolates produced antibiotics against gram positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*. Fish meal extract agar was the most suitable medium for antibiotic production. The isolates were not sensitive to rifampicin, cephaloridin, dimethyle chlortetracycline and penicillin G, but were sensitive to gentamycin, neomycin and oleandomycin.

4-5 Production of enzymes:

The isolate produced lipolytic, keratinolytic, celluolytic and pectinolytic enzymes. It also produced nitrate, hydrogen sulphide, coagulated and peptonized milk. The isolate degrade hypoxanthine, guanine, L-tyrosine, tween 80, starch, urea, gelatin, and glycogen but could not degrade testosterone.

4-6 Identification of isolate No. R-4:

Consulting the references of the taxonomy of actinomycetes and on the basis of the obtained biological characteristics, isolate No.R-4 was identified as *Streptomyces cineropurpucrus* (Fahmi, 1986).

Table (6): Cultural characteristics of 14-day-old culture of isolate No. R – 4 on different agar media.

Colour / Medium			
	Aerial mycelium	**Substrate mycelium**	**Medium**
Starch nitrate	Pale grey	Non pigmented	Non pigmented
Soybean meal	Medium grey	Reddish pink	Pink
Glycerol asparagine	Pale grey	Non pigmented	Non pigmented
Fish meal extract	Red grey	Reddish pink	Pink

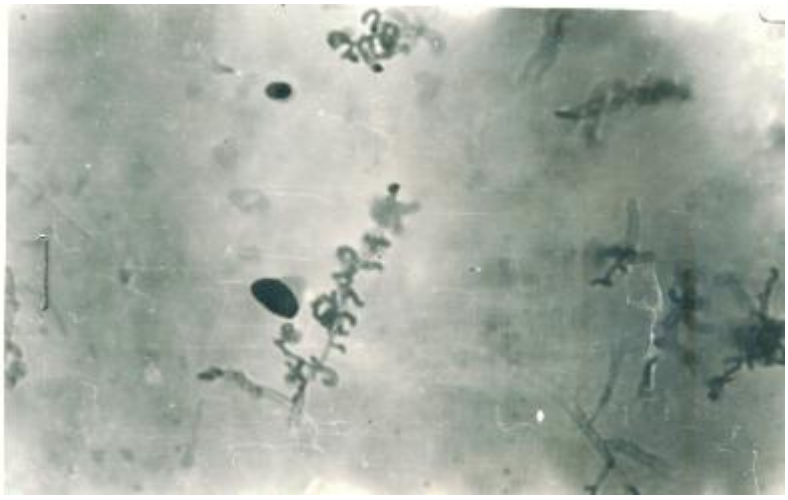


Fig (20): Microphotograph of spore chains of isolate No. R – 4 (x 400).

Fig (21): Electron micrograph of spore chains of isolate No. R– 4 (25,000 X).

Section (III)

Biocontrol of dermatophytes

1- The ability of actinomycetes isolates for controlling the dermatophytes in vitro:-

Three actinomycetes species namely ***Nocardiopsis halotolerans, Streptomyces cineropurpucrus,*** and ***Streptomyces diastaticus*** were proved to exhibit potential antagonism to dermatophytes namely: ***Trichophyton violaceum, Trichophyton rubrum*** and ***Microsporum canis***. Three methods were applied as follows:-

(a)Streak method (Johnson *et al*., 1960):

Obtained results had shown that the three actinomycetes species effectively inhibited the growth of *T. violaceum, T. rubrum* and *M. canis* as shown in Tables (7 - 12) and Fig. (22 - 27).

(b)Reversed layer method (Waksman, 1919 & Hisegawa *et al*, 1990):

In general, the three actinomycete species had shown well expressed antifungal activity against the tested species of dermatophytes. The effectiveness of the isolates were greatly affected by the type of media as shown in Tables (7 - 12) and Fig. (22 - 27), which indicated that the antifungal potentiality of *N. halotolerans* against *T. violaceum, T. rubrum* and *M. canis* was more effective on starch nitrate agar media which produced inhibition zones of 22, 35 and 22 mm in diameter respectively.

While the antifungal activity of *S. cineropurpucrus* against *T. violaceum, T. rubrum* and *M. canis* showed a greater effect on glycerol asparagine agar medium and produced inhibition zones of 25, 42 and 45 mm respectively.

The antifungal substances produced by *S. diastaticus* were more effective against *T. violaceum,* T. *rubrum* and *M. canis* on Yeast malt extract agar medium, they produced inhibition zones of 25, 35 and 35 mm respectively.

(c) Bore diffusion method (Waksman, 1959):

Culture filtrates of the three tested actinomycetes species grown on starch nitrate, soy bean meal, yeast extract and glycerol asparagine liquid media and were obtained similarly when grown on the synthetic starch nitrate medium with different carbon and nitrogen source, different incubation temperatures and different pH values. Culture filtrates of *N. halotolerans*, *S. cineropurpucrus* and *S. diastaticus* grown on soy bean meal media gave the most effective antagonism against the tested dermatophyte. *N. halotolerans* produced inhibition zones against *T. violaceum* and *M. canis* at 45 and 42 mm, where it produced an inhibition zone at 51 mm against *T. rubrum.*

S. cineropurpucrus produced inhibition zones against the tested dermatophyte at 50, 47 and 48 mm respectively, while *S. diastaticus* produced inhibition zones at 45, 40 and 50 mm against the dermatophyte (Fig. 23, 25, 27 & Tables 8, 10, 12).

Table (7): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Trichophyton violaceum* using different agar media.

Media types	Inhibition zone in mm		
	N.halotolerans	*S. cineropurpucrus*	*S.diastaticus*
Starch nitrate	22	24	22
Soy bean meal	12	13	13
Yeast malt extract	17	13	25
Glycerol asparagine	15	25	24

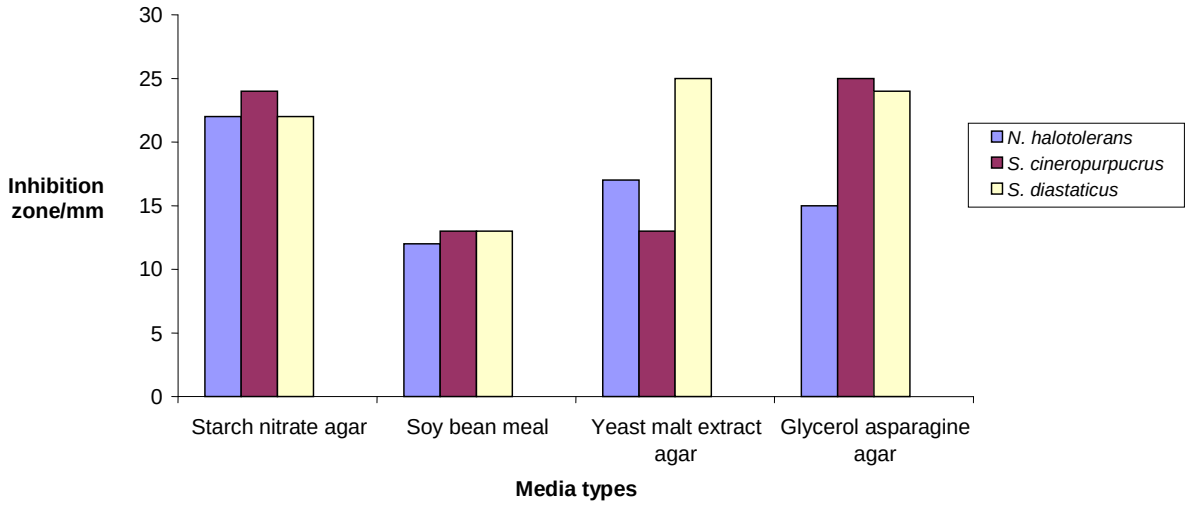


Fig (22): Inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Trichophyton violaceum* using different agar media.

