RESULTS

4.RESULTS

4.1. Isolation of soil microorganisms.

The data represented in Table (2) and Fig. (1), show that, four kinds of microorganisms isolated from the three soil samples, (Toukh, Nubaria and El-Hamol) of which, the count of bacteria, actinomycetes , fungi and yeast range from 23-40, 5-20, 2-15 and $5x10^3$ CFU/gm dry soil.

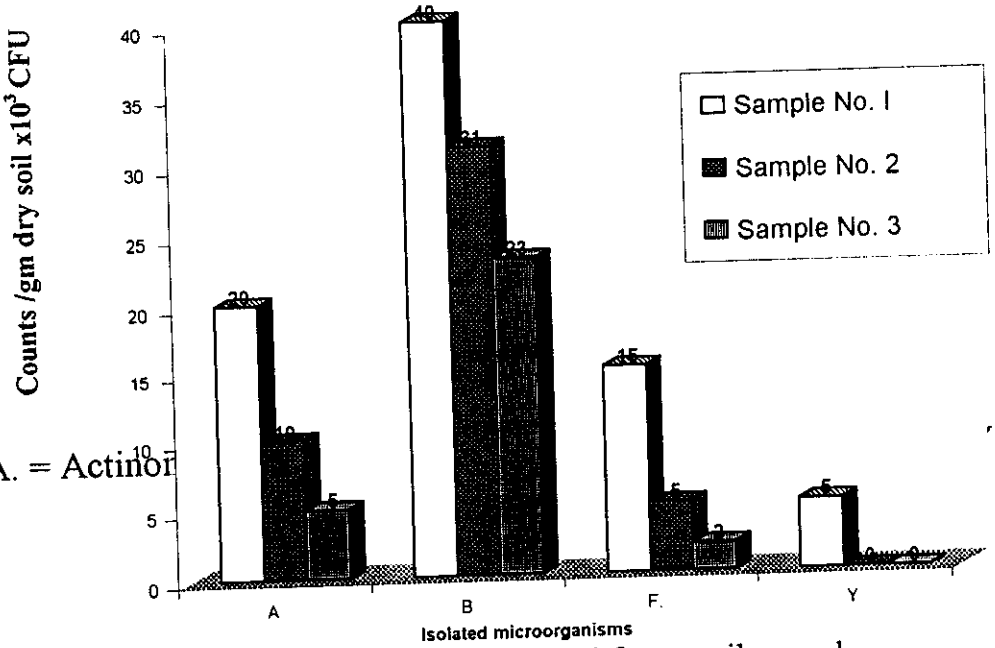
- ***Isolation of microorganisms tolerance to fungicide.***

As it clear from the results given in Table (3) and Fig. (2), the isolated microorganisms were highly decreased in culture amended with fungicide of which only 6 isolates of actinomycets, 8 isolates of fungi, 3 isolates of bacteria and only one isolate of yeast (for each one gram dry weight) were capable of growing in the presence of fungicide (kocide 101) at concentration of 0.15 gm/ml.

Table (2): Microorganisms isolated from different soil samples.

Soil samples No.	Location	Description	Isolated microorganisms[a]			
			Actinomycetes	Bacteria	Fungi	Yeast
Sample No.1	Toukh, Kalubia	Cultivated	20	40	15	5
Sample No.2	Nubaria, El-Behera,	Cultivated	10	31	5	-
Sample No. 3	El-Hamol, Kaffer El-Sheikh	Cultivated	5	23	2	-
Total isolates			35	94	22	5

a) Counts /gm dry soil x 10^3



A. = Actinoi 'east

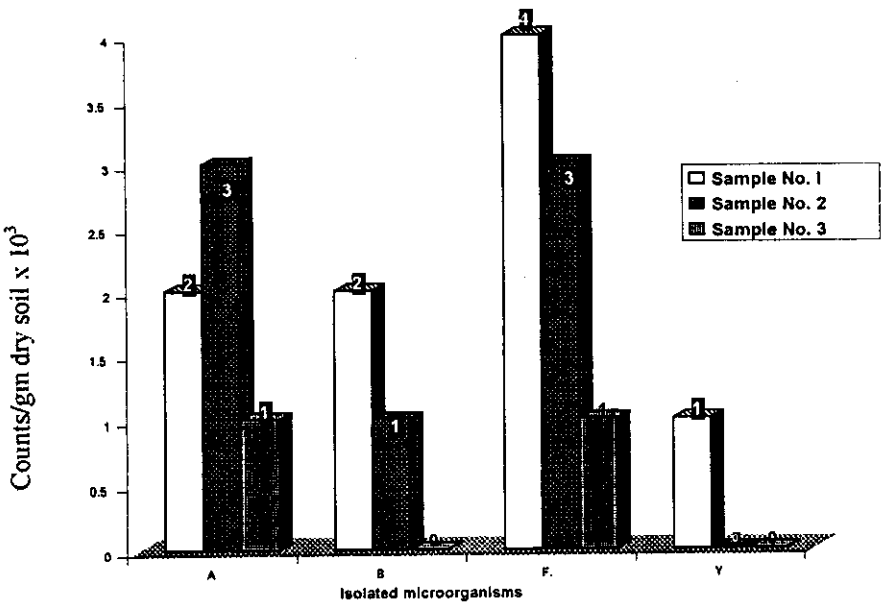
Fig. (1): Microorganisms isolated from soil samples.

Table (3): Microorganisms isolated in the presence of fungicide (Kocide 101).

Sample No.	Location	Counts[(a)] of microorganisms			
		Actinomycetes	Bacteria	Fungi	Yeast.
1	Toukh	2	2	4	1
2	Nubaria	3	1	3	0
3	El-Hamol	1	0	1	0
Total		6	3	8	1

a) Counts /gm dry soil x 10^3 CFU

Initial concentration of fungicide is 0.15 gm /100ml.



A. = Actinomycetes B. = Bacteria F. = Fungi Y.=Yeast

Fig. (2): Microorganisms isolated in the presence of fungicide (Kocide 101).

- ***Effect of different concentration of fungicide on the growth of isolated actinomycete.***

From the results given in Table (4), the data reveal that, the isolate T_{118} was the only isolate, which could grow in the presence of all concentrations used (0.05-0.25%) of fungicide within relatively short period i.e. 3-5 days. Some other isolates failed to grow in the presence of only 0.15% of the used fungicide, and all isolates except isolate T_{118} failed to grow at concentration of 0.2%. While all the used isolates show growth after one day incubation in the control plates (0.0% fungicide).

Table (4): Effect of different concentrations of fungicide (kocide 101) added to ISP-2[(a)] on the isolated actinomycetes growth.

Fungicide (w/v) / Actinomycete isolates	Incubation time (days) [(b)]					
	D_{118}	D_{123}	D_{204}	D_{205}	D_{290}	D_{307}
0.05 %	3	3	4	4	3	4
0.10%	4	5	7	7	5	6
0.15%	4	7	-ve	- ve	7	8
0.20%	5	-ve	-ve	-ve	-ve	-ve
0.25%	5	-ve	-ve	-ve	-ve	-ve
0.0 %	1	1	1	1	1	1

a)Yeast extract Malt extract[(5)].

b) Incubation time after which growth appeared in the first subculture.

- ve The isolate failed to show any growth.

4.2. Isolation from faba bean and pathogencity test of *Botrytis* isolates

4.2.1. Isolation of the causal organism of chocolate spot disease.

The pure isolates obtained from the samples of Kalubia,(sample No1), El –Beheira ,(sample No2) and Kafr El-Sheikh,(sample No3) were characteristic to *Botrytis faba* (3 isolates), *Botrytis cinerea* (4 isolates) and *Alternaria alternatn* (8 isolates)

4.2. Pathogencity test of Botrytis sp.

This was a preliminary test for the pathogenicity of seven *Botrytis* sp. isolates, (3of *Botrytis fabae* and the rest are *Botrytis cinerea)* which are responciple for chocolate spot disease on faba bean, Table (5).

The three cultivars (c.v) used were Giza 40, Giza 3 and Giza 716. The results given in (Table 5) show that, the seven isolates were pathogenic to the three cultivars, symptoms were appeared after 3 days from inoculation.

Results revealed that, the used isolates were able to infect the three tested cultivars, but in different severity of infection. Significant difference was observed between the three cultivars at each isolate. The isolates of *B.fabae* (1,3and 6) were more virulent than those of the *B.cinerea.* (2,4,5and 7). The most virulent isolate among those of *B.fabae* was No. 3 which isolated from faba bean plants grown in Nubaria area resulting in an average disease severity of 5.0 and 5.2 on detached leaves and on potted plants, respectively it show significant difference to the rest isolates therefore it used for further studies

While both isolates 1and 6 didn't show significant difference, showed disease severity of 3.9 and 4.3 for isolate No. 1 while isolate

No.6 resulting in disease severity of 3.7, on both detached leaves and on potted plants.

The least virulent on the three cultivares was isolate No. 7 isolated from plants grown in El-Hamol in which showed disease severity of 1.6 and 1.9 on detached leaves and on potted plants respectively, the rest isolates of *B.cinerea* No. 4 & 5 (with no significant difference) obtained from infected plants grown in Nubaria area while isolates No. 2 & 7 isolated from Toukh and El-Hamol doesn't show significant difference between each other but the isolates 4 & 5 show significant difference to isolates 2 &7. Also the tabulated data in the Table reveled that, the used three faba bean cultivares were different in their reaction to chocolate spot disease where, Giza 40 was susceptible, Giza 3, was moderate while Giza 716 was resistance.

Table (5): Visrulance of *Botrytis* sp. isolates on three fabae bean cultvars using detached leaves technique and on potted plants..

Source of isolates	Isolate No.	Disease severity (Scale 0-9)							
		Detached leaves				On potted plants			
		G 40	G 3	G 716	Mean	G40	G 3	G716	Mean
Toukh	1*f*	5.2	3.8	2.8	3.9	6.8	3.4	2.6	4.3
Nubaria	3 *f*	6.8	4.8	3.6	5.0	8.5	4.9	3.2	5.2
El-Hamol	6 *f*	4.6	3.6	2.8	3.7	5.8	2.9	2.5	3.7
Toukh	2 *c*	2.4	1.8	1.0	1.7	3.3	2.1	1.1	2.2
Nubaria	4 *c*	3.0	2.0	1.6	2.2	3.1	2.1	1.1	2.1
Nubaria	5 *c*	2.8	2.0	1.8	2.2	3.1	2.2	1.3	2.2
El-Hamol	7 *c*	2.2	1.6	1.0	1.6	3.1	1.7	1.1	1.9

(L.S.D. 5%) Isolates (I) 0.5 0.3

Cultivars (c) 0.3 0.2

I x C 0.8 0.5

f = *Botrytis fabae* *c* = *Botrytis cinerea*

4.3. Anti-microbial potentialities of actinomycetes isolated from faba bean soil.

In this work, soil samples were used for isolating some actinomycetes cultures, these cultures were purified and tested for their antibiotic activities against Gram-positive and Gram-negative bacteria, and some phytopathogenic fungi.

Thirty-five actinomycetes cultures were isolated from the used sampling soil using the methods have been described previously. The anti-microbial activities of the previous isolates were evaluated by using the classical diffusion methods which have been described before.

Generally, this methods is based on the formation of inhibition zones of the test organism growth on the agarized seeded medium by the test organism.

4.3.1. Survey of anti-microbial activities.

The results in Table (6) appeared that, the chosen 14 actinomycetes isolates which found to produce anti-microbial activities, were classified according to their anti-microbial activities into five groups.

Group(I) of which isolates show anti-microbial effect against G+ve bacteria, that contain (T_{101}, T_{105}, T_{112}, T_{118}, T_{123}, N_{204},N_{205}, N_{290}, H_{302} , H_{304} and H_{315}) of which isolate T_{118} was the most one produced anti-microbial activity.

The group(II) according to anti-microbial activities against G-ve bacteria it contains isolates T_{101}, T_{112}, T_{113}, T_{118}, N_{290} H_{304} and H_{315} , it found to produce anti-microbial activities, of which the isolate T_{118} appeared the highly anti-microbial effect.

Group(III) that contains isolates T_{101}, T_{112}, T_{118}, H_{304} and H_{315} which produced anti-microbial activities against both G+ve and G-ve bacteria. The isolate T_{118} was also the best isolate.

Group(IV) which included isolates T_{101}, T_{105}, T_{118}, T_{123} , N_{204} and H_{304} that have the capability to produce anti-microbial activities against fungi and G+ve bacteria,

Group(V), included only the isolate T_{118} which produced a wide spectrum anti-microbial effect, for this reason this isolate was used for further studies.

From the previous results, it was observed that, the six isolates of actinomycetes (T_{118}, T_{123} , N_{204}, N_{205}, N_{290} and H_{307}) were the most active isolates that showed anti-fungal effect , as well as their capability to tolerate the fungicide. For this reason, these isolates were shosen for testing their effect for reducing chocolate spot diseases on faba bean caused by *Botrytis fabae* .

Table (6): Anti-microbial activities of selective actinomycetes isolated from soil.

Tested Organism / Isolate No.	Mean diameter[a] of inhibition zones (mm) against.													
	Bacteria						Fungi							
	G+ve			G- ve			Unicellular			Filamenteus*				
	B.s	*M.l*	*R.e*	*S.t*	*P.a*	*E.c*	*S.c*	*C.a*	*G.c*	*B.f*	*B.c*	*F.o*	*F.s*	*F.m*
I101	15	0.0	0.0	18	0.0	0.0	0.0	17	13	0.0	0.0	0.0	0.0	0.0
I105	0.0	0.0	14	0.0	0.0	0.0	0.0	12	0.0	0.0	0.0	0.0	0.0	0.0
I112	18	15	17	11	8.0	13	0.0	16	14	0.0	0.0	0.0	5.0	2.0
I113	3.0	0.0	4.0	12	14	15	8.0	18	12	0.0	0.0	0.0	6.0	4.0
I118	12	19	18	14	18	10	0.0	14	15	20	20	25	13	15
I120	0.0	0.0	11	0.0	0.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
I123	11	13	18	0.0	0.0	0.0	0.0	9.0	8.0	10	12	7.0	5.0	4.0
I204	8.0	7.0	11	0.0	0.0	0.0	6.0	8.0	7.0	8.0	11	9.0	6.0	5.0
I205	9.0	12	13	0.0	0.0	0.0	3.0	5.0	6.0	0.0	0.0	3.0	2.0	3.0
I290	10	10	8.0	11	13	11	0.0	0.0	5.0	4.0	5.0	0.0	0.0	0.0
I302	8.0	8.0	6.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
I304	0.0	7.0	0.0	0.0	5.0	0.0	0.0	8.0	6.0	0.0	3.0	8.0	6.0	5.0
I307	0.0	0.0	0.0	8	0.0	0.0	0.0	0.0	0.0	6.0	5.0	8.0	7.0	6.0
I315	7.0	9.0	8.0	10	9.0	9.0	0.0	0.0	3.0	2.0	3.0	6.0	0.0	0.0

B.s = *Bacillus subtilis* NCIB 3610
R.e = *Rhadococcus equi* ATCC 6939
P.a = *Pseudomonas aeruginosa* ATCC10145
C.a= Candida albicans ATCC 10231
S.c = *Saccharomyces cervisiae* ATCC2601
G.c =*Geotrichm candium* ATCC 34614
F.o = *Fusarium oxysporum*
M.l=*Micrococcus leteus* NCIB 190
S.t = *Salmonella typhie* NCTC4111
E.c = *Echericha Coli* NCIB 9132
B.f =*Botrytis fabae*
F.s. = *Fusarium solani*
B.c.= *Botrytis cinerea*
F.m. = *Fusarium moniliforme*

* Isolated fungi during the experimental work.