Table (8): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Trichophyton violaceum* using different liquid media.

Media types	Inhibition zone in mm		
	N.halotolerans	*S. cineropurpucrus*	*S.diastaticus*
Starch nitrate	30	32	35
Soy bean meal	45	50	45
Yeast malt extract	30	22	35
Glycerol asparagine	32	27	33

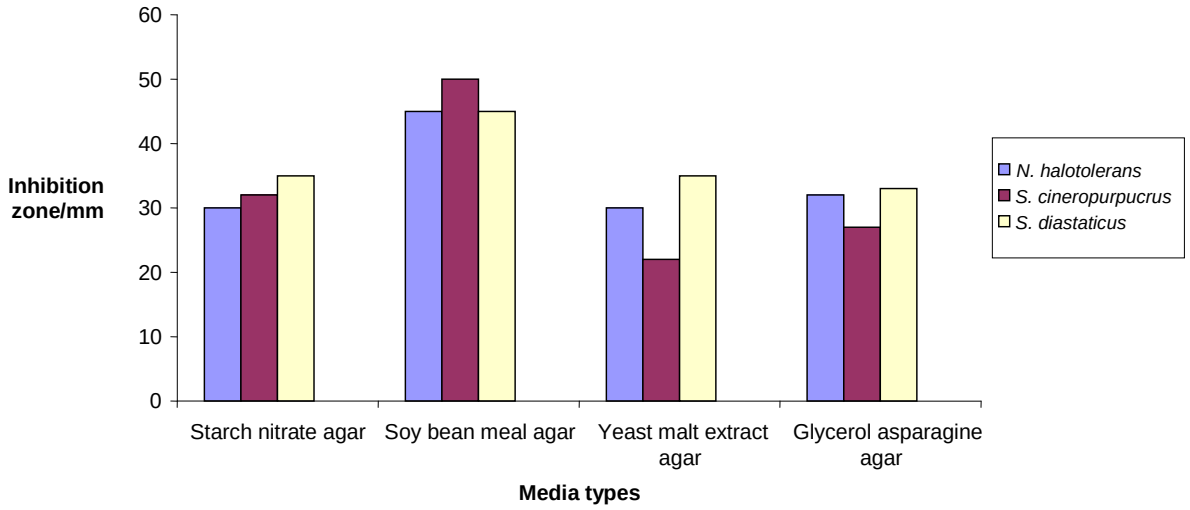


Fig (23): Inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Trichophyton violaceum* using different liquid media.

Table (9): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Trichophyton rubrum* using different agar media.

Media types	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S. diastaticus*
Starch nitrate	35	25	32
Soy bean meal	14	34	22
Yeast malt extract	14	28	35
Glycerol asparagine	14	42	22

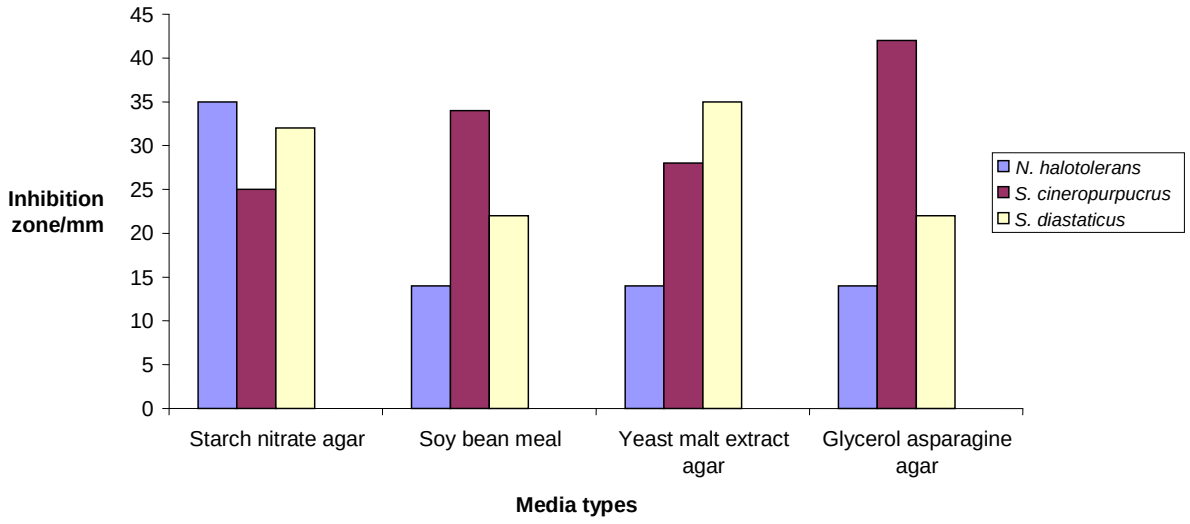


Fig (24): Inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Trichophyton rubrum* using different agar media.

Table (10): The inhibition effect of *Nocardiopsis halotolerans, Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Trichophyton rubrum* using different liquid media.

Media types	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S. diastaticus*
Starch nitrate	40	38	30
Soy bean meal	51	47	40
Yeast malt extract	22	25	20
Glycerol asparagine	33	41	38

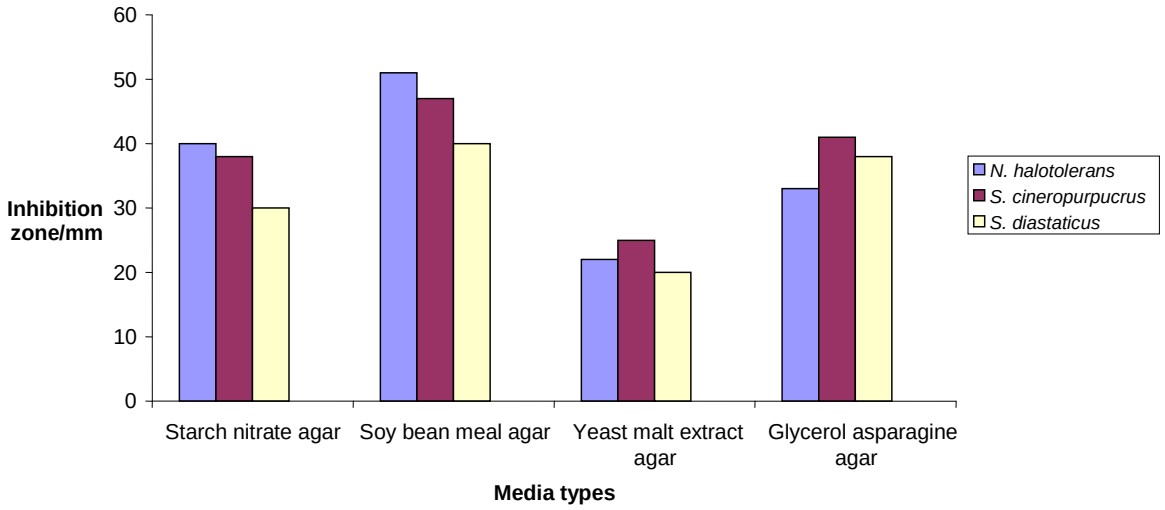


Fig (25): Inhibition effect of *Nocardiopsis halotolerans, Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Trichophyton rubrum* using different liquid media.