(a) Mean diameter of three replicates.

3.4.2. Antagonistic activities of six actinomycetes isolates against *Botrytis fabae.*

The results in Table (7) and Fig.(3) showed the effect of six actinomycetes (tolerant to the fungicide) on the mycelium growth of the causal agent of chocolate spot disease on faba bean (*B.fabae*). The results revealed that, significant difference between the isolate T_{118} and the rest isolates, while both isolates T_{123} and N_{290} didn't show significant difference as well as the two isolates N_{204} and N_{205}. However, isolate T_{118} was the highly effective one, it have been succeeded to reduce the radial growth of the pathogen with inhibition zone 25 mm, while the fungal growth have filed the hole plate surface in the control.

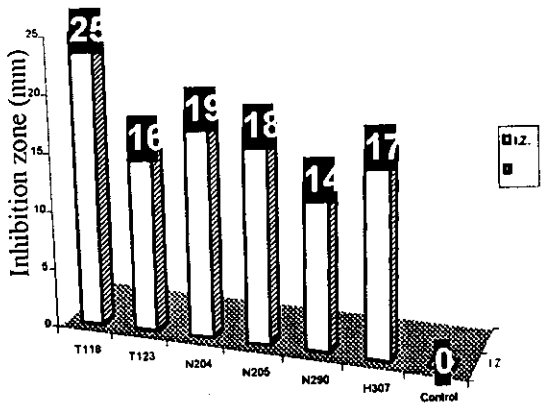
Also, Fig. (4) illustrated, the antagonistic activity of isolate T_{118} against *Botrytis fabae* on plates containing Potato Dextrose Agar (PDA) medium[6]. The isolate succeeded to reduce the mycelium growth of *B.fabae* in dual culture (A)compared to the control plate (B).

Also, Fig. (5) (A, B, and C) illustrated, the hyphal interaction between isolate T_{118} and *B.fabae* under light microscope. It's clear that, the effect of the antagonistic actinomycete T_{118} on the growth of the pathogen (*B.fabae)*. The pathogen hyphae have small rounded swellings at the zone near the antagonistic activity (Fig. 5B) compared to the control (Fig. 5A), also after six days incubation lysis of hyphae was observed (Fig. 5C).

Table (7): Antagonistic activities of six actinomycete isolates (tolerant to fungicide) against *Botrytis fabae* on PDA plates.

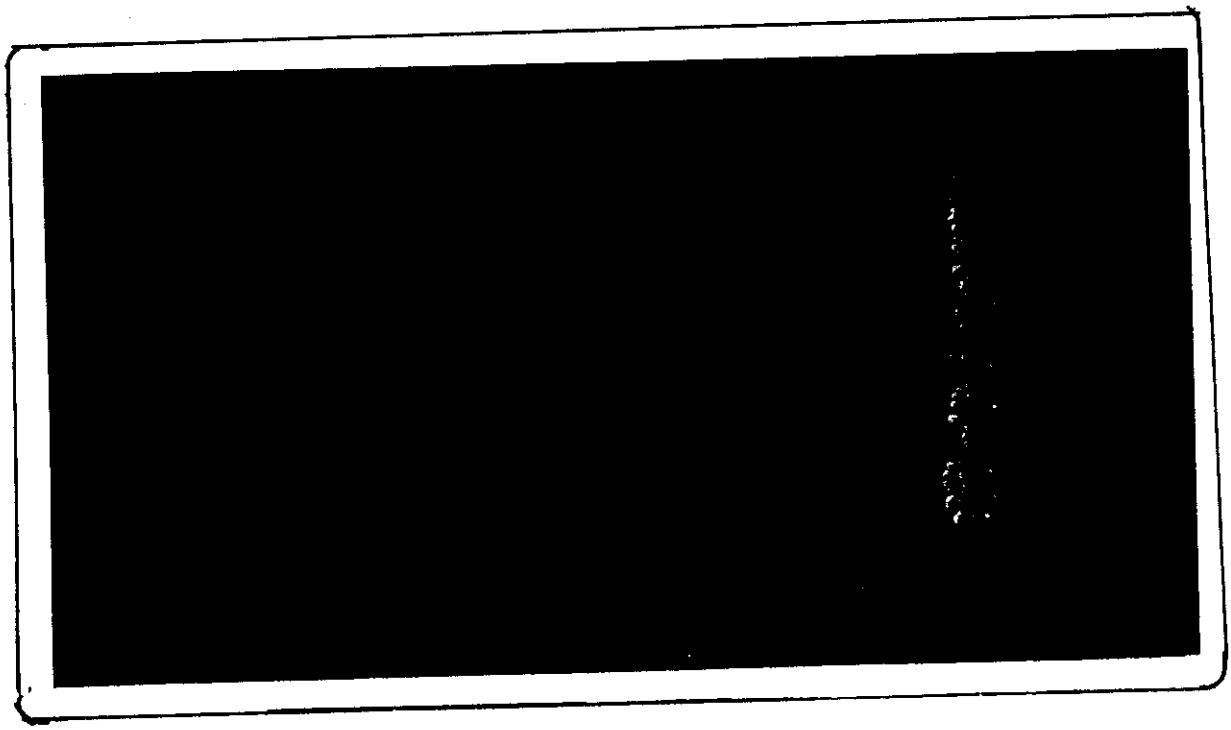
Actinomycetes isolates	Inhibition zone (mm)
T_{118}	25
T_{123}	16
N_{204}	19
N_{205}	18
N_{290}	14
H_{307}	17
Control *	0.0
(L.S.D.0.05)	2.09

* *Botrytis fabae* only.
+ ve Result was recorded as I.Z. > 10 mm.



Actinomycetes isolates

Fig. (3): Antagonstic activities of six actinomycetes isolates (tolerant to fungicide) against *Botrytis fabae* on PDA plates



(A) *(B)*

Fig. (4): The antagonstic activity of actinomycete isolate T_{118} against *Botrytis fabae*. A, *B.fabae* (control) and B, selective isolate T_{118} and *B.fabae* in dual culture which illustrate the inhibition of mycelial growth of *B.fabae*.

(A) *(B)* *(C)*

Fig. (5): Hyphal interaction between actinomycete isolate T_{118} and *Botrytis fabae* in dual culture, A) non effected hyphae (normal branching), B) swollen at hyphal tip due to the effect of the isolate after 2 days, C) As early as six days after incubation the *B.fabae* hyphae exhibited very thin at regions near the diffusate substance and lysis the hyphae.

4.3.3. *Bio-activities of six actinomycete isolates for controlling chocolate spot disease.*

I- On detached leaves.

Data presented in Table (8) and Fig. (6) showed, the effect of 6 isolates of actinomycetes (tolerant to fungicide) for controlling chocolate spot disease on faba bean (c.v. Giza 3) using detached leaves. It revealed that, during incubation time 24 h. the two isolates T_{118} and T_{123} show significant difference between each other for controlling the disease, by incubation time 48,72 and 96h. a significant difference was observed, in general the isolate T_{118} was the best one for reducing mycelium growth (disease severity was 0.6) compared to control (disease severity was 7.2) after 96h., followed by T_{123} (disease severity was 1.2), the rest isolates were less effective for controlling the disease.

II- On plants in pots

With regarde to the disease severity on faba bean plants (in pots), Table (9) and Fig. (7) showed that, by incubation time 3 and 7 days both isolates T_{118} and T_{123} didn't show significant difference between each other but show significant difference to the rest isolates, also isolate T_{118} was the effective one for reducing the disease. The disease severity after 3-weeks was 2.0, while control was 9.0, followed by the isolate T_{123} by which the disease severity was 2.8, while in case of the remaining isolates disease severity ranged between 4.2-5.8 compared to control (disease severity of 9).

These results revealed that, the isolate T_{118} is the most active one, since it controlled chocolate spot disease on faba bean. For this reason it was chosen for other studies.

Table (8): Bio-activity of the used six actinomycetes isolates for controlling chocolate spot disease on faba bean plants (cultivar Giza 3) using detached leaves.

Actinomycetes Isolates / Incubation time	Disease severity[a] on scale (0-9) after			
	24 h.	48 h.	72 h.	96h.
T_{118}	0.0	0.2	0.2	0.6
T_{123}	0.2	0.6	1.0	1.2
N_{204}	2.8	3.2	4.0	4.6
N_{205}	2.8	3.4	4.4	4.4
N_{290}	2.4	2.8	3.0	3.4
H_{307}	2.6	2.8	3.2	3.4
Control[b]	2.8	3.4	5.8	7.2

(L.S.D.0.05) Incubation time (A) 0.2, Isolates (B) 0.3 A x B 0.6

(a) Mean value of five replicates.

(b) *Botrytis fabae* spores only with distilled water.

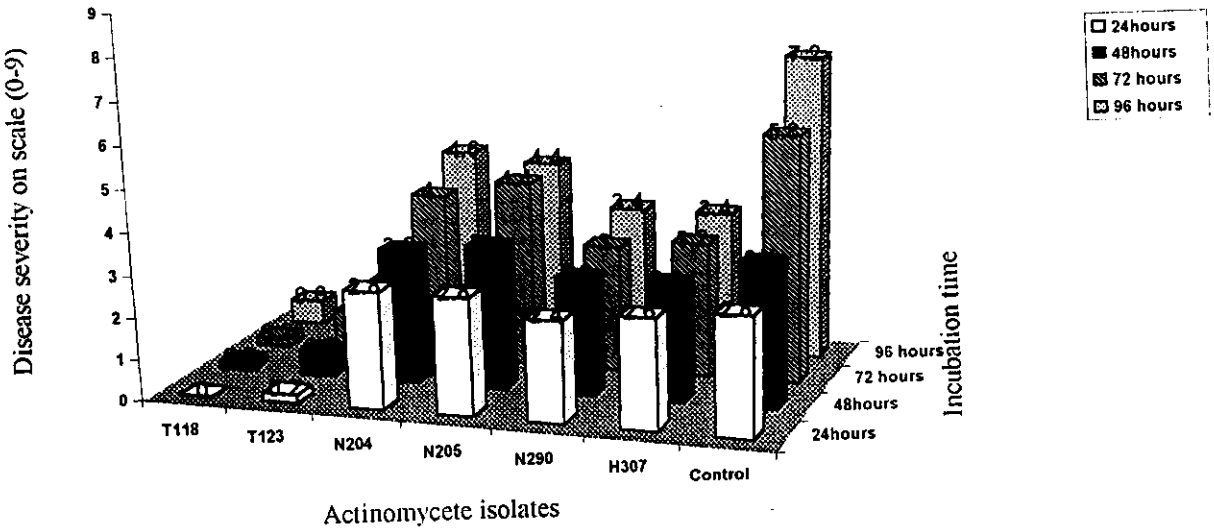


Fig. (6): Bio-activity of the six actinomycetes isolates for controlling chocolate spot disease on detached leaves.

Table (9): Bio-activity of the six actinomycetes isolates for controlling chocolate spot disease on faba bean plants cultivar Giza 3 (in pots) under green house conditions.

Actinomycetes Isolates / Incubation time	Disease severity[a] on scale (0-9) after			
	3 days	7 days	14 days	21 days
T_{118}	1.0	1.2	1.6	2.0
T_{123}	1.0	1.4	2.4	2.8
N_{204}	2.6	3.0	3.8	5.4
N_{205}	3.6	3.6	4.4	5.8
N_{290}	3.5	3.2	3.6	4.2
H_{307}	3.4	3.8	4.4	4.8
Control[b]	4.6	7.4	8.4	9.0

(L.S.D. 0.05) Incubation time (A) 0.3 Isolates (B) 0.4 A x B 0.7

(a) Mean value of five replicates.

(b) Plants treated with *Botrytis fabae* spore suspension only.

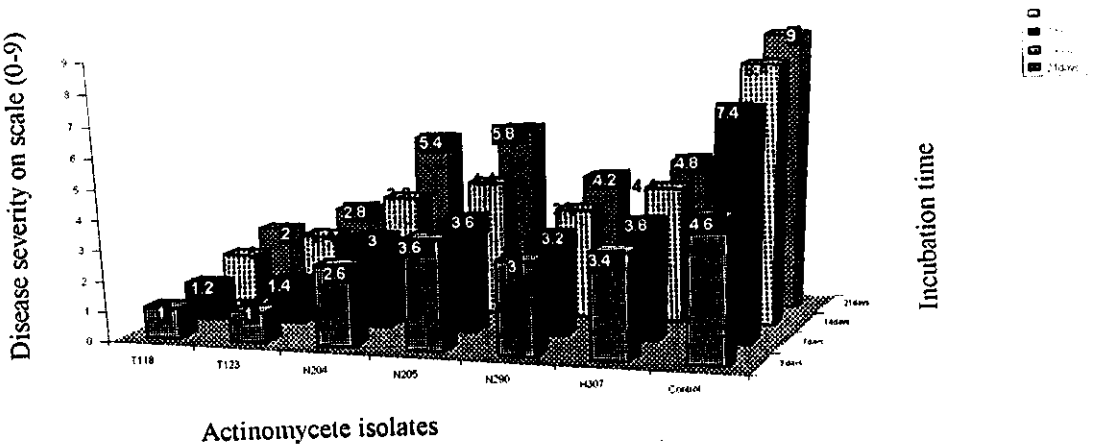


Fig. (7): Bio-activity of the six actinomycetes isolates for controlling chocolate spot disease on faba bean plants under greenhouse conditions.

- **Effect of culture filtrate of isolate T_{118} on percentage of spore germination and lengths of germ tubes of *B. fabae.***

The effect of culture filtrate of isolate T_{118} on *B.fabae* spore germination and lengths of germ tubes were studied *in vitro*. Data in table (10) show that , all tested culture filtrate concentrations were significantly decreased of spore germination percentage and lengths of germ tubes compared with the control . The effect of culture filtrate was increased with increased of culture filtrate (100%) was the most effective on spore germination (0.0%) and length of germ tube (0.0µm) .Also data indicated that , no significant difference between 75% and 50% (V/V) concentration on length of germ tube which gaive 13.4 and 17.7 µm . respectively .

Table (10) . Effect of culture filtrate concentrations of isolate T_{118} on spore germination and lengths of germ tubes of *B.fabae* (after 24 hr.).

Concentration %	Germination of B.fabea spores (%)	Total length of germ tube (µm)
0.0	97.7	15.6
25	53.9	44.6
50	18.6	17.7
75	8.6	13.4
100	0.0	0.0
L.S.D.0-05	2.4	4.5

4.4. Taxonomical and biological studies of the most potent actinomycetes isolate (T_{118}) producing anti-microbial metabolites.

Isolate No. T_{118} which was the most effective isolate against the tested organisms was used for anti-microbial production after have been identified for such the identification was used on the following:

4.4.1. The detection of Diamino-pimelic acid in the cell wall hydrolysate of isolate T_{118}.

Results were shown that, Diamino-pimelic acid (DAP) in the cell wall hydrolysate is LL –DAP type, while sugar pattern not detected , this revealed that isolate T_{118} belong to *Streptomyces* spp.

4.4.2.Morphological characteristics of the isolate T_{118}.

Isolate T_{118} showed a good growth of aerial mycelium at 30°C, after incubation period for 14 days on all of the following agar media, glucose casin yeast extract beef extract , inorganic salts starch, yeast extract-malt extract, oat meal and fish meal extract . The morphological characteristics of the *Streptomyces* spp. T_{118} was examined and recorded. Fig. (8) illustrate the spore chain which was spiral form.