Table (11): The inhibition effect of *Nocardiopsis halotolerans, Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Microsporum canis* using different agar media.

Media types	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S. diastaticus*
Starch nitrate	22	42	34
Soy bean meal	21	30	30
Yeast malt extract	18	33	35
Glycerol asparagine	18	45	28

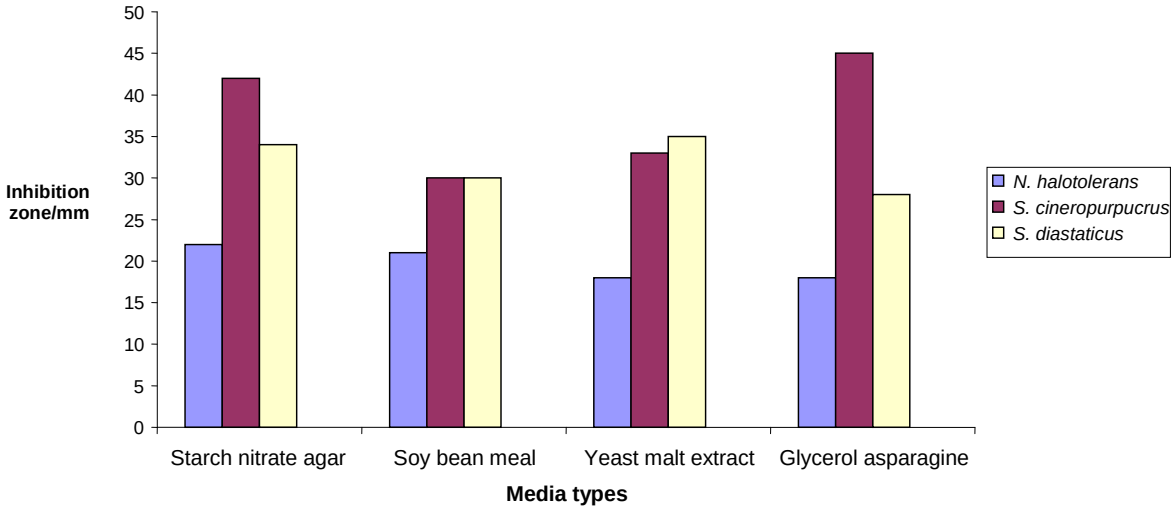


Fig (26): Inhibition effect of *Nocardiopsis halotolerans, Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Microsporum canis* using different agar media.

Table (12): The inhibition effect of *Nocardiopsis halotolerans, Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Microsporum canis* using different liquid media.

Media types	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S.diastaticus*
Starch nitrate	38	40	33
Soy bean meal	42	48	50
Yeast malt extract	18	22	30
Glycerol asparagine	33	35	28

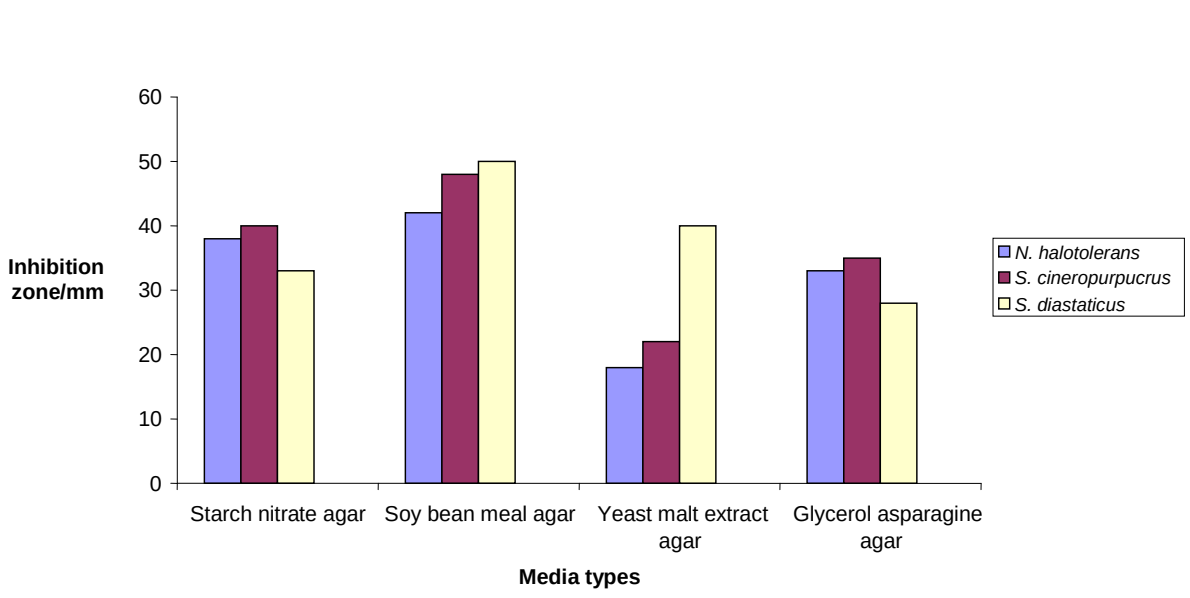


Fig (27): Inhibition effect of *Nocardiopsis halotolerans, Streptomyces cineropurpucrus* and *Streptomyces diastaticus* on *Microsporum canis* using different liquid media.

The antifungal potentiality of *N. halotolerans* and *S. cineropurpucrus* against *T. violaceum* were much more effective on starch nitrate agar; however *S. diastaticus* gave its maximum inhibitory effects on glycerol asparagine agar.

The antifungal agent effect of *N. halotolerans* against *T. rubrum* was greater on starch nitrate agar, however, *S. cineropurpucrus* showed more effect on glycerol asparagine agar, and *S. diastaticus* gave a maximum inhibitory response on Yeast malt extract.

The inhibitory effect of antifungal products of the three tested actinomycetes species against *M. canis* was greater on starch nitrate agar.

2- Effect of different carbon sources on the production of antifungal products:

The results given in tables (13-15) and represented in Fig (28-29) indicate that the antifungal potentiality of *N. halotolerans* and *S. cineropurpucrus* against *T. violaceum* was much more effective when cultivated on starch as the sole carbon source while *S. diastaticus* gave maximum inhibition on glycerol asparagine agar.

The inhibitory effect of the antifungal agents produced by *N. halotolerans* and *S. cineropurpucrus* was greater when glycerol was used as the carbon source.

The effect of antifungal potentiality of the three tested actinomycetes species against *M. canis* was much more effective on starch as a sole carbon source (Table 15).

Table (13): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different carbon sources against *Trichophyton violaceum*.