The color of aerial mycelium was pink-gray to pink-white and the color of substrate mycelium was yellowish gray to browenish gray. Scleratia were not present as well as sporulation on substrate mycelium. Fig. (9)illustrate the electronmicroscop photography of spores, it ellipsoid to oval shaped, with smooth surface, it's related to colour series produced on both ISP1 and Glucose Aspragine agar media. The colour name charts were used for colour determination of both aerial and substrate mycelia. The colour of reverse side of it's colony was bale yellow on the used ISP media while bale-brown to brown on Waksman's media.

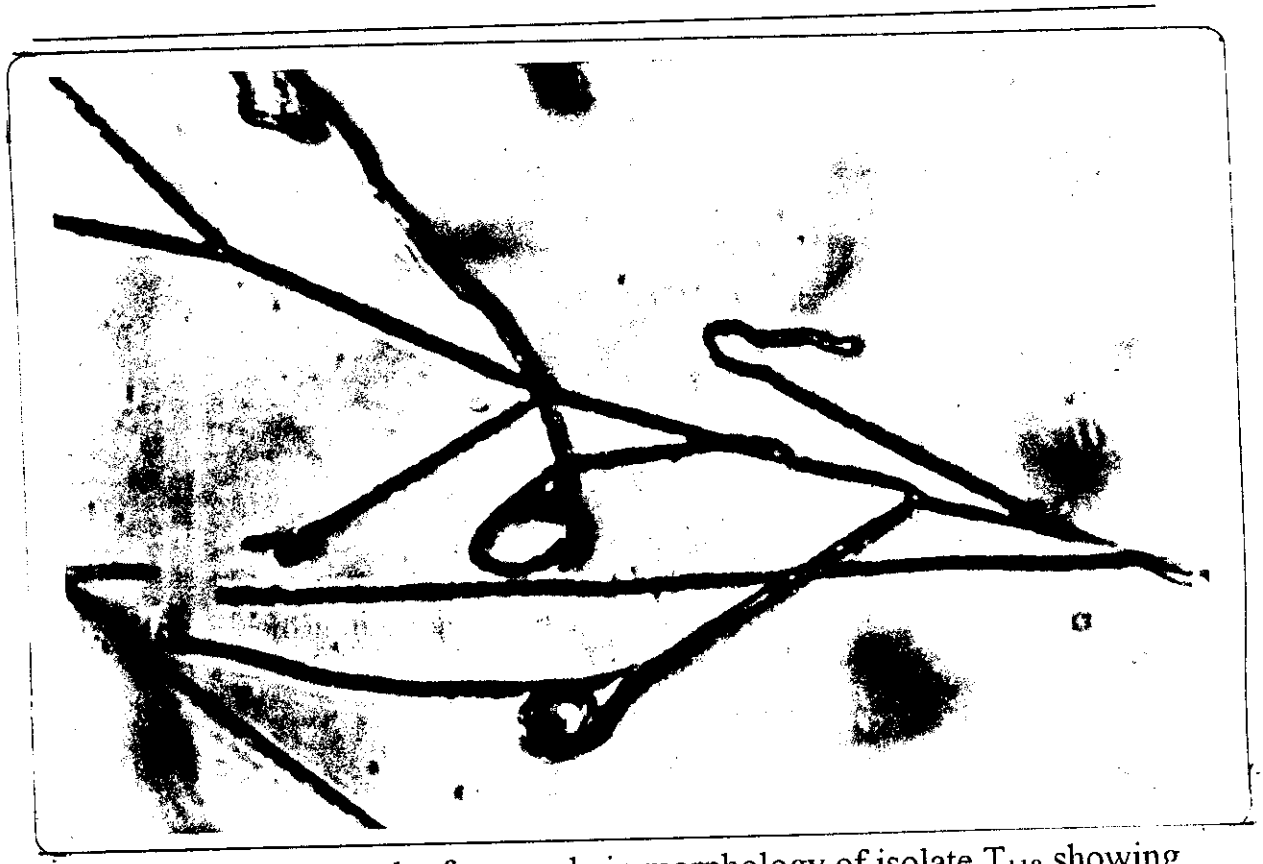


Fig. (8): A photograph of spore chain morphology of isolate T_{118} showing spiral formation (x 1500)

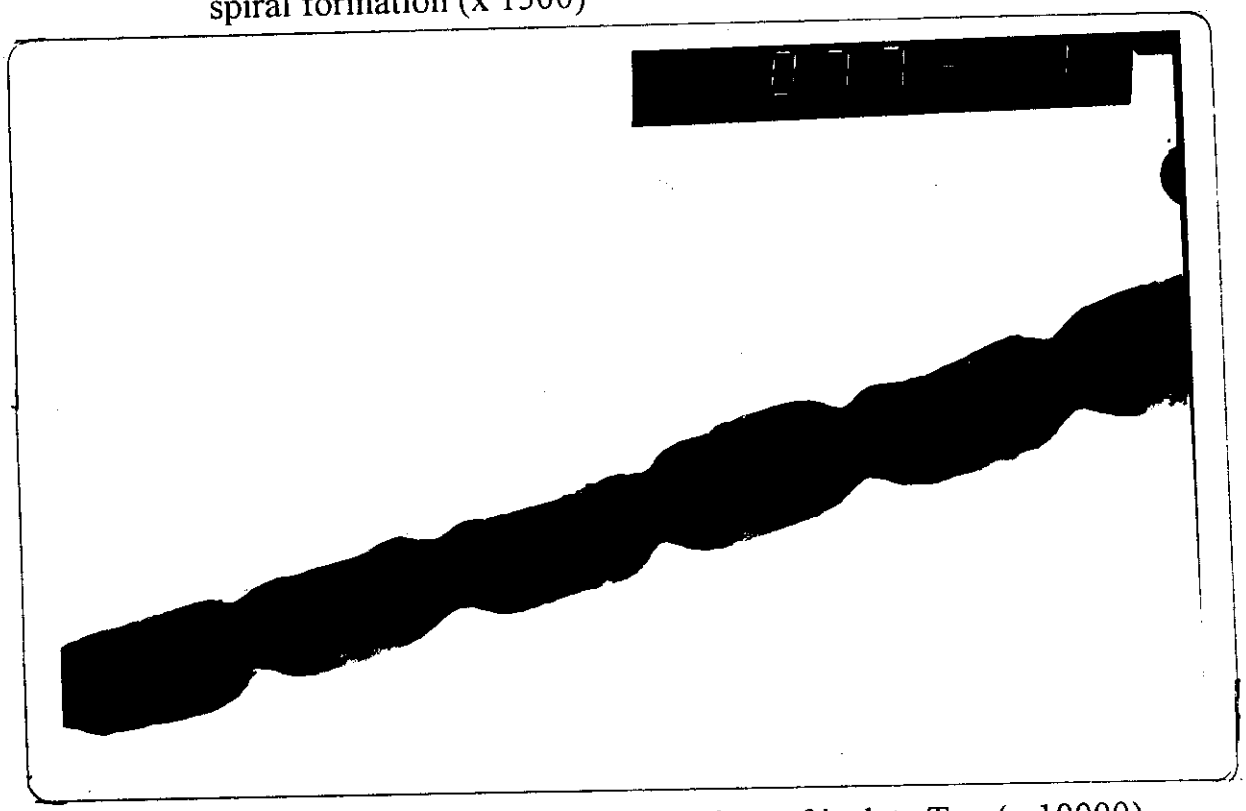


Fig. (9): Electron micrograph of spore surface of isolate T_{118} (x 10000).

4.4.3. Cultural properties

The study of cultural properties of the isolate Streptomyces spp D_{118} showed that, tested organism appeared very good growth on, yeast extract –Malt extract, fish meal, and starch nitrate agar media, while ranged between good, moderate and poor growth on the other used media.

Aerial mycelium and substrate mycelium were pink gray and yellowish brown, respectively. The used organism don't produced any diffusible pigments (Table 11).

4.4.4. Physiological and biochemical characteristics of the used isolate (T_{118}).

- **Utilization of different carbon sources.**

As it is clear from the data recorded in Table (12), the tested organism Streptomyces spp T_{118}, succeeded to utilize all the used carbon sources with a different degrees (except D-xylose, inulin, and xylitol). The four carbon sources i.e. Mannitol, D-fructose, raffinose and D-galactose were the best carbon sources that utilized by the tested organism (very good growth). The results also showed that, the organism gave a good growth with sucrose, L-rhamnose, D-manose D-lactose, cellobiose sodium citrate and sodium pyruvate, while exhibited a moderate and poor growth with the rest used carbon sources.

Table (11): Cultural properties of *Streptomyces* (T_{118}).

Media type	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
Yeast extract-Malt extract ISP –2*	Very good	Pink gray	Yellowish-brown	-
Oat-meal agar ISP –3*	Good	Pinkish-white	Red-gray	-
Inorganic salts-starch agar ISP-4*	Good	Pink-gray	Grayish-yellow	-
Glycerol-Asparagin agar ISP-5*	Poor	-	-	-
Sucrose nitrate agar**	Poor	-	-	-
Potato-Dextros agar**	Good	Pinkish-white	Grayish-brown	-
Fish meal agar	Very good	Pinkish-white	Brownish-yellow	-
Nutrient agar**	Moderate	Pink-gray	Yellowish-gray	-
Glucose-casein yeast extract beef extract agar**	Good	Redish-gray	Pink-gray	-
Czepeak's Dox solution agar	Moderate	Pinkish-white	Pink-gray	-
Starch nitrate agar	Very good	Pink gray	Graish-brown	-
Glycerol nitrate agar**	Poor	Poor	-	-

* Media recommended by ISP.

** Media recommended by Waksman (1961).

- Not detected.

Table (12): Utilization of different carbon sources expressed as a growth yield by *Streptomyces* (T_{118}).

Carbon source	Growth yield	Carbon source	Growth yield
L-Arabionse	+ +	Trehalose	+ +
Sucrose	+ + +	D-Melibiose	+ +
D-xylose	±	Dextran	+ +
Meso-Inositol	+ +	D-Galactose	+ + + +
Mannitol	+ + + +	Cellobiose	+ + +
D-fructose	+ + + +	Xylitol	±
L-Rhamnose	+ + +	Sodium acetate	+ +
Raffinose	+ + + +	Sodium citrate	+ + +
D-Melezitose	+ +	Sodium malonte	+ +
D-Mannose	+ + +	Sodium propionate	+
D- Lactose	+ + +	Sodium pyruvat	+ + +
Inulin	±		
Adonitol	+ +		
Salicin	+		

± ≡ not detected

+ + ≡ moderate growth

+ + + + ≡ very good growth

\+ ≡ poor growth

+ + + ≡ Good growth

- **Utilization of different nitrogen sources.**

The tested streptonycetes isolate (T_{118}) exhibited a very good growth only with L-valine, while it gave a good growth with L-cysteine, L-phenylalmine, L-methonine and L-hydroxyproline. The organism failed to appear any growth with Dl-amino-n-butyric acid and appeared a moderate and poor growth with the remaining used nitrogen sources. (Table 13).

- **Other physiological and biochemical properties.**

The results represented in Table (14) showed that, the tested organism succeeded to produce melanin pigment on both peptone yeast extract and tyrosine agar media, as well as some degree of catalase production, lecithinase Lipolysis and proteolysis activities, hydrolysed all of pectin, chitin, hippurate and reduce nitrate to nitrite, in addition to production of hydrogen sulfied gas. The isolate also had the ability to breakdown certain complex compounds as starch, Hypoxnathine, xanthine, guanine, L-tyrosine, adenine, xylan, casein, gelatin, aesculin and both tween 20 and 80. The organism failed to degrade all of DNA, RNA, urea arbutin, Elestin, Keratin and Testostrone.

- **Resistant to certain antibiotics.**

It was obvious from the results recorded in the Table (15) that, the six antibiotics (ampiclin, sulfa methoxazole, topramycin, mikacin, cephololexin and microfura nitrofurmation) prevents the microbial growth while the rest of the antibiotics failed to inhibit the growth of the tested isolate (T_{118}).

Table (13): Utilization of certain nitrogen sources expressed as growth yield by *Streptomyces* (T_{118}).

Nitrogen sources	Growth yield
DL- amino- n-butyric acid	-
Potassium nitrate	+ +
L- cysteine	+ + +
L- valine	+ + + +
L- threonine	+ +
L- serine	+ +
L-phenylalanine	+ + +
L-Methionine	+ + +
L- Histidine	+ +
L- Arginine	+
L-Hydroxyproline	+ + +

+ ≡ poor growth

+ + ≡ Moderate growth

+ + + ≡ Good growth

+ + + + ≡ very good growth

- ≡ not detected

Table (14): Other physiological and biochemical properties of *Streptomyces*.(T_{118}).

Tests	Results	Tests	Results
Melanin pigment production on :		Starch	+ ve
• Peptone yeast extract	+ ve	Hypoxanthine	+ ve
• Tryosine agar	+ ve	Xanthine	+ ve
Enzyme activity		Guanine	+ ve
• Lecithinase	+ ve	DNA	- ve
• Protoysis	+ ve	RNA	- ve
• Lipolysis	+ ve	L-tyrosine	+ ve
Hydrolysis of		Adenine	+ ve
• Pectin	+ ve	Xylan	+ ve
• Chitin	+ ve	Casein	+ ve
• Hippurate	+ ve	Urea	- ve
Nitrate reduction	+ ve	Arbutin	- ve
Hydrogen sulfide production	+ ve	Elastin	- ve
Catalase production		Gelatin	+ ve
• **Degrative tests**	+ ve	Aesculin	+ ve
Tween 20	+ ve	Keratin	- ve
Tween 80		Testosterone	- ve

+ ve ≡ positive result

- ve ≡ Negative result

Table (15): The ability of *Streptomyces*. (T_{118}) to resist certain antibiotics.

Antibiotics	Result
Gentamycin	- ve
Raffampicin	- ve
Ampicilin	+ ve
Penicillin G	+ ve
Tetracycline	- ve
Sulfa methoxazole	+ ve
Amilcacin	- ve
Topramycin	+ ve
Mikacin	+ ve
Cepholoiexin	+ ve
Microfura nitrofurmation	+ ve

+ ve ≡ inhibited growth

- ve = normal growth

- **Growth tests.**

Data of table (16) revealed that the organism under investigation grow at temperature ranged between 15 to 37°C, while doesn't grow at 4°C, 10°C and 45°C. With regard to pH, the isolate (T_{118}) grow at pH 7.0 but failed to grow at pH 4.3. The results also revealed that, the used isolate grow at NaCl concentration of 4 and 7% w/v, while it failed to grow at 10 and 13 % w/v of NaCl. The used concentrations of sodium azide (0.01 and 0.02% w/v) were suitable for growth. Also the results show that, the tested organism didn't succeeded to grow in the presence of the rest used chemical inhibitors.

- **Identification of the isolate (T_{118}).**

On the basis of morphological properties, cell wall hydrolysate, physiological and biochemical characteristics summarized in Table (17), the *streptomyces* isolate T_{118} identified as *streptomyces violaceus* gave the name *S. violaceus* T_{118}.

Table (16): Growth of *Streptomyces* (T_{118}) at different temperature degrees, pH values and in the presence of certain chemical inhibitors.

Tests	Growth	Tests	Growth
Growth at temperature °C		10	-ve
4	- ve	13	- ve
10	± ve	Sodium azide	
15	+ ve	(0.01) & (0.02)	+ve
20	+ ve	Phenyl ethanol	
37	+ ve	(0.01) & (0.03)	- ve
45	- ve	phenol (0.1)	- ve
pH dvalues		Postassium tellurite	
4.3	- ve	(0.001) & (0.01)	- ve
7.0	+ ve	Thallus acetate	
Sodium chloride (% w/v)		(0.001) & (0.01)	-ve
4	+ ve	Crystal violet (0.001)	- ve
7	+ ve		

+ ve ≡ Show growth

- ve ≡ Fail to grow

Table (17): Characteristic properties of *Streptomyces* (T_{118}).

Characteristics	*Streptomyces* spp. T_{118}
Morphological characteristic	
- Spore chain	Spiral
- Spore mass	Gray red
- Spore surface	Smooth
- Motility	non-motile
- Substrate mycelium	Yellow to brown
- Diffusoible pigment	-
• **Cell wall hydrolysate**	
- DAP	LL-DAP
- Sugar pattern	Not detected
• **Physiological&Biochemical Characteristics**	
- Lecithinase activity	+
- Lipolysis	+
- Pectin hydrolysis	+
- Nitrate reduction	+
- H_2S production	+
• **Melanin pigment on**	
-Peptone-yeast iron agar	+
-Tyrosine agar	+
Growth at 45°C	-
• **Growth at (% w/v)**	
-Sodium chloride 7.0	+
-Sodium a zide (0.01%)	+
-Phenol (0.1)	-
-Potassium tellurite (0.001)	-
-Thallous acetate (0.001)	-

4.5. Production, extraction, purification and characterization of the anti-microbial product produced by *Streptomyces violaceus* T_{118}.

4.5.1. Effect of certain factors on the anti-microbial product produced by Streptomyces violaceus T_{118}.

4.5.1.1. The effect of different incubation periods on growth rate and anti-microbial activities of Streptomyces violaceus T_{118}.

As it's clear from the data presented in Table (18) and Fig. (10). The growth of *S.violaceus* T_{118} was appeared after two days of incubation, then gradually increased until 6 days of incubation at which the maximum growth was obtained (0.53 gm / 50 ml medium). The growth was slightly decreased after this period reaching 0.23 at 9^{th} day. Significant difference among the incubation time and the bio-activities of the anti-microbial product (inhibition zone) was observed.

With regarded to anti-microbial product by *S.violaceus* T_{118} , it was observed from the data in Table (18) that, at the first and second day of incubation there was no anti-microbial activities, while it was started at a low amount causing inhibition zone of 0.4 cm, at the third day of incubation, then it increased to reach a maximum value of 1.8 cm at 6^{th} day incubation.

Table (18): Effect of incubation periods on growth rate and anti-microbial activities of *Streptomyces violaceus* T_{118} against *Botrytis fabae* grown on PDA medium at 20 ± 2°C

Incubation time[a]	Growth (dry weight)[b]	Inhibition zone[c]
1	0.0	0.0
2	0.032	0.0
3	0.093	0.4
4	0.25	0.8
5	0.39	1.30
6	0.53	1.80
7	0.48	1.53
8	0.35	1.30
9	0.23	0.80
(L.S.D. 0.05)	0.027	0.174

(a) Incubation time in days.

(b) Growth dray weight gm / 50 ml medium.

(c) Mean diameter of inhibition zone in cm.

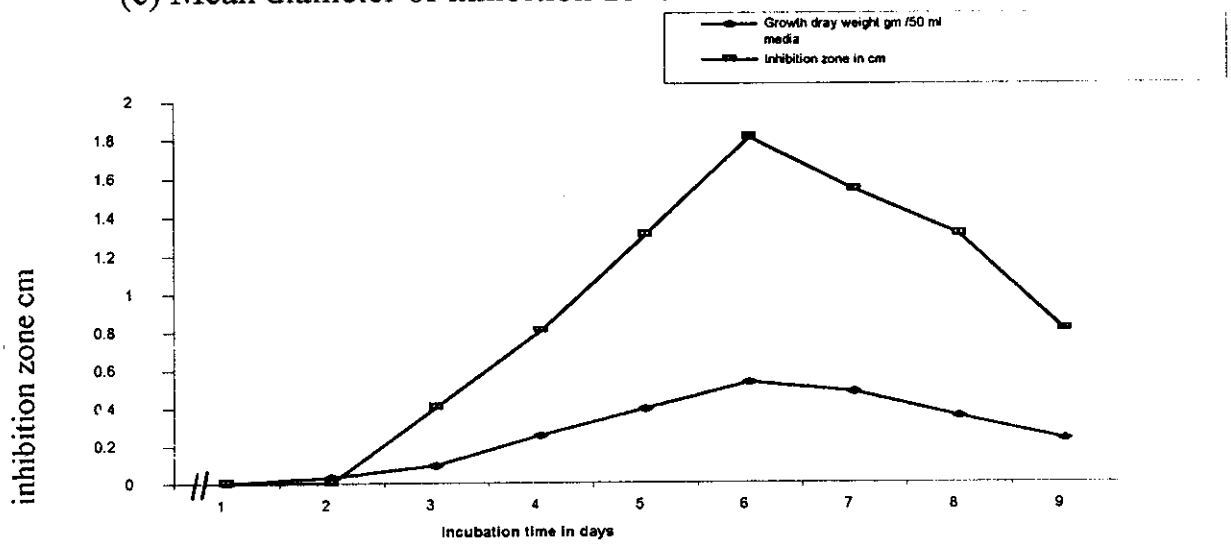


Fig. (10): Effect of incubation periods on growth rate and anti-microbial activities of *Streptomyces violaceus* T_{118} against *Botrytis fabae* grown on PDA medium at 20 ± 2°C

4.5.1.2. ***The effect of different Hydrogen ion concentrations (pH) on growth rate and antimicrobial activities of Streptomyces violaceus T_{118}.***

The data in Table (19) and Fig. (11) showed that, the hydrogen ion concentration play an important role for growth of *S.violaceus* T_{118} and their bio-activity against *Botrytis fabae.* At pH value of 3.0 and 4.0 there was no growth, while at pH 5.0 the growth was shown (0.32 gm / 50 ml medium) and also the bio-activity against *Botrytis fabae* (expressed as inhibition zone) was detected (0.87 cm). Significant difference was shown between the used pH degrees. However the maximum growth and the maximum anti-microbial activities (expressed as inhibition zone of *B.fabae*) were obtained at pH 7.0 of which the growth was 0.63 gm / 50 ml medium and inhibition zone 1.88 cm. The growth and anti-microbial production of the studied organism were gradually decreased at pH higher than 7 until disappeared completely at pH 10.

Table (19): Effect of different hydrogen ion concentrations (pH) on growth rate and anti-microbial activities of *Streptomyces violaceus* T_{118} against *Botrytis fabae* grown on PDA medium at 20 ± 2°C

PH	Growth dry weight[a]	Inhibition zone[b]
3	0.0	0.0
4	0.0	0.0
5	0.32	0.87
6	0.51	1.55
7	0.63	1.88
8	0.58	1.20
9	0.42	0.90
10	0.33	0.63
(L.S.D. 0.05)	0.023	0.17

(a) Mycelium dry weight gm/50ml medium.

(b) Mean diameter of inhibition zone in cm.

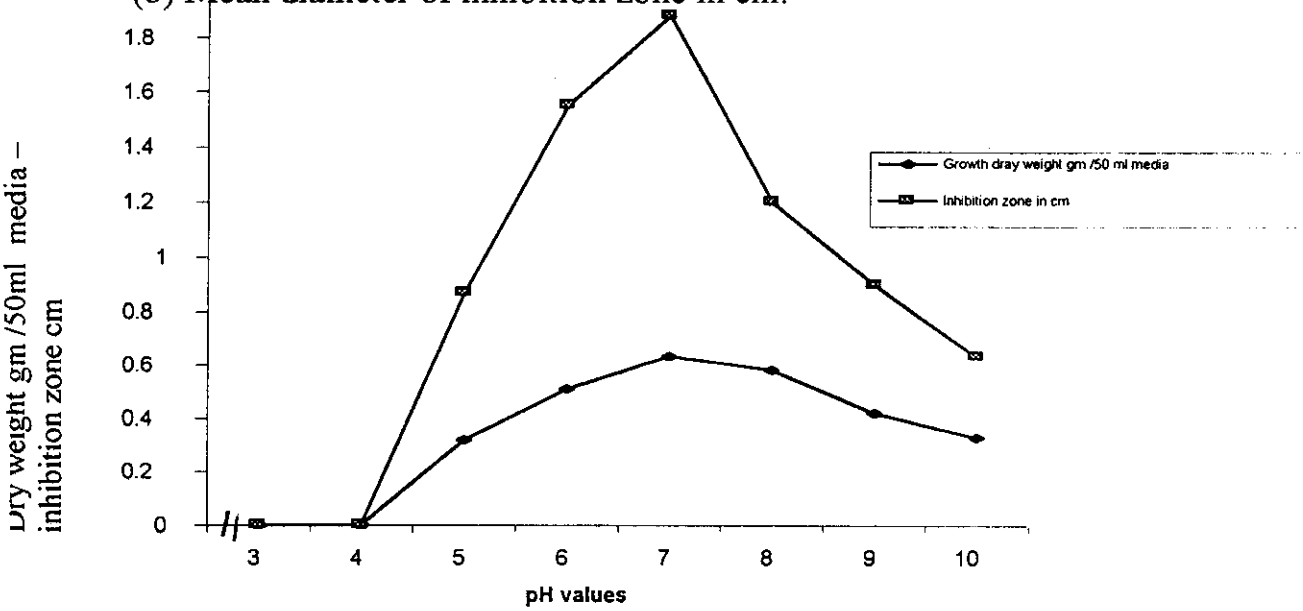


Fig. (11): Effect of different hydrogen ion concentrations (pH) on growth rate and anti-microbial activities of *Streptomyces violaceus* T_{118} against *Botrytis fabae* grown on PDA medium at 20 ± 2°C

4.5.1.3. Effect of different temperature degrees on growth rate and antimicrobial activities of Streptomyces violaceus T_{118}.

As it is shown in Table (20) the growth of *Streptomyces violaceus* T_{118}, not appeared at 10°C, while it was started to grow at 15°C then gradually increased to reach its maximum at 30°C (0.58 gm/ 50 ml medium). At 35°C the growth rate was slightly decreased (0.51 gm/50ml medium) and markedly decreased at 40 °C, at 45°C there was no growth. The results were revealed a significant difference between the temperature values.

With regard to the bio-activities of the product expressed as inhibition zone, the results represented in the same table also revealed that, at 30°C the maximum diameter of the inhibition zone was 2.0 cm while there was no inhibition zone 10°C or 45°C. From these results it was concluded that there was no growth and consequently bio-activities (inhibition zone 0.0) at 10°C and 45°C, while the optimum temperature was 30°C for both growth and bio-activity Fig. (12).

Table (20). Effect of different temperatures on growth rate and bio-activities of *Streptomyces violaceus* T_{118} against *Botrytis fabae* grown on PDA medium at 20 ± 2°C

Temperature °C	Growth (dry weight)[a]	Inhibition zone[b]
10	0.0	0.0
15	0.21	0.06
20	0.35	0.73
25	0.49	1.1
30	0.58	2.0
35	0.51	1.6
40	0.42	0.77
45	0.0	0.0
(L.S.D. 0.05)	0.02	0.171

a) Mycelium dry weight gm/ 50 ml media

b) Mean diameter of inhibition zone in cm.

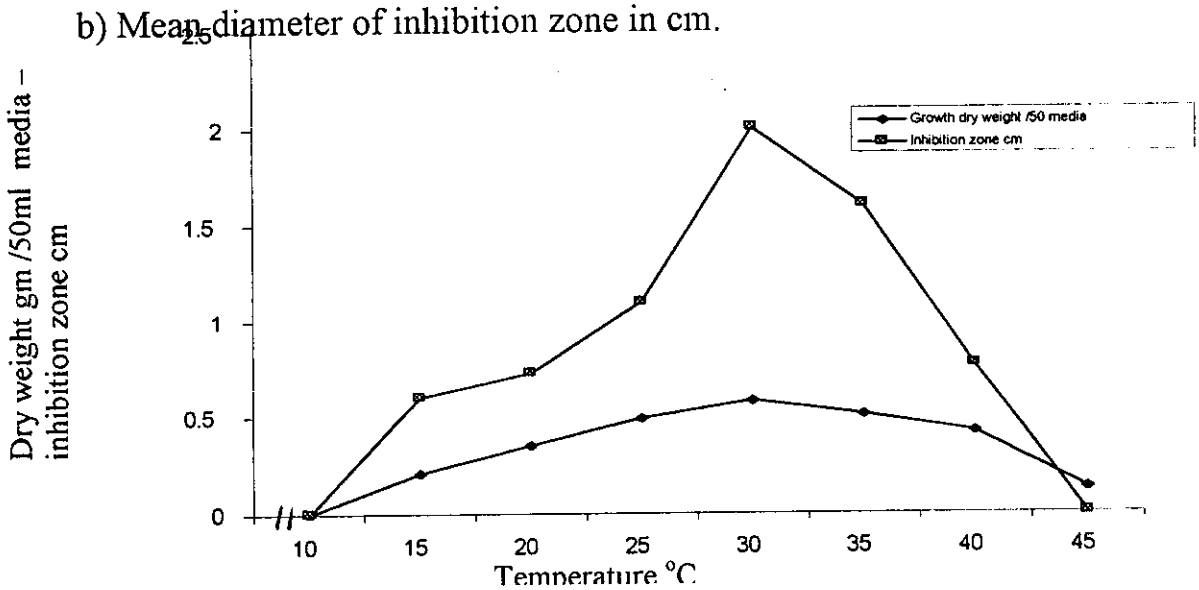


Fig. (12). Effect of different temperatures on growth rate and bio-activities of *Streptomyces violaceus* T_{118} against *Botrytis fabae* grown on PDA medium at 20 ± 2°C

4.5.1.4. *Production of the antimicrobial product in shaken cultures and fermentor.*

- ***Effect of different media on the bio-activity of the anti-microbial substance produced by Streptomyces violaceus T_{118}.***

The data presented in Table (21) and Fig. (13) revealed that, the isolate *Streptomyces violaceus* T_{118} produced an anti-microbial substance on starch nitrate medium, that inhibit the growth of Gram positive bacteria *i.e. Bacillus subtils NCIB 3160, Micrococcus leteus NCIB 190 and Rhadococcus equii ATCC 6939* with inhibition zone ranging from 13 mm to 19 mm, while it showed inhibition zone against Gram-negative bacteria *i.e. Salmonella typhie NCTC 4111 Eschericha coli NCIB 9132 and Psudomonas aeruginosa. ATCC 10145* between 10mm to 18 mm. On the other side the produce antimicrobial product suppressed the fungal growth by inhibition zone about 14mm to 27.5 mm for some fungal isolates. (*i.e. Candida albicans ATCC 10231, Geotrichum candium ATCC 34614, Fusarium solani, F. oxysporium, F.moniliforme, Botrytis cinerea and B.fabae).* It also noticed that, the product didn't show any anti-microbial activities against *Saccharomyces cervisiae ATTC 2601.* Generally among the used three media (starch nitrate, fish meal and oat meal) starch nitrate medium proved to be the best medium for the production of the antimicrobial substance.

Table (21): Effect of different media on the bio-activity of the anti-microbial product produced by *Streptomyces violaceus* T_{118}.