Carbon sources	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S. diastaticus*
Starch.	38	33	37
Glucose.	10	12	0
Sucrose.	13	20	22
Glycerol.	22	30	40

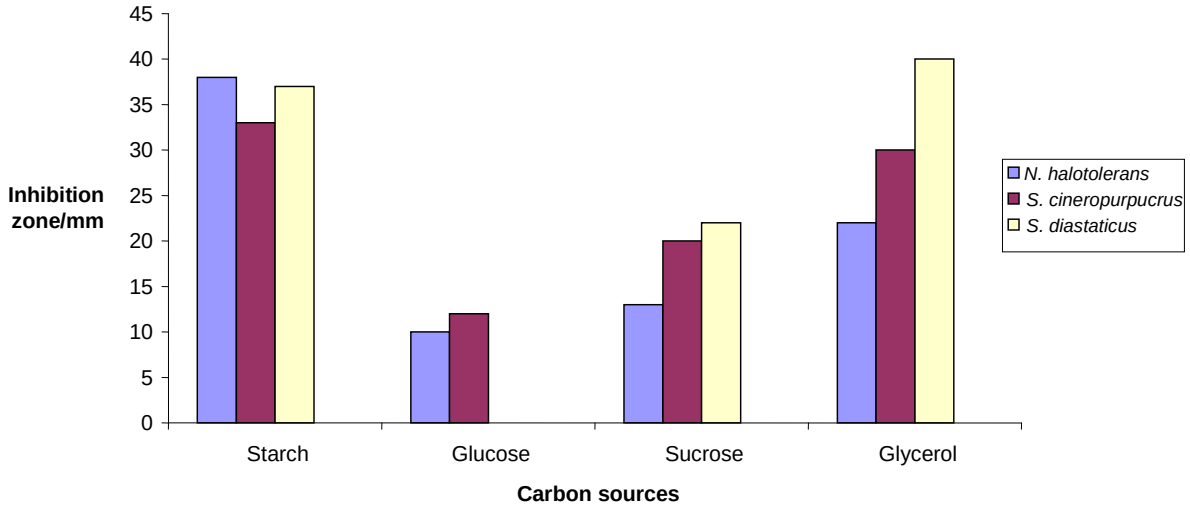


Fig (28): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch media with different carbon sources against *Trichophyton violaceum*.

Table (14): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different carbon sources against *Trichophyton rubrum*.

Carbon sources	Inhibition zone in mm		
	N.halotolerans	*S. cineropurpucrus*	*S. diastaticus*
Starch.	38	23	25
Glucose.	10	20	20
Sucrose.	11	15	18
Glycerol.	36	32	12

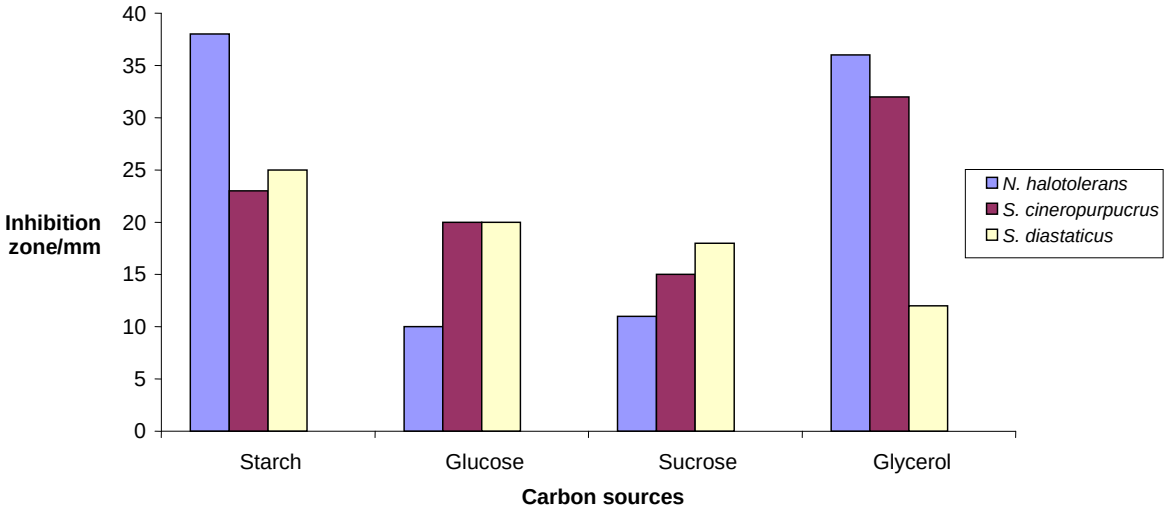


Fig (29): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch medium with different carbon sources against *Trichophyton rubrum*.

Table (15): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different carbon sources against *Microsporum canis*.

Carbon sources	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S.diastaticus*
Starch.	35	30	36
Glucose.	10	18	12
Sucrose.	0	12	0
Glycerol.	15	10	13

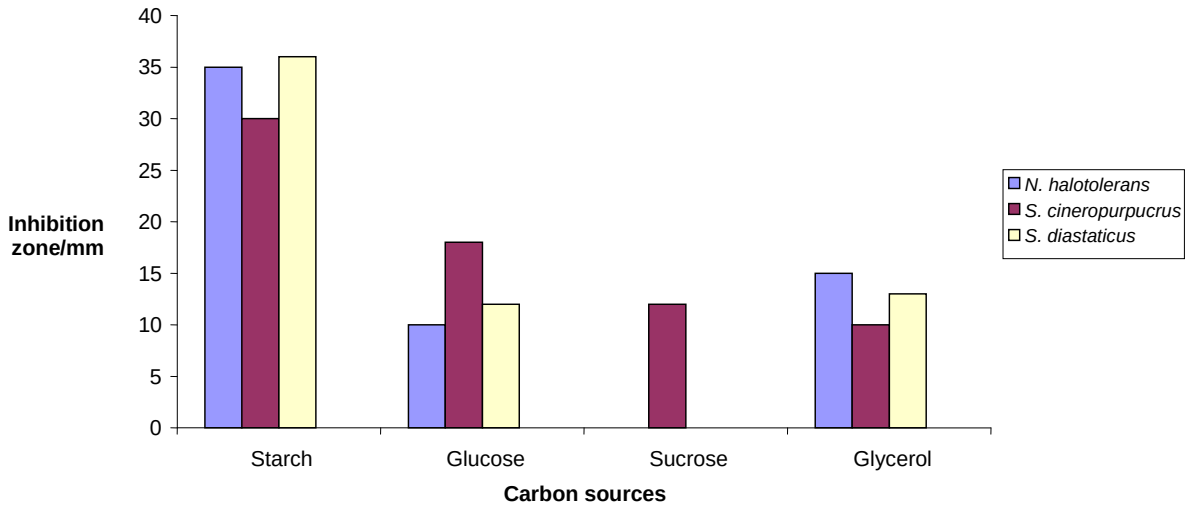


Fig (30): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch media with different carbon sources against *Microsporum canis*.

3- Effect of different nitrogen sources on the production of antifungal substances:

The results represented in Tables (16 - 18) and Fig (31 - 33) showed that the antifungal potentiality produced by *N. halotolerans*, *S. cineropurpucrus* and *S. diastaticus* against the tested species of dermatophytes was much more effective when potassium nitrate was used as the sole nitrogen source.

4- Effect of different incubation temperatures on the antifungal biosynthesis:

The data recorded in Tables (19 - 21) and Fig. (34 - 36) revealed that the best production of antifungal biosynthesis was produced by the tested actinomycetes species against dermatophytes species were achieved at 28ºC and 30ºC.

While, the production of antifungal biosynthesis by the tested actinomycete species took place from 25ºC to 35ºC, but no production at 40ºC or 50ºC.

Table (16): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different nitrogen sources against *Trichophyton violaceum*.

Nitrogen sources	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S. diastaticus*
Potassium nitrate	35	32	38
Ammonium sulfate	18	21	15
Peptone	12	10	12

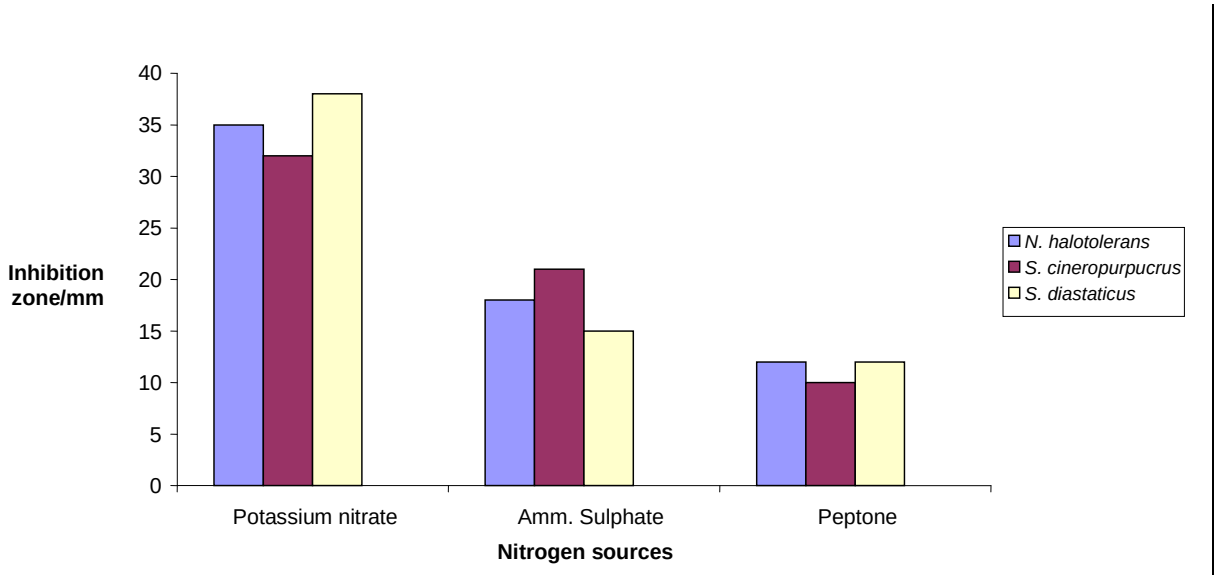


Fig (31): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different nitrogen sources against *Trichophyton violaceum*.

Table (17): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different nitrogen sources against *Trichophyton rubrum*.

Nitrogen sources	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S.diastaticus*
Potassium nitrate	28	35	32
Ammonium sulfate	21	18	22
Peptone	10	15	11

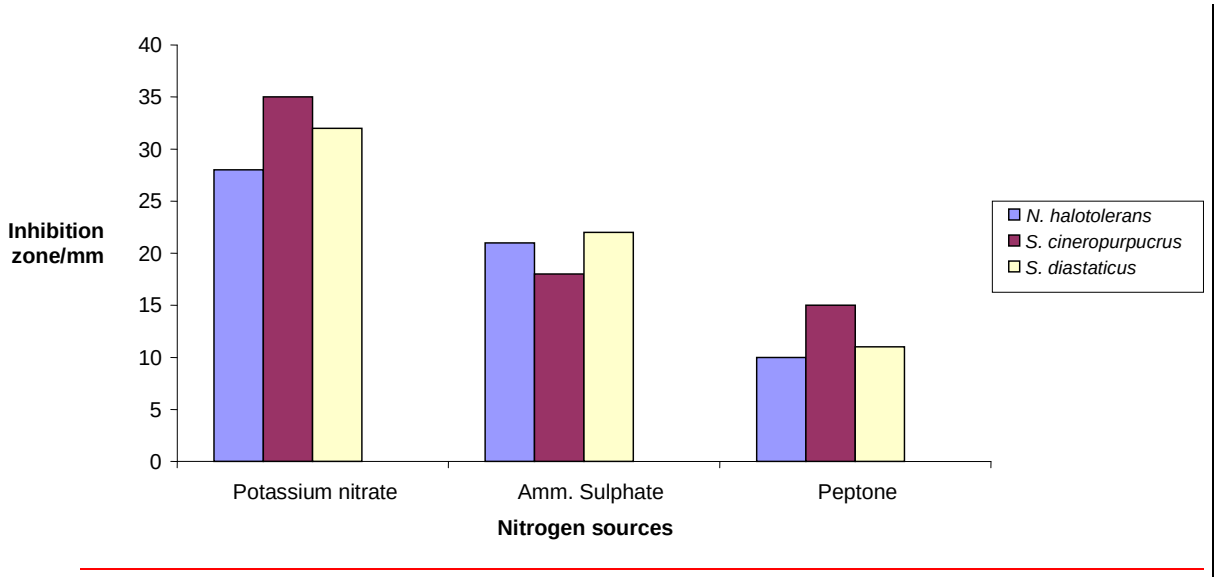


Fig (32): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch media with different nitrogen sources against *Trichophyton rubrum*.

Table (18): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different nitrogen sources against *Microsporum canis*.

Nitrogen sources	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S.diastaticus*
Potassium nitrate	22	33	31
Ammonium sulfate.	18	12	15
Peptone	11	15	30

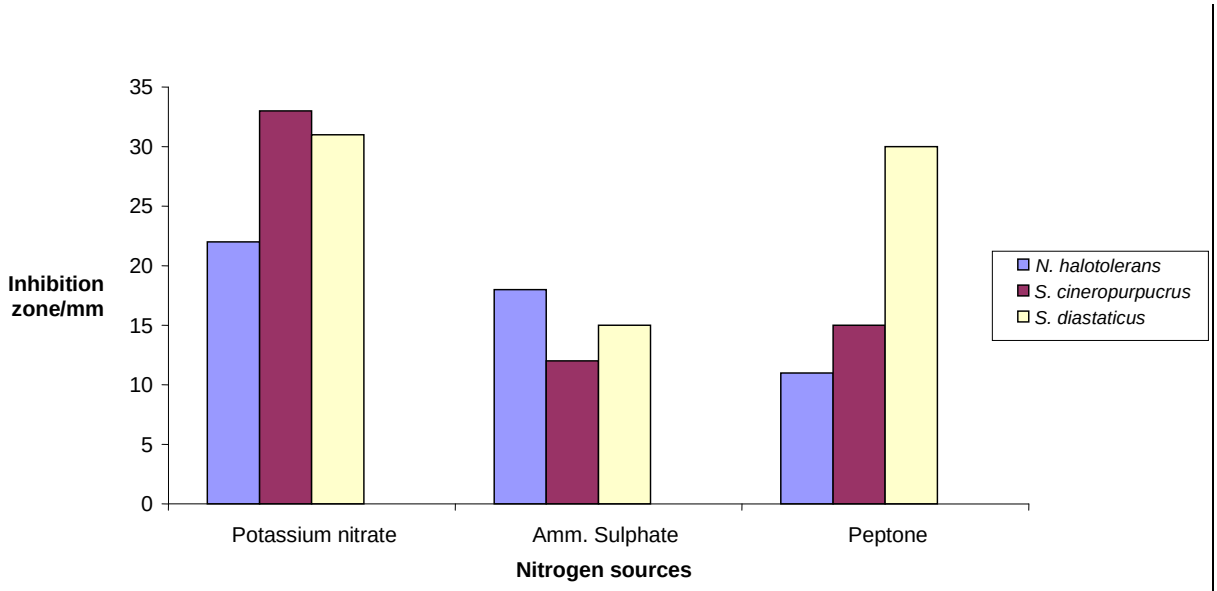


Fig (33): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch media with different nitrogen sources against *Microsporum canis*.