'est organism	[(a)]Mean diameter of inhibition zone in mm.		
	Starch nitrate	Fish meal	Oat meal
'acillus subtilus NCIB 3610	13	10	8.0
flicrococus luteus NCIB 190	19	11	8 .0
'hadococcus equii ATCC 6939	18	10	7 .0
'almonella typhi NCTC 4111	14	8.0	5 .0
'shericha coli NCIB 9132	10	7 .0	5 .0
'sudomonas aeruginosa ATCC 10145	18	11	6 .0
'andidia albicans ATCC 10231	14	9 .0	7 .0
'eotrichum candium ATCC 34614	15	8 .0	5 .0
accharomyces cervisia ATCC 2601	0.0	0.0	0.0
*'usarium solani**	19	15	12
*'. oxysporum**	27.5	20	18
*'. moniliforme**	23	15	18
*'otrytis fabae**	18	14	10
*'otrytis cinerea**	15	10	10

(a) Mean value of five replicates

* Isolated fungi during the investigation.

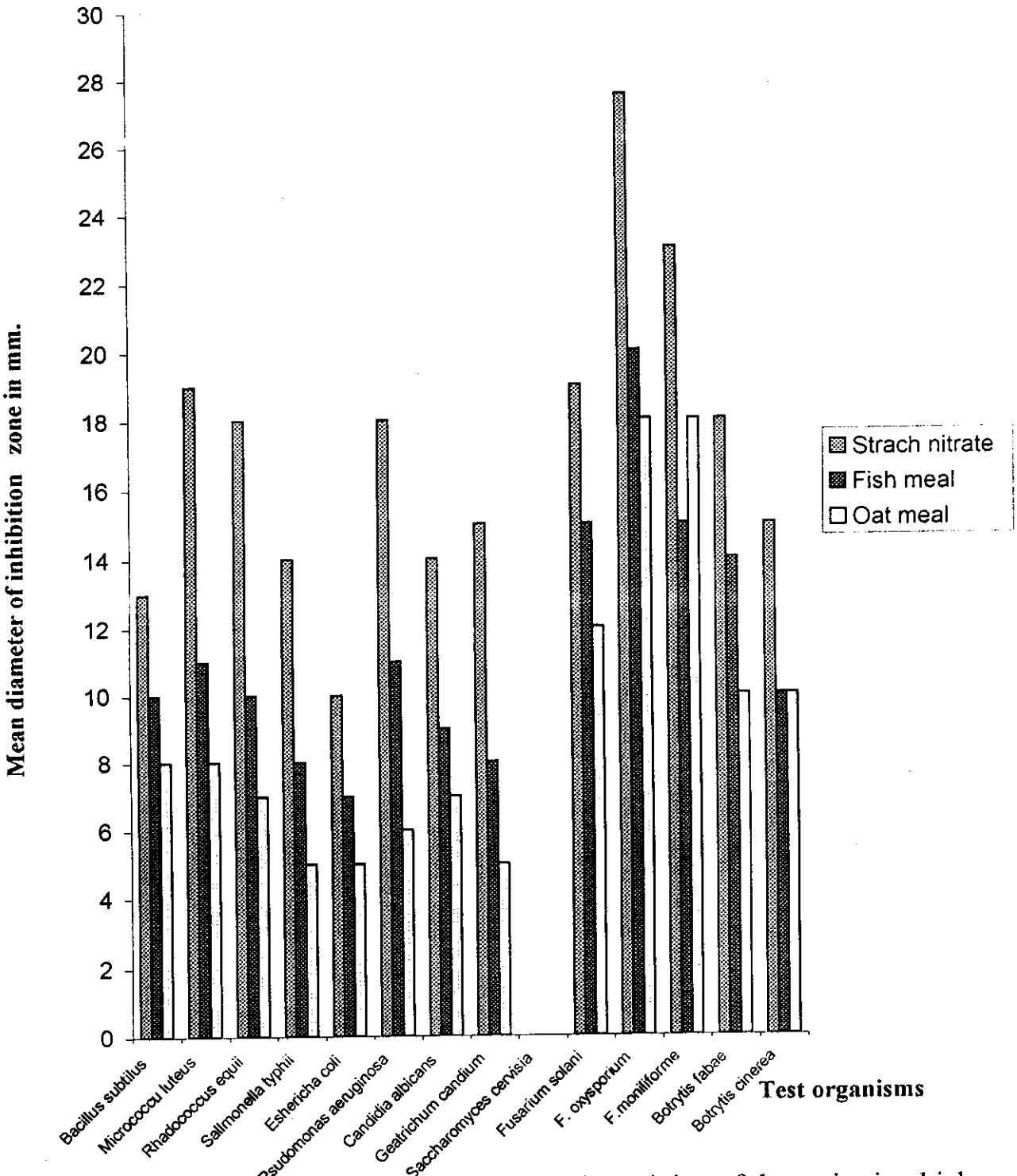


Fig. (13): Effect of different media on the bio-activity of the anti-microbial product produced by *Streptomyces violaceus* T_{118}.

4.5.2. Extraction and purification of the anti-microbial product.

4.5.2.1. The Bio-autography of the obtained anti-microbial.

It's clear from the results present in Table (22) and Fig. (14) that, the chromatographic spectrum was determined in fifteen solvent systems. Ten out of the fifteen solvent systems were positive, in which the rate of flow (R_f) ranged from 0.05 to 0.75, the highest value was recorded with ethyl acetate (0.75) followed by 0.70 with 3% NH_4Cl in water and n. Butanal acetic acid water 2 : 1 : 1 by which R_f was 0.60. The rest solvent system showed R_f value ranged between 0.05 to 0.30.

4.5.2.2. The anti-microbial potentialities of the product.

The Data in Table (23) showed that, the antibiotic produced by the tested organism (*Streptomyces violaceus* T_{118}) expressed anti-bacterial activities against Gram-positive and Gram negative with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) between 7.5 to 35 µg/ml. Also it showed antifungal activities by MIC 20 µg/ml to 100 µg/ml .

Table (22): The different rate of flow (R_F) values of the purified anti-microbial product obtained from *Streptomyces violaceus*, T_{118} culture, when biochromatograph with various developing solvents.

Solvent	R_F
1- Methanol	0.2
2- Carbon tetra chloride	0.1
3- Ethyl acetate	0.75
4- Chloroform	0.05
5- Acetone	0.00
6- Petroleum ether	0.00
7- Iso- propanol	0.00
8- Benzen	0.10
9- Water saturated with N-butanol	0.25
10- N.Butanol saturated with water	0.30
11- 3% Ammonium chloride(in water)	0.70
12- Diethyl ether	0.00
13- N.Butanol-acetic acid water 2:1:1	0.60
14- N.Butanol pyridine water 1: 0.6:1	0.10
15- N-propanol	0.00

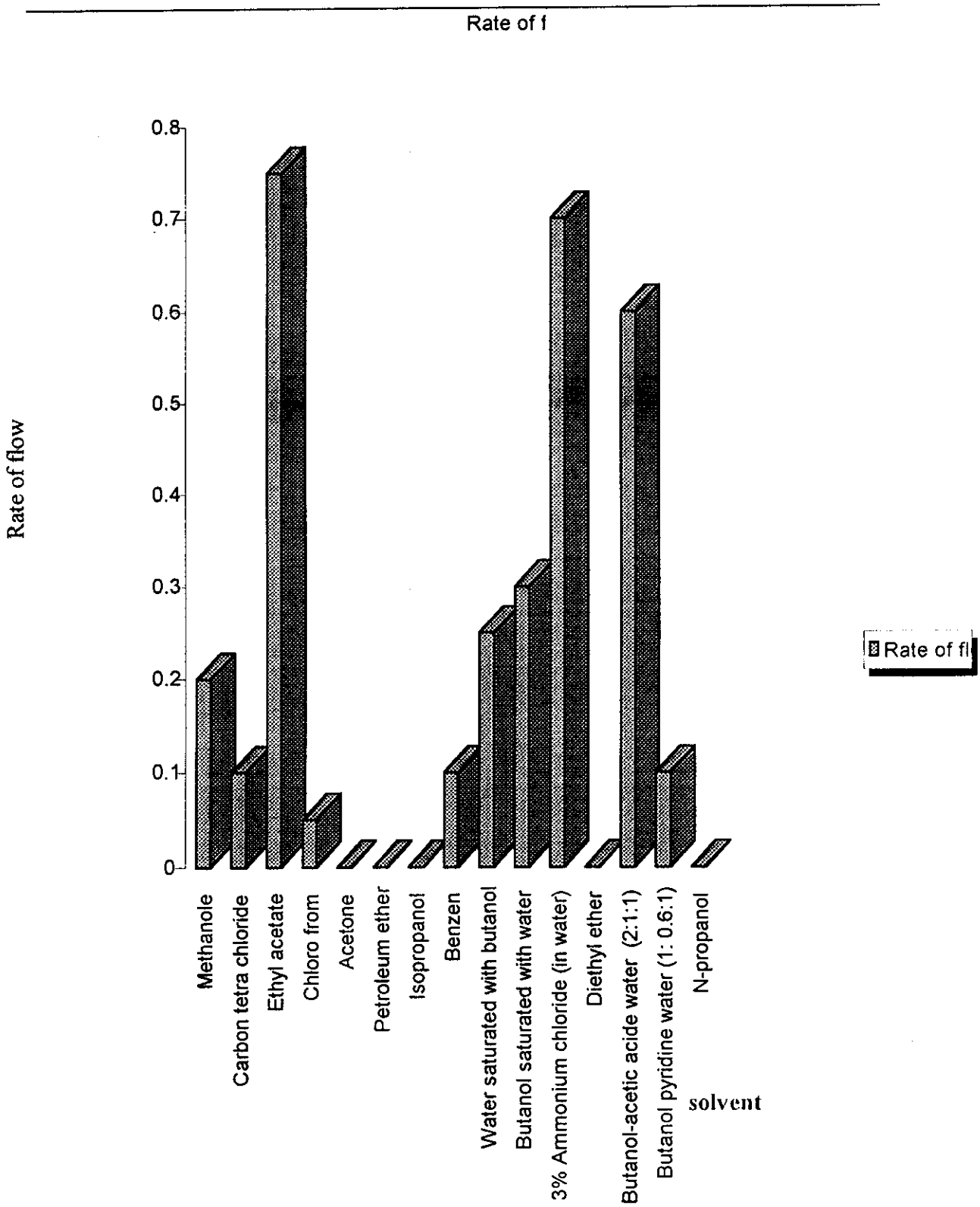


Fig. (15): The different rate of flow (R_F) values of the purified anti-microbial product obtained from *Streptomyces violaceus*, T_{118} culture, when bio-chromatograph with various developing solvents.

Table (23): The anti-microbial potentialities of the antibiotic produced by *Strepotomyces violaceus* T_{118}.

Test organism	MIC (µg/ ml)	MBC (µg/ ml)
Bacillus subtilis NCIB 3610	7.5	10
Micrococus leteus NCIB 190	10	15
Radococcus equi ATCC 6939	7.5	10
Escherichia coli NCIB 9132	30	35
Pseudomonas aeruginosa ATCC 10145	20	25
Salmonella typhi NCTC 4111	30	35
Condida albicans ATCC 10231	15	20
Geotrichum candium ATCC 34614	20	25
Botrytis fabae*	90	-
Fusarium oxysporum*	> 100	-
F. moniliforme*	65	-
F. solani*	65	-

*Isolated fungi from faba bean plants during the experimental work.

MIC = Minimum Inhibitory Concentration.

MBC = Minimum Bacericedial Concentration.

4.5.3. Control of chocolate spot disease by using the anti-microbial product produced by S. violaceus T_{118}.

- **Detached leaves**

The data recorded in Table (24) and Fig. (15) showed that, there was an inhibition effect of the anti-microbial substance which produced by *Streptomyces violacues* T_{118} when it was applicated on detached leaves. The used anti-microbial product reduced the bio-mass of the pathogen after 24 h. however many conidial germ tubes invaded the host tissues and disease symptoms appeared, symptom severity on treated leaves with *Botrytis fabae* spore suspension only (control) was high necrotic which the disease severity was developed from 2.4-8.2 during the experimental time, while the treated leaves with both *B.fabae* spore suspension and the product showed less necrotic effect (the disease severity was ranging of 1.0 to 1.6 and 0.8 to 1.4 by using 85 and 90 mg/100ml respectively). However the two concentrations didn't show significant difference between each and other Fig. (17).

- **Plants in pots.**

Table (25) and Fig. (17) show, the bio-activities of the used anti-microbial substance produced by *Streptomyces violaceus* T_{118} against chocolate spot disease on faba bean plants C.V. Giza 3 under greenhouse conditions (plants in pots). All the used concentrations succeeded to reduce the disease severity. The sporulation potential (sprouting of condiophores of *Botrytis fabae*) in the control treatment (*B.fabae* spore suspension only in addition distilled water) covered the range between 3.2 to 9.0 on leaf surface. Significant difference was clearly observed between the used concentration (100 mg/100 ml) and the rest concentrations after 21 days.

The best treatment was obtained with 100mg /100 ml which resulting the reduce disease severity of 2.0 compared with the control of which the disease severity was 9 after 21 days from application.

Table (24): Effect of antimicrobial substance produced by *Streptomyces violaceus* T_{118} on chocolate spot disease using detached leaves on cultivar Giza.3.

Treatment / Incubation time	Mean[(a)] of disease severity using scale (0-9) after[(b)]			
	24	48	72	96
85 mg / 100 ml	1.0	1.2	1.4	1.6
90 mg / 100 ml	0.8	1.0	1.2	1.4
control[(c)]	2.4	4.2	5.6	8.2

(L.S.D. 0.05) Incubation time (I) 0.4 Treatment (T) 0.4 I x T 0.7

(a) Mean value of five replicates.

(b) Incubation time in hours.

(c) *B.fabae* spore suspension in addition to distilled sterilized water.

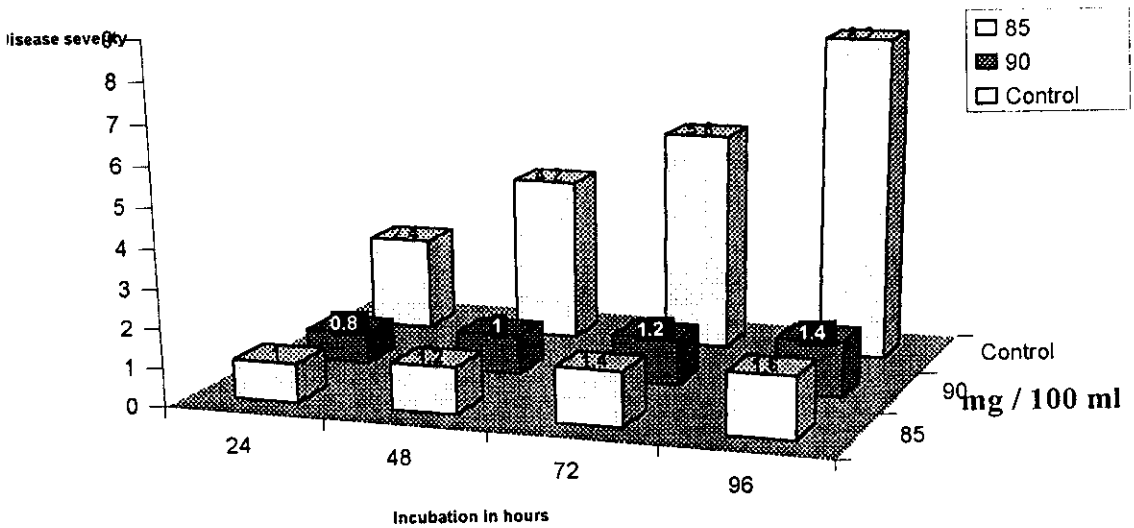


Fig. (16) : Effect of antimicrobial produced by *Streptomyces violaceus* T_{118} on chocolate spot disease using detached leaves.