Table (19): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different incubation temperature against *Trichophyton violaceum*.

Temperature (°C)	Inhibition zone in mm		
	N.*halotolerans*	*S. cineropurpucrus*	*S.diastaticus*
25	18	22	20
28	38	42	33
30	33	30	22
35	18	22	24
40	0	0	0
50	0	0	0

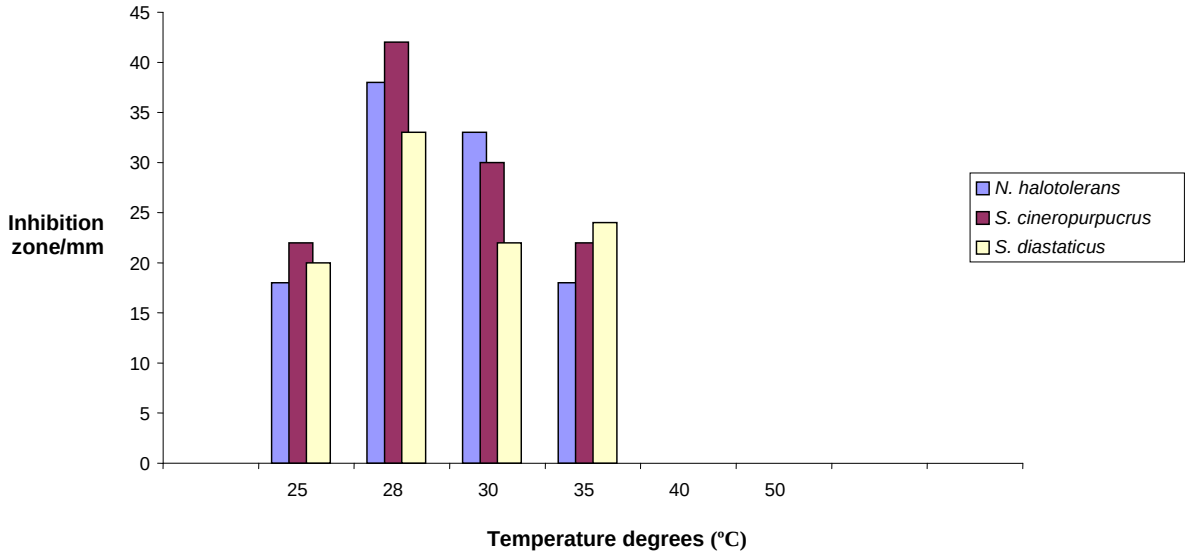


Fig (34): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate with different incubation temperature against *Trichophyton violaceum*.

Table (20): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different incubation temperature against *Trichophyton rubrum*.

Temperature (°C)	Inhibition zone in mm		
	N. *halotolerans*	*S. cineropurpucrus*	*S.diastaticus*
25	21	18	18
28	38	44	40
30	40	35	33
35	22	12	18
40	0	0	0
50	0	0	0

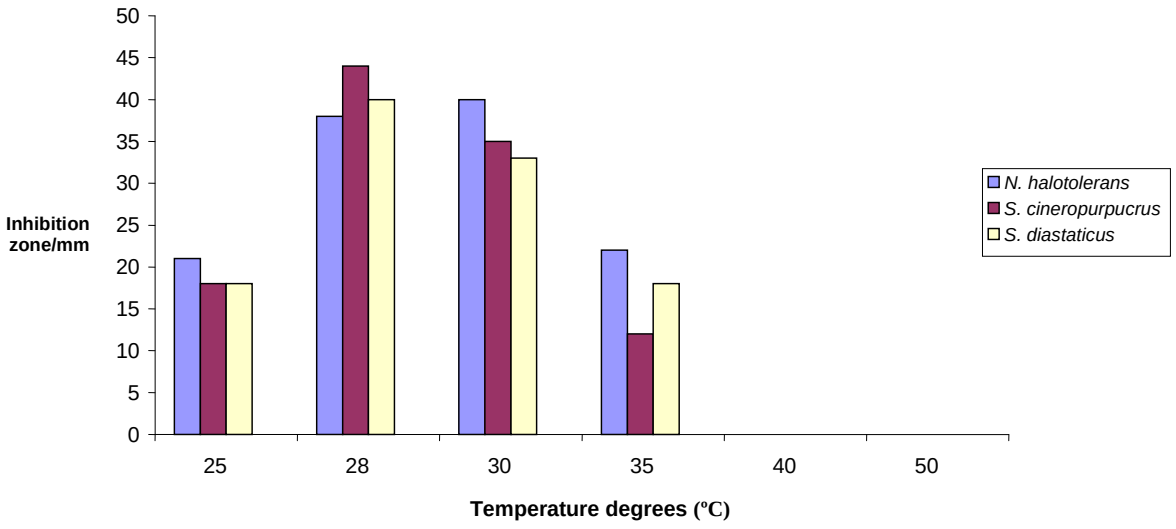


Fig (35): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different incubation temperature against *Trichophyton rubrum*.

Table (21): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different incubation temperature against *M. canis*.

Temperature (ºC)	Inhibition zone in mm		
	N.halotolerans	*S. cineropurpucrus*	*S. diastaticus*
25	18	22	20
28	34	40	42
30	30	33	34
35	15	12	14
40	0	0	0
50	0	0	0

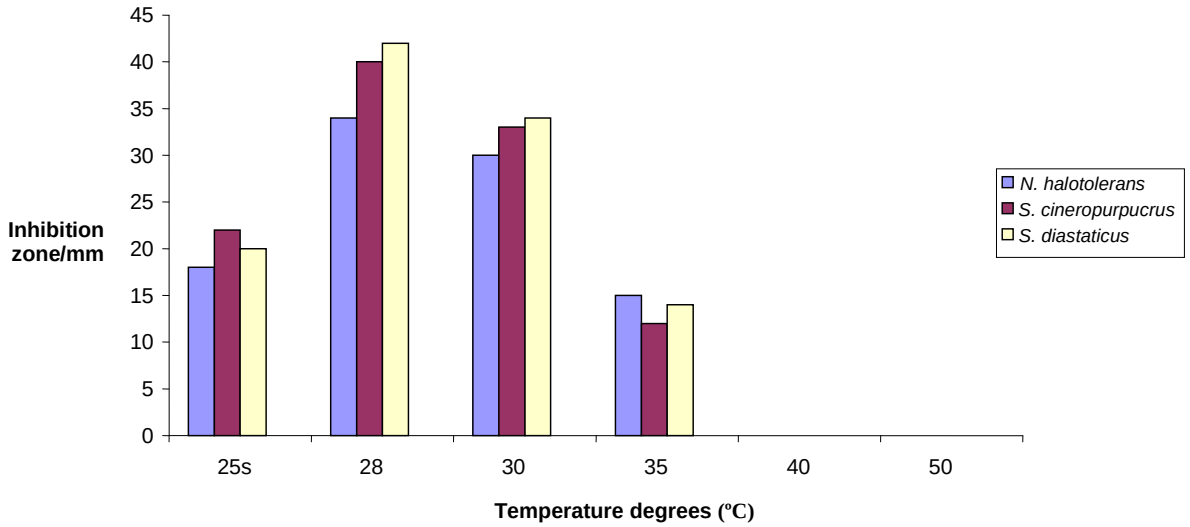


Fig (36): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different incubation temperature against *Microsporum canis*.

5a. Effect of pH on the production of antifungal substances produced by actinomycetes isolates against *T. violaceum*.

Effect of different pH values on the antagonism of actinomycetes isolates against *T. violaceum* was also studied. Data obtained were presented in Table (22) and Fig (37), which indicates that *S. cineropurpucrus* and *S. diastaticus* showed the maximum antagonistic effect at neutral or slightly alkaline media at pH 7-7.5. The third actinomycete *N. halotolerans* showed the maximum antagonistic effect under alkaline pH 8.5. While the extreme acidic (pH 5-5.5) and extreme alkaline (pH 10) were inhibited the production of antifungal agent.

5b. Effect of pH on production of antifungal substances produced by actinomycete isolates against *T. rubrum.*

The effect of pH values on the antagonistic abilities of actinomycete species against *T. rubrum* was also studied. Data obtained from Table (23) and Fig. (38) indicate that the maximum production of antifungal products for *N. halotolerans* was at pH 8.5, while *S. diastaticus* and *S. cineropurpucrus* was at pH 7, and the production failed at pH 5 and 5.5 pH for all actinomycete isolates.

5c. Effect of pH on production of antifungal substances produced by actinomycete isolates against *M. canis*.

The effect of pH on antifungal production by actinomycetes was studied to find out the optimum pH values for their process. The obtained results were listed in Table (24) and Fig. (39). The pH values for antifungal production by *N. halotolerans* ranged from 6.5 to 10. As the pH value increased, there was a corresponding increase in metabolic antifungal production at pH 10, while pH ranged from 5.5 to 7.5 for *S. cineropurpucrus* and *S. diastaticus*. The maximum production of antifungal substances was at pH 7 for both organisms.

Table (22): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different pH values against *Trichophyton violaceum*.

pH values	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S. diastaticus*
5.0	0.0	0.0	0.0
5.5	0.0	0.0	0.0
6.0	5.0	18	15
6.5	8.0	21	12
7.0	18	40	40
7.5	21	20	38
8.0	31	5.0	10
8.5	35	0.0	0.0
9.0	20	0.0	0.0
9.5	5.0	0.0	0.0
10.0	0.0	0.0	0.0

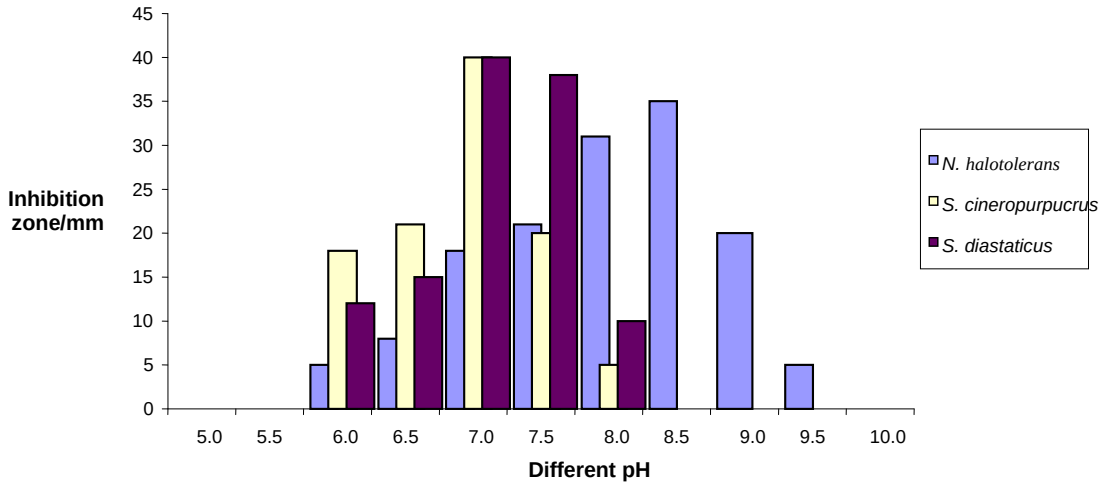


Fig (37): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different pH values against *Trichophyton violaceum*.

Table (23): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different pH against *Trichophyton rubrum*.

pH values	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S. diastaticus*
5.0	0.0	0.0	0.0
5.5	0.0	0.0	0.0
6.0	8.0	10	12
6.5	10	24	22
7.0	22	38	37
7.5	30	20	30
8.0	32	0.0	0.0
8.5	40	0.0	0.0
9.0	34	0.0	0.0
9.5	18	0.0	0.0
10.0	8.0	0.0	0.0

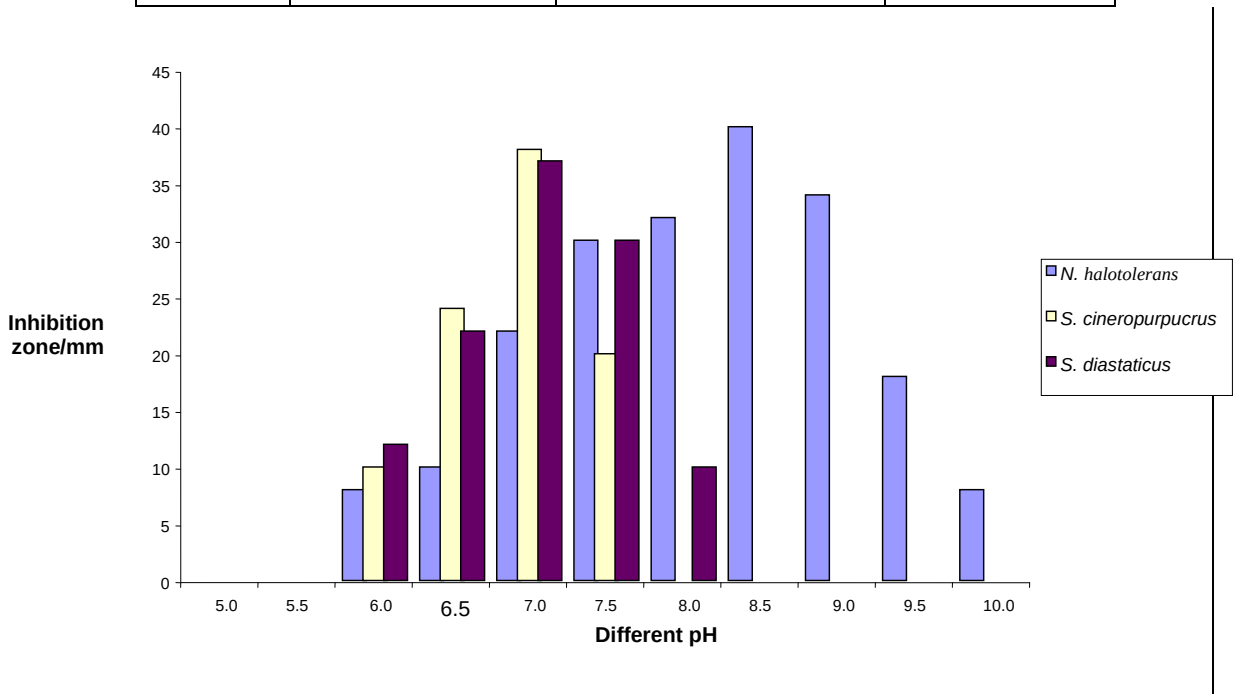


Fig (38): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different pH against *Trichophyton rubrum*.