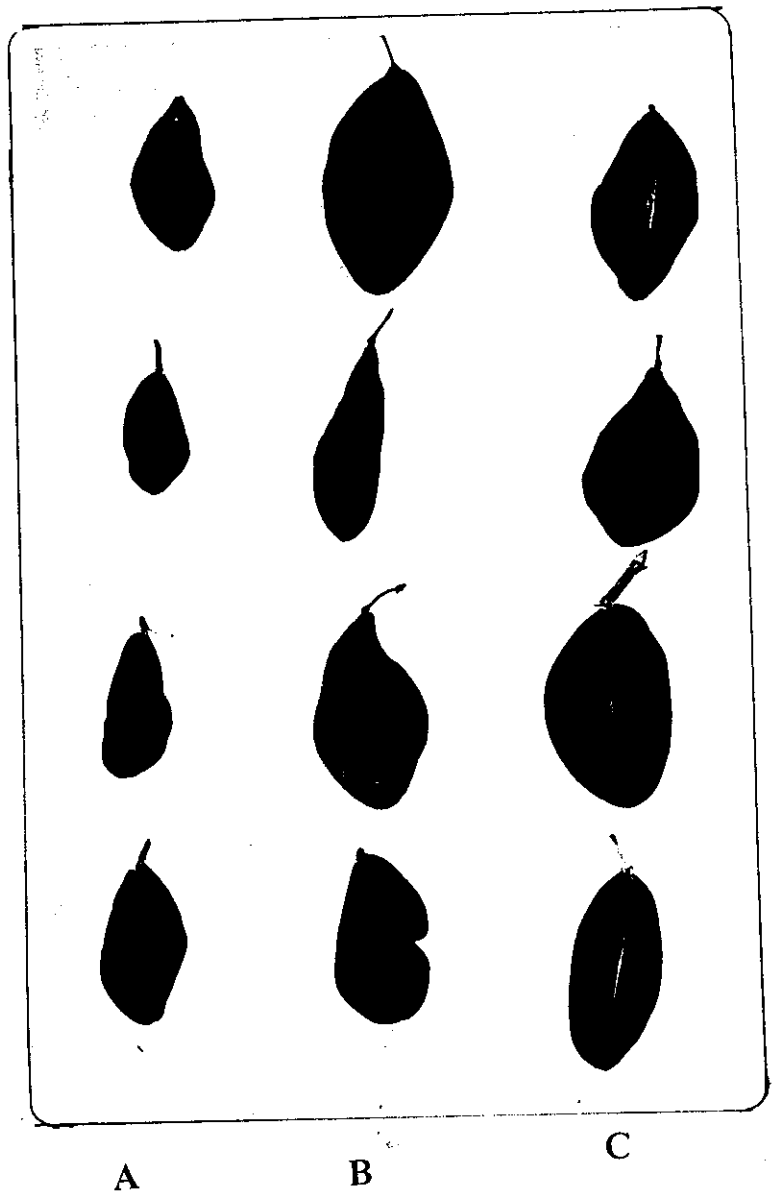


Fig. (17): Effect of anti-microbial product produced by *Streptomyces violaceus* T_{118} on chocolate spot disease (using detached leaves).

A) Control (nontreated)

B) 85 mg/100ml

C) 90 mg/100ml

Table (25): Effect of the anti-microbial product produced by *Streptomyces violaceus* T_{118} on chocolate spot disease under green house conditions.

Treatment / Incubation time	Mean[a] of disease severity using scale (0-9) after[b]			
	3	7	14	21
85 mg / 100 ml	1.0	3.0	3.2	3.2
90 mg / 100 ml	0.8	2.0	2.0	2.6
100 mg / 100 ml	0.8	1.0	1.8	2.0
control[c]	3.2	7.4	9.0	9.0

(L.S.D. 0.05) Incubation time (I) 0.3 Treatment (T) 0.3 I x T 0.6

(a) Mean value of five replicates

(b) Incubation time in days.

(c) *Botrytis fabae* spore suspension in addition to distilled sterilized water.

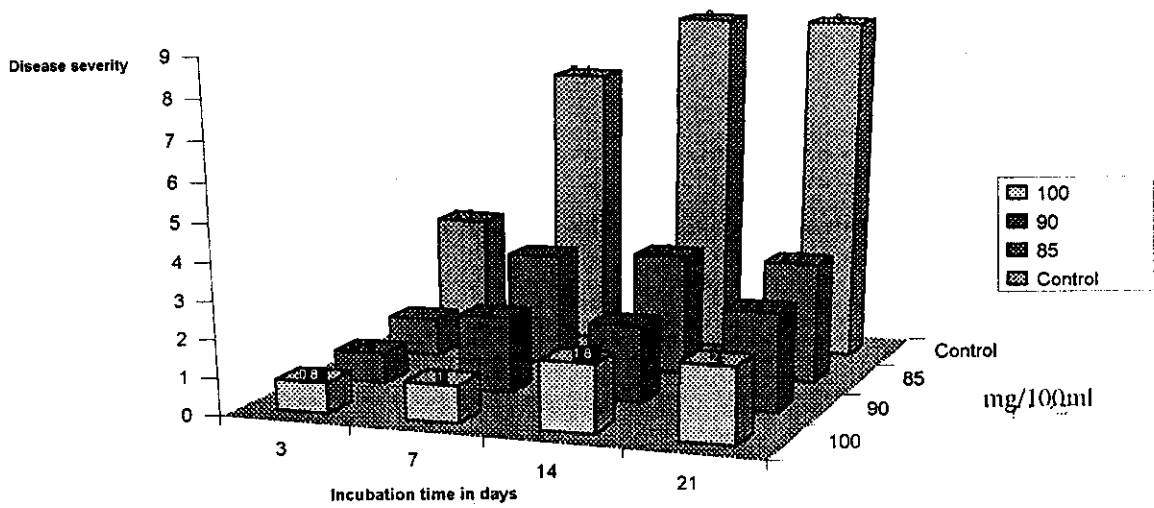


Fig. (17) : Effect of the anti-microbial product produced by *Streptomyces violaceus* T_{118} on chocolate spot disease under green house conditions.

- **The effect of the product on percentage of spores germenation and lengths of germ tubes of *B.fabae*.**

Data in table (26) indicated that , all tested antimicrobial product concentrations were significantly reduced spores germination percentage and lengths of germ tubes compared with the control . The antimicrobial product produced by *S. violacues* T_{118} completely inhibited of spores germination and germ tube development at the concentration of 100 mg / 100 ml H_2O . Also the effect on spores germination and lengths of germ tubes were decreased by decreasing the concentration of antimicrobial product.

Table (26) . Effect of antimicrobial product of *S.violacues* T_{118} on spores germination percentage and lengths of germ tubes of *B. fabae* &(after 24 hr).

Concentration Mg / 100ml	Germination of *B.fabae* spores (%)	Total length of germ tube(µm)
0.0	97.7	151.5
680	22.5	20.7
90	3.1	16.0
100	0.0	0.0
L.S.D.0.05	**1.8**	**4.4**

4.6. Characterization of the anti-microbial product produced by Sterptomyces violacues T_{118}.

4.6.1. The physicochemical characteristics of the purified anti-microbial product.

4.6.1.1. The physical properties.

The purified compound was found to be yellow powder with no characteristic odor, soluble in chloroform, n.butanol and dimethyl sulfoxide but sparingly soluble in water and acetone while insoluble in cyclohexan and peteroleum ether. Melting point was 195 ~ 198°C.

4.6.1.2. The Spectroscopic analysis of the purified anti-microbial product.

Elemental analysis of the product showed that it contain carbon 77,2% hydrogen 6.6% nitrogen 2.6% and oxygen 13.6% which agrees with the formula $C_{32}H_{38}N_2O_7$, however molecular weight was 562gm. Fig (18) illustrate the Ultraviolet data which show broad peak at λ_{max} 244 n.m., its mass spectra showed the presence of peak at λ 550 (10%), the most important peaks are m /z (abundance) 90.0 (26.97), 131.00 (4.49), 133.15 (14.61), 147.05 (64.66), 172.05 (2.25), 242.25 (2.25), 266.2 (100.00) and 390.45 (1.12) Fig. (19). The infrared spectrum I.R. of the anti-microbial product showed absorption indicating the presence of OH and/or NH groups (3417.6cm^{-1}), aromatic C-H (3020 cm^{-1}), alephatic C-H (2962 cm^{-1}), ν >C = O (1735.8 cm^{-1}) ν – CO –NH or C=N (1651.0 cm^{-1}) and ν C=C (1519.8 cm^{-1}) Fig.(20). Analysis of proton magnetic resonance (H^1 NMR) showed aromatic CH at σ 7.26 (s), 7.51 (m), 7.73 (m), CH at σ 4.2 (m), CH_2 at σ 2.2 (s), CH_3 at σ 1.36 (q) and CH at σ 0.924 (l) Fig.(21).

4.6.2. The chemical analysis of the product.

4.6.2.1. Detection of amino acids content.

It was found that, amino acids not detected in the acid hydrolysate of the used anti-microbial product.

4.6.2.2. Detection of sugar content.

It was found that, the hydrolysate of the used anti-microbial product contained non reducing glucose as a sugar moiety

4.6.2.3. Determination of proteins.

The protein measurements in the sample was 0.4125 μg/ml.

4.6.3. The behaviour of the anti-microbial product towards certain chemical tests and nomeclature the product.

Data presented in Table (27) show the behaviour of the purified anti-microbial material produced by *Streptomyces violaceus* T_{118}. Positive results was shown with all of Molish's reaction, Sakaguchi's reaction, ninhydrin test, nitroprusside reaction and Tollen's reaction which indicate the presence of sugar moiety, arganine, free amine group (NH_2), and aromatic amine respectively. However, the remaining chemical tests showed negative result.

Table (27): Remarks on the behaviour of the anti-microbial obtained from *Streptomyces violaceus* T_{118} culture towards certain chemical tests

Chemical	Results	Remarks
Molish's Reaction	+ ve	Presence of sugar moiety
Sakaguchi's Reaction	+ ve	Arganine is present
Ninhydrin test	+ ve	Free-NH_2 groub is present
Nitroprusside Reaction	+ ve	Amines is found
Millon's Reaction	-ve	Tyrosine is absent
Ferric chlorid Reaction	- ve	Di-ketanes or enalic group aren't present
Fehling Reaction	- ve	Free aldhyde and / or keto sugare are absent
Mayer's Reaction	- ve	Nitro group is absent
Tollen's Reaction	+ ve	Aromatic amine is present
Lead sulphide reaction	-ve	Amino acids containing sulpher are absent

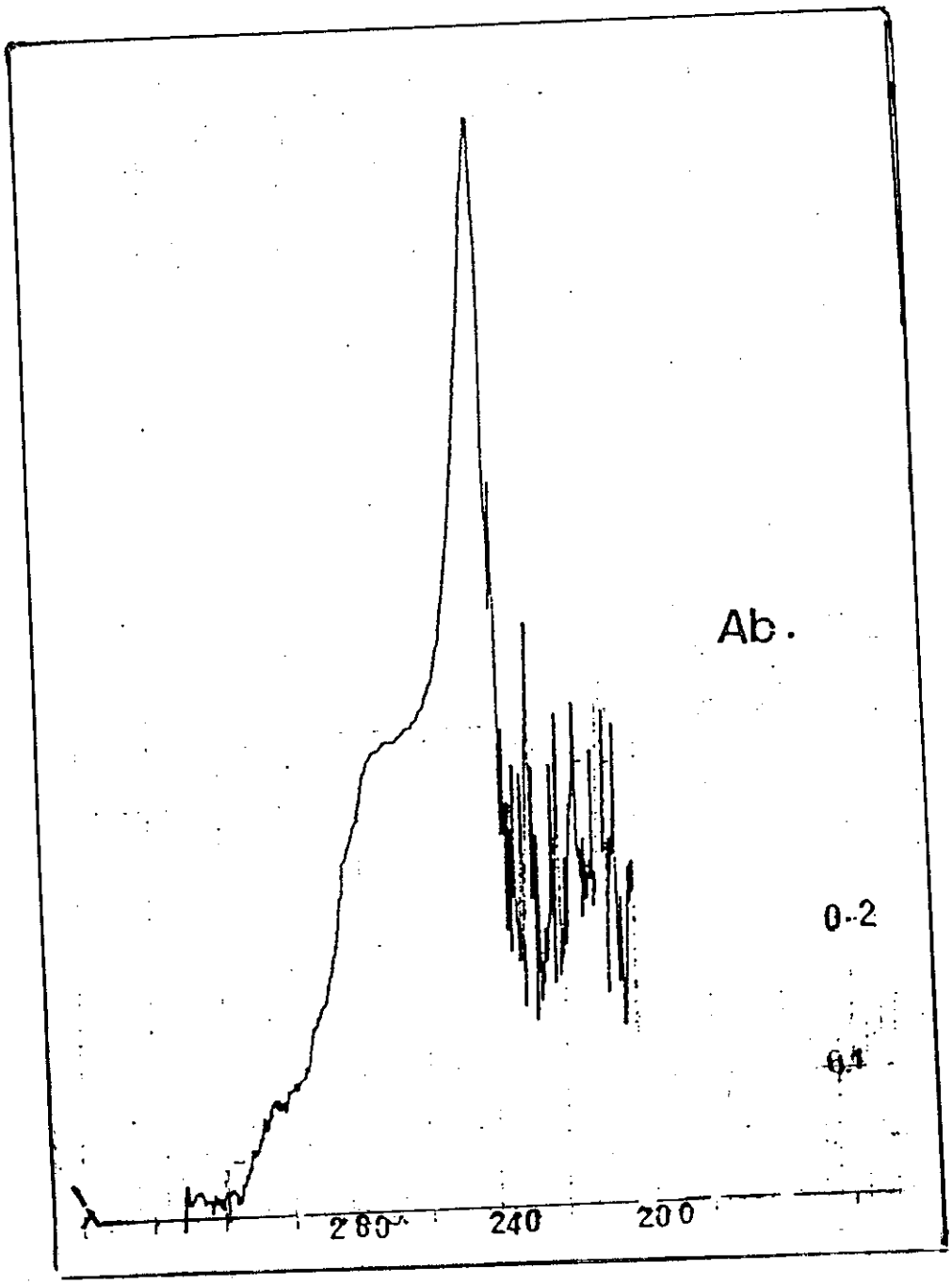


Fig. (18): Ultraviolet spectrum of the antibiotic produced by *Streptomyces violaceus* T_{118}.