Table (24): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different pH against *Microsporum canis.*

pH values	Inhibition zone in mm		
	N. halotolerans	*S. cineropurpucrus*	*S.diastaticus*
5.0	0.0	0.0	0.0
5.5	0.0	10	8.0
6.0	0.0	13	10
6.5	18	30	18
7.0	22	32	40
7.5	38	10	35
8.0	44	0.0	5.0
8.5	48	0.0	0.0
9.0	50	0.0	0.0
9.5	40	0.0	0.0
10.0	38	0.0	0.0

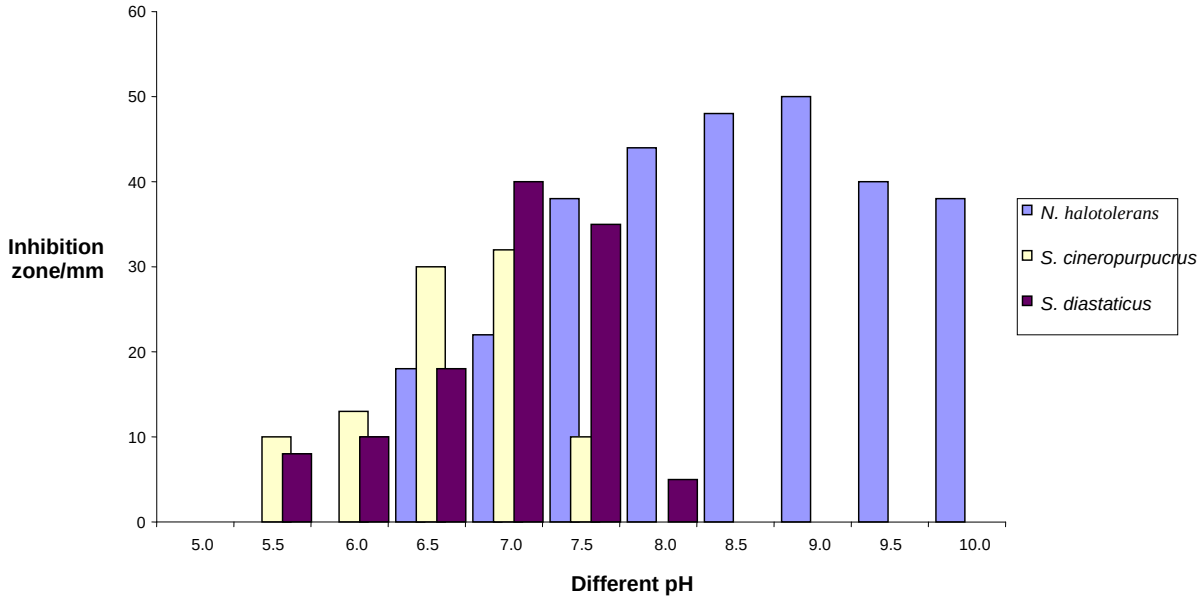


Fig (39): The inhibition effect of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* grown on starch nitrate medium with different pH against *Microsporum canis.*

6- Minimum Inhibitory Concentration (MIC) values of the supernatant of actinomycetes against dermatophytes isolates:-

The minimum values of supernatant of *N. halotolerans* ranged from 84 mg/ml for *T. rubrum*, 120 mg/ml for *M. canis* and 128 mg/ml for *T. violaceum*. However, minimum values of supernatant of *S. cineropurpucrus* ranged from 120 mg/ml to 180 mg/ml. As for the supernatant of *S. diastaticus*, the minimum values ranged from 128 mg/ml to 140 mg/ml. See Table (25) and Fig. (40).

Table (25): Minimum Inhibitory Concentration (MIC) values of the culture supernatant of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* against Dermatophytes isolates.

Actinomycetes isolates	Minimum inhibitory Conc.(MIC) mg/ml		
	T. violaceum	***T. rubrum***	***M. canis***
N. halotolerans	128	84	120
S. cineropurpucrus	180	180	120
S. diastaticus	130	140	128

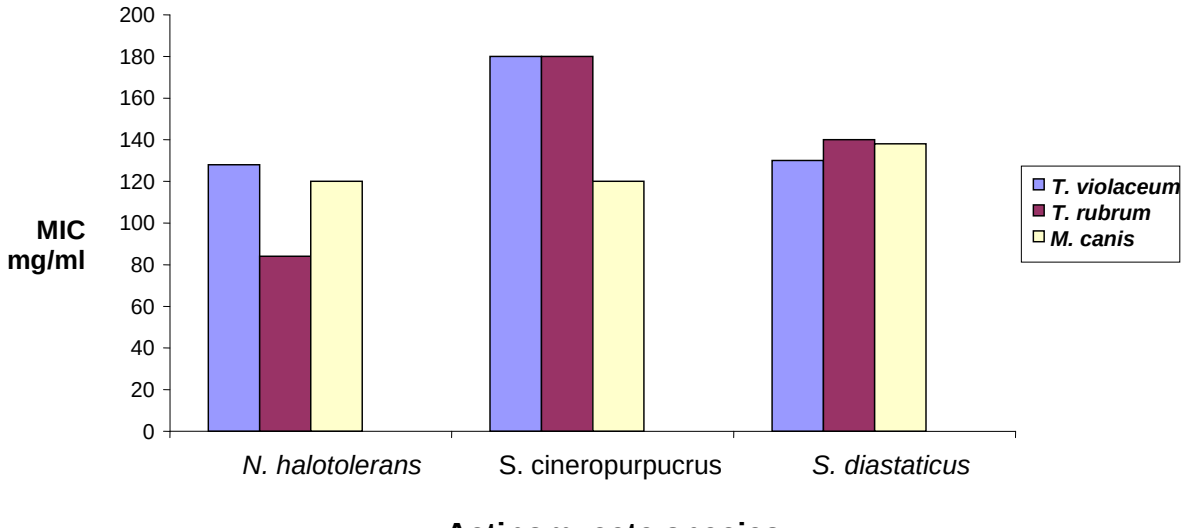


Fig (40): Minimum Inhibitory Concentration (MIC) values of the culture supernatant of *Nocardiopsis halotolerans*, *Streptomyces cineropurpucrus* and *Streptomyces diastaticus* against Dermatophytes isolates.