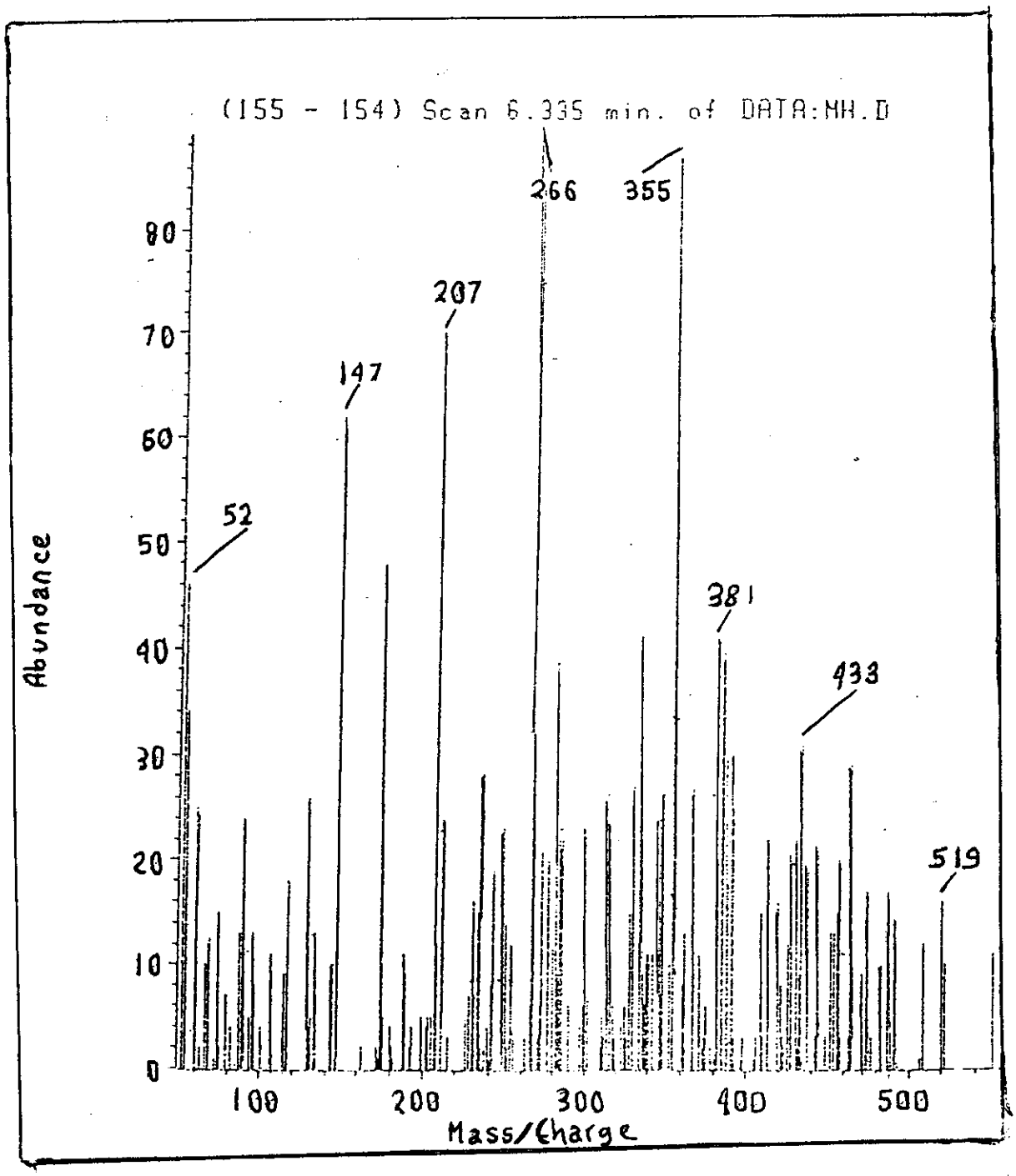


Fig. (19): Mass spectrum of the antibiotic produced by *Streptomyces violaceus* T_{118}.

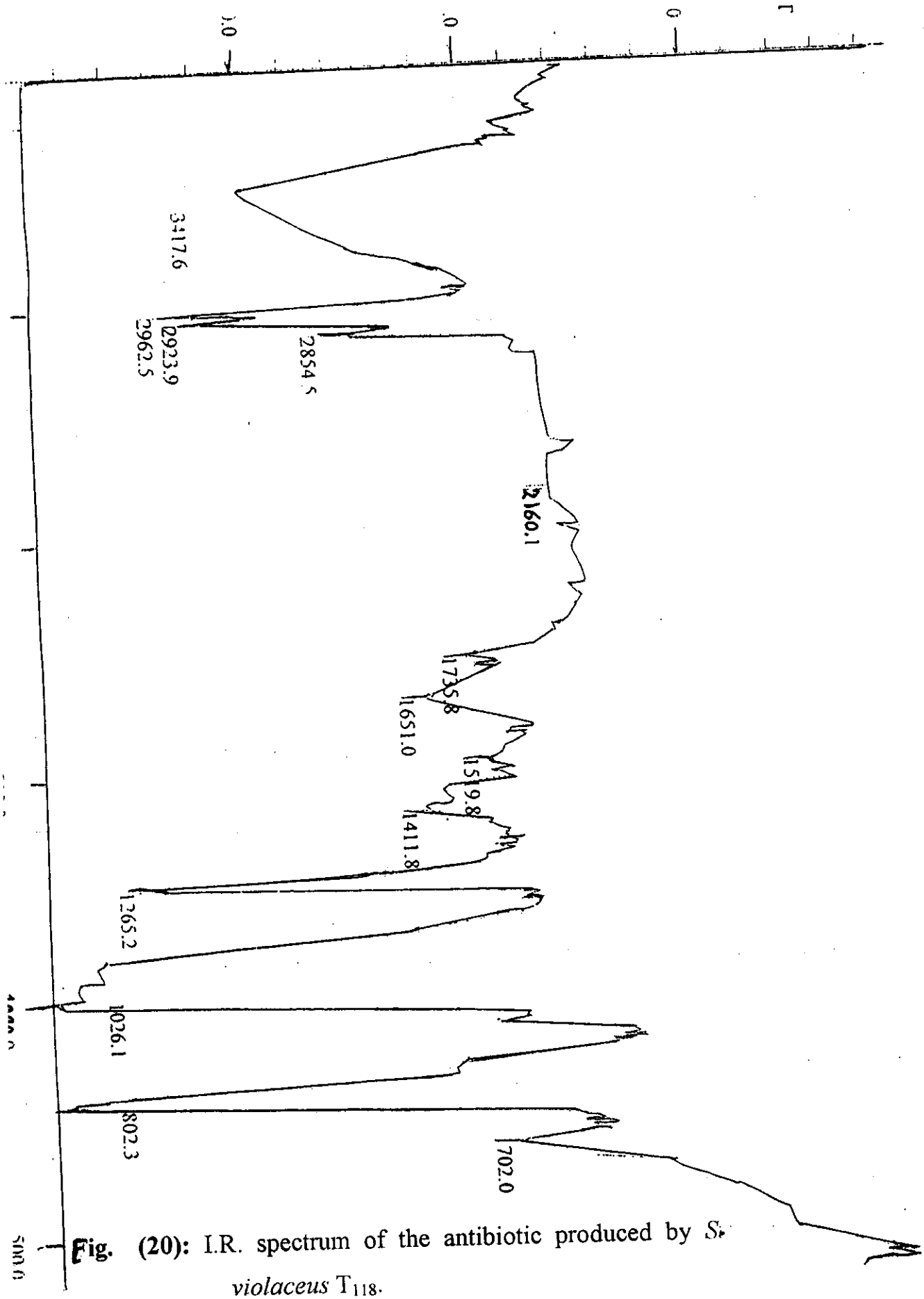


Fig. (20): I.R. spectrum of the antibiotic produced by *S. violaceus* T_{118}.

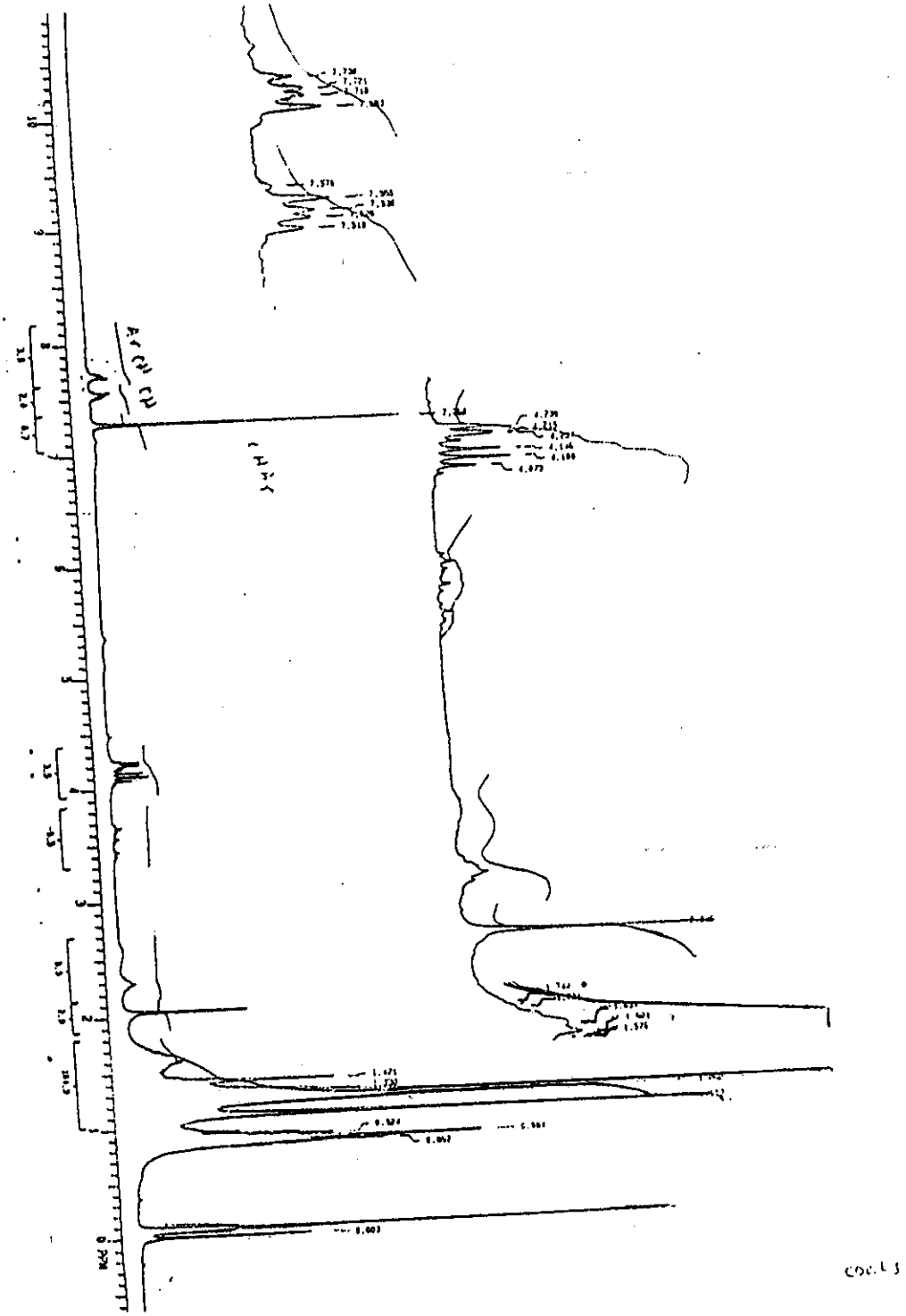
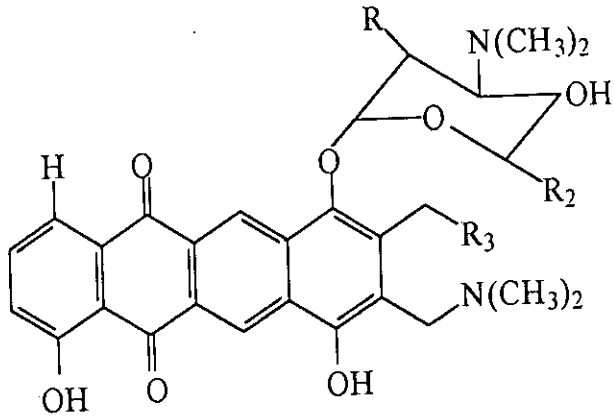


Fig. (21): Proton nucleus magnetic resonance of the antibiotic produced by *Streptomyces violaceus* T_{118}.

- **Nomenclature the produced antimicrobial compound.**

.According to both chemical and physical analysis it may be classified as anthracyclines compound which named Anthracyclin T_{118}.

From the listed data of the spectrum analysis the proposed chemical structure of such compound illustrated by Fig. (22).



$R_1 = H$

$C_{32}\ H_{38}\ N_2\ O_7$ (M.wt. 562gm). R_2 & $R_3 = CH_3$

Fig. (22). Anthracyclin T_{118}

DISCUSSION

4-DISCUSSION

Faba bean (*vicia faba* L.) is considered one of the most important and economic food legume crop in Egypt. It's important is mainly due to its highly protein value (about 28%). The increase in the consumption from year to anther needs corresponding increase in the production of faba bean. The aerial parts surfaces of the plants are subjected to fluctuations as temperature, radiation, relative humidity (RH), surface wetness, gases and air movement, ***(Burrage, 1971).*** These conditions may effect on modifying leaf characteristics, *e.g.* metabolic state, morphology ***(Cutter, 1976)*** and surface chemistry.***(Hallan & Juniper, 1971).*** On the plant surface nutrients are necessary for the growth of saprophytes and pathogens that have an epiphytic growth phase before penetration ***(Sol, 1967).***

Chocolate spot disease on faba bean can be caused by either *Botrytis fabae* or *Botrytis cinerea* ***(El-Helaly, 1938).*** However *B.cinerea* causes epidermal infection, while *B.fabae* causes necrosis of the mesophyll ***(Leach, 1955 & Harrison,1983).*** Chocolate spot disease caused by *Botrytis fabae* is considered the most destructive agent which causes highly crop losses, about 22-25% reduction of the seed yield **(*Khalil et al.*, 1984).**

The procedure for the isolation of microorganisms from its habitat varies with (a) the nature of the microorganism and (b) the number of germs relative to the other microbes within the habitat. Isolation of actinomycetes requires other enrichment and/or use of more or less selective media. The methods used for the isolation of streptomycetes

3- Encouragement of the development of streptomycetes on isolation plates by choosing carbon and nitrogen sources preferred by these organisms.

4- Inhibition of the accompanying flora by the incorporation of selective substances into the nutrient agar used for isolation ***(Kützenr,1981).***

5-

In the course of searching for microbial fungicide degrades. The work began by the isolation and purification of microbes which are capable of growing on fungicide as a secondary substrate, form different soil samples collected from different locations. All microbial isolates obtained during this part were isolated on specific isolation media containing utilizable carbon source in addition to fungicide. During this work we have been isolated so many different microorganism from the used soil samples these micro-organisms tested their capable of growing in the presence of fungicide . No microbe can grow on fungicide as a primary substrate, on the other hand the number of microbes that managed to grow on fungicide as a secondary substrate was relatively smaller than expected which showed small number of both bacteria and yeast, this may due to the toxic effect the Cupper of fungicide (CuOH), However both isolated actinomycets and fungi were recorded highest number, that could be due to their ability to bio-degradation which referred to as "cometabolism" due to their enzymatic system and their physiological properties and/or detoxification process ***(Dalton &Stirling,1982).*** Also it may results in tolerance to fungicide which may be a result of one or a few stable mutations in the genome ***(Yourman & Jeffars, 1999).***. Actinomycetes isolates exhibited growth on fungicide as secondary substrate this may due to detoxification process" ***(Bohonos et al ,1977).***

During the isolation from faba bean leaves the domenant fungi were *Alternaria alterenata* and Botrytis spp. classified into *B. cinerea* and *B. fabae* type while the rest were *fabae* type.

Results show differences in virulence among the isolates of *B.fabae* and *B.cinera*, which agree with ***El-Helaly (1938), Naguib (1948) and Abou-Zeid et al., (1985),*** they have been mentioned that chocolate spot disease of faba bean can be caused by both *Botrytis* types. However the spots caused by *B.cinerea* is localized on the epidermal cells while ones caused by *B.fabae* are mesophyll necrosis ***(Leach, 1955*** and ***Harrison,1983)*** that in agree to ***Abou-Zeid et al., (1990 & 1998)*** they reported that *B. fabae* is the fingus causes the most destructive value and the most virulent. Moreover, the pathogenic behavior of both two types is similar under dry condition, where lesion accrued by the two types remain non aggressive and localized ***(Wilson, 1937)***.

Both *Botrytis* spp. cause chocolate spot under field conditions but *B.fabae* isolates were the most virulent and aggressive thane the isolates of *B.cinerea*, that agree with ***Harrison(1988),*** he found that chocolate spot disease under field conditions caused by *Botrytis* spp. but *B.fabae* seemed to be more pathogenic and more frequent than *B.cinerea*, *B.fabae* isolated from plants growing in Nubaria was the most destructive among the *fabae* type that agree with ***Abou -Zeid; et al., (1998)*** they found a differences in virulence among isolates of B. fabae where isolates obtained from Nubaria were more virulent than those obtained from other governorate, may be due to the high humidity at leaf surface (due to geographic climate), thereby this isolate produce toxins which diffuse throughout the leaf tissue, that also agree with ***Harrison (1988)*** he has reported that, phytotoxein produced in bean tissues infected with *Botrytis*

are phytotoxic. The toxins produced by *B.fabae* in chocolate spot lesion may diffuse throughout the leaf at high humidity, while it concentrated between the infected area and the edge of the lamina at lower humidity, also at low humidities desiccation of the flaccid cells surrounding the infected zone of the infected zone itself may inhibit fungal growth. The cells surrounding the infected tissue may be die but only became partially dehydrated, thus reducing the growth rate of the fungus. Alternatively, toxins may have killed cells at infection site and other substances from these dead cells may have diffused out words and caused the flaccidity, after inoculation when lesions have become well established toxin levels within the uninfected leaf tissues surrounding a lesion may be high enough to suppress phytoalexin production.

In the present investigation, the varietal reaction of some faba bean cultivatrs against *B.fabae* was studied on G3, G40 and G716 which are moderate resistant, susceptible and resistant, respectively such results are agree with ***Abou-Zeid et al., 1998.*** They reported that G716 (resistant) were the less in average disease severity, while G 3 cultivar was moderate in average disease severity to chocolat disease.

Biological control with an organism introduced only once or occasionally, would be the ideal biological control since suppression of the target pathogen would be more or less permanent within the agroecosystem (s) where the organisms is released.

In recent years, there has been spectacular development in using an alternative non toxic means of plant disease control. Biological control of folaiar fungal pathogens involved several strategies such as.

a) Microbial suppression of infection, the major of naturally occurring as well as introduced yeasts or hyphal fungi seems to removal of infection stimulating exogenous nutrients.

b) Microbial suppression of pathogen sporulation. Suppression of the dissemination of the pathogen will reduce the progression of epidemics, this approach allows a long interaction period between the antagonist and the pathogen and is successfully applied in the control of mildews and rusts using mycoparasites.

c) Microbial suppression of pathogen survival. Mycoparasites may interfere with the formation and vitality of sclerotia in infected above ground plant tissue and crop remains. Depending on the importance of initial inoclum, treatment of infected plant material may reduce the severity of the disease ***(Cook & Baker ,1983* and *Cook,1993).***

Therefore the objective of this study was to reduce chocolate spot disease severity and hence the amount of losses in faba bean seed yield. Many methods have been used for such purpose as chemical treatment with Dithane-M.45 ***(Abou-Zeid et al., 1981),*** by production of resistant cultivars ***(Abou–Zeid et al., 1986)*** or using other alternative bio-control agents (***Abou-Zeid* and *Hassanein 2000).*** The practical part was began by survey for microorganisms which reduce the mycelial and bio-mass growth of the pathogen and also have the ability to tolrat the fungicide *i.e.*Bio-remidiation agent. The antimicrobial activity of the isolated actinomycetes has been classified based on their anti-microbial activity.. which show that, the isolate T_{118} of actinomycetes (*Streptomyces violaceus*) could suppress the mycelium growth of *B.fabae* on agar plate, it was the most effective isolate to reduce the mycelial growth causing the highest inhibition zone where the inhibitors substances have diffused through the medium compared to the control plates . also appeared to be

as antogonstic activities against several phytopathogenic fungi *in vitro*. Also the data illustrate the interaction between isolate T_{118} and *B.fabae* using light microscope, which represented that, the fungal mycelium appeared flaccid and collapsed at the line near the isolate, the antagonistic activity of the used isolate was due to the production of wide spectra anti-fungal secondary metabotites which diffused throughout the media these results are in agreement several investigators ***Pierson & Weller, 1994, Rosales et al., 1995, Sedra & Maslouly 1995, Yeomjurip et al., 1995*** and ***Ashour & Affify 2000.*** The data also illustrate the lysis of the mecleum in addition to the disintegration of the cell wall and released its cytoplams. Similar results were reported by ***Cherif*** and ***Benhamou (1990)***. The results are in similar to those published by ***Janisiewicz*** and ***Roitman (1988)***, who summarized that, the principal mode of action of the isolate of *Pseudomanas cepacia* appeared to be antagonism by the production of pyrrolnitrin a powerful anti-fungal compound which inhibited *Botrytis cinerea* and *Alternaria alternata*. The destructive at the tip may be due to the production of anti-fungal compound that secreted into the medium ***(Janisiewics and Roitman, 1988*** and ***Cherif*** and ***Benhamou 1990)*** also could be due to a secretion of enzymes, such as an effect suggested by ***Milling and Richardson (1995).***

Based on the data *in vitro* the actinomycetes isolates were tested against *B.fabae* on detached leaves, isolate T_{118} was the most effective one on controlling chocolate spot disease. The disease severity was not similar on faba bean leaves inoculated with *B.fabae* spore suspension with and without the isolates the first 20h., after inoculation. After 24 h. in case of isolate T_{118} the pathogen bio-mass was reduced by 100% (disease severity 0.0) while the non treated was 2.8, it may be due to the

effect on germ tube elongation and to a lesser extent on germination rate, ***(Zimand et al., 1996).***

Both the disease severity and the bio-mass of pathogen were reduced in the presence of the bio-control agent at the end of the experiment it progressed much more slowly on the treated leaves. However disease severity on the control leaves and plants were developed rapidly, this could be due to the reduction in bio-mass during the initial 20h., after inoculation also could be due to the effect of the used isolate on the activities of the pathogen, such an effect has been suggested by ***Milling* and *Richardson (1995)*** they suggested the mode of action for pyrimethanil against *Botrytis* spp. The effect could be due. *to* secretion of proteolytic enzymes that affect the pathogen's enzymes. (***Pieter et al., 1994)*** .

In general the reduction of mycelium growth and disease severity by *Botrytis fabae* attributed to suppression of spore germination due to the effect of anti-microbial product which inhibit the growth of hyphae of *B.fabae* and other fungi on leaf surfaces, we surmise that the used product is probably more active against spore germination or germ tube development and inhibiting the early perpetration of pathogen infection .Similar results were scored by ***Zhang & Yuen (1999).*** They found that the suppressive of the bacterial strain C_3 to leaf spot was active against spore germination or germ tube development and the biocontrol agent inhibitingthe early perpetration stages of pathogen infection.

Antagonists used in biological control aimed at the reduction of sporulation by *Botrytis* spp. or other nectrotrophic pathogens on necrotic leaf tissue should colonize the substrate rapidly under field conditions,

survive in the substrate during unfavorable conditions and compete successfully with the pathogen and other saprophyts when conditions become favorable for fungal growth. A high competitive ability is a prerequisite for saprophytic antagonists introduced to senscing or necrotic leaf tissue under field conditions.

Only strong competitors have the ability to colonize necrotic tissue of lesions induced and already partly colonized by the pathogen or to protect necrotic tissue from external colonization by *Botrytis* spp. ***(Köhl et al., 1995)***. Besides competition with the pathogen, the introduced antagonist has to compete with naturally occurring saprophytes ***(Sivan and Chet, 1989).*** Competition for space and nutrients is thought to control *Botrytis* spp. because the pathogen requires exogenous nutrients for germination and germ tube elongation over a period of several hours on the phyllosphere before penetrating the host plant ***(Charles & Michal 1989).*** A strong saprophytic competitive ability could be based on high enzymatic activity for substrate utilization and adaptation to harsh microclimatic conditions in the phyllosphere. Mycoparasitism has been suggested by several investigators ***(Eladet al., 1984; Chet, 1987*** **and** ***Benhamou & Chet, 1993).***

The results showed that the control of the pathogen due to "antibiosis" the product produced by the used *Streptomyces violaceus* D_{118} which showed anti-microbial activities, that was agree with ***(Lewis & Papavizas, 1985; Claydon, et al., 1987*** **and** ***Edwards & Sedden, 1992)***,they have found that, the control of the pathogen may due to antibiosis *i.e* production of inhibitory compound or antibiotics. Also suppression of sporulation and the effect of extraneous substances, leaf diffusats, antagonistics action and/or induced resistance ***(Köhl,et al., 1992, and Pieter, et al., 1994).*** However effective bio-control may

(Bergey's ,1994) so the species of *S.violaceus* is the nearest species to the present isolate D_{118} and it was suggested as a variant of *S.violaceus* vary in growth at NaCl 7%, phenol (0.1%), potassium tellurite (0.001%) and thallous acetate (0.001%), also *S.violaceus* is resistant to penicillin G (10µl). According to the obtained results, previously recorded for *Streptomyces violaceus*, its considered to be a variety and it was given the name *Streptomyces violaceus* T_{118}.

In the present work, an attempt was carried out to determine the optimum conditions (incubation period, incubation temperature, and pH value) and different media as factors for maximum production of the anti-microbial agent. Streptomycetes producing antibiotics are cultivated at temperature ranging from 26 to 30°C, although some streptomycetes may grow at lower temperatures (from 0 to 18°C) and also some streptomycetes grow at high temperatures (55-60°C). Deviation of temperature from the optimum range will effect on the microorganism growth ***(Ahmad, 1990).***

This can be explained by the substantial effect of temperature on the activity of the enzyme, the activity of the transport systems, and other important physiological and biochemical function of the microbial cell, when a penicillin producing strain was grower at 30°C and shifted to 20°C, ***(Owen* and *Johnson, 1955).***

Therefore, searching for the optimal incubation temperature for production high yield of antibiotic from microorganisms still needs further investigation. Hence, the following deals with the effect of different incubation temperature on the biosynthesis of antibiotic by actinomycete isolate . Streptomycetes producing antibiotics are cultivated at temperature ranging from 26 to 30°C, although some streptomycetes may grow at lower temperatures (from 0 to 18°C) and also some

streptomycetes grow at high temperatures (55-60°C). Deviation of temperature from the optimum range will effect on the microorganism growth ***(Ahmad, 1990).*** This can be explained by the substantial effect of temperature on the activity of the enzyme, the activity of the transport systems, and other important physiological and biochemical function of the microbial cell, when a penicillin producing strain was grower at 30°C and shifted to 20°C, ***(Owen* and *Johnson,1955).***

Therefore, searching for the optimal incubation temperature for production high yield of antibiotic from microorganisms still needs further investigation. Hence, the following deals with the effect of different incubation temperature on the biosynthesis of antibiotic by actinomycete isolate, in this work we used three different media to investigate which is the best for anti-microbial production .Starch nitrate was the best one to support highly anti-microbial production, that was agree with ***Hussein et al ,1998*** ,they used starch nitrate for antibiotic production by isolate 97 in shaken flasks .For the anti-microbial agent biosynthesis, *Streptomyces violaceus* T_{118}, was grown in a sproulating medium .

The active metabolites were extracted by counter current distribution using different water immasbial solvents at various pH values. From the tested solvents it was found that n-butanol the most efficient for the anti-microbial extraction

Bio-autographic technique was used for the detection of the active spot and to determine the R_F values of the antibiotic mobility on paper strip chromatograms using different solvent systems the syrup showed one definite inhibition zone and this indicate the antibiotic is one component. The antimicrobial compound isolated from a cultures broth of Streptomyces violacevs T118 have been dermined by U.V. HNHR, IR and mass analyses which showed the compound is related to macrolide

antibiotics, the proposed structure of the compound closely resemble anther a cycline-Members of anthracycline family of antibiotics are structurally anthracyclinone glycoside in which the glycosidic-specific aminosugar is linked at C-7 or C-10 or both.

Spectrophotometry, chemical characteristics such as acid hydrolysis, and chemical reaction of its active specific groups as well as its biological activities were studied. Among the main characteristics of the obtained antibiotic, one can enumerate that it is freely soluble in chloroform, n-butanol and dimethyl sulfoxide. It is sparingly soluble in water and acetone but insoluble in petroleum ether, n-hexan, benzene and carbon tetrachloride. It's yellow colour with no characteristic odaur and melting point (M.P) 195~198°C.

It gives positive reaction with Molish's reagent indicates the presence of sugar moiety, but not free sugar where Fehling reaction gives negative result. Ferric chloride reaction gives negative result indicates the absence of diketons or enolic group. It is of positive Tollen's reaction that indicates the presence of aromatic amines.

It gives positive reaction with ninhydin test, that indicate the presence of NH_2 group, and Sakoguchi's reaction, that indicate the presence of Arganine but negative Millon's reaction and lead sulphide reaction which means that it does not contain amino acids, and/or peptides. Therefore, the obtained antibiotic is related to an aromatic or cyclic compound with sugar moiety. By carrying out the spectrophotometry the results showed only one sharp band at λ 244 nm.that also in agree with bioautgraphy results which revealed the product is only one compound. Whereas the inspection of the IR

SUMMARY

SUMMARY

Some of fungicideshave been successfully used for controlling some plant disease but due to their harmful effect in environmental pollution, their toxic effect on human body and carcinogenic diseases, it became dangerous-on man health-for using. In the last periods more efforts have been made for producing new substances instead of these chemical substances (pesticides) via using some micro-organisms which have the ability to produce some antibiotic for controlling some plant diseases. It was found that, the biological control, is the best mean that replace the chemical control, but it was needed more investigation. The present work deals with isolation of some microorganisms from Egyptian soil and study its potentiality for producing some antibiotics which affect on the growth of some fungi that causes some disease to faba bean plants. The examination of the ability of these microorganisms on growth in the presence of certain concentrations from the fungicide (koside 101) which used for controlling some foliar fungal disease of some agricultural plants as faba bean which used by 0.20 %.was also studed.

In this research some microorganisms were isolated from three regions of Egyptian soil and purified, and also from faba bean plant which cultivated in different regions of Egypt.

The causal agent of chocolate spot disease was also identified as it is a type of fungi. The study also extended to make the pathogencity test for the isolated fungi on three faba bean cultivars Giza 40, G.3 and G. 716. The produced antibiotic was also extracted, purified and identified.

also tested for recognized their effect on controlling chocolate spot disease.

The following results can be obtained:

(1) One hundred and fifty six colonies of microorganisms were isolated from some Egyptian soil .The identified as 94 bacterial isolates, 35 actinomycete isolates, 22 fungal isolates and only 5 isolates of yeast.

(2) It was found that in the presence of the fungicide the microorganisms was reduced of which only 8 fungal isolates and 6 actinomycete isolates were succeeded to grow.

(3) The results revealed the some isolates of actinomycetes have the ability for growing in the presence of some concentrations of the applied fungicide while the isolate T_{118} of actinomycete could grow with all the used concentrations during incubation period 3-5 days at 30°C.

(4) The causal agent of chocolate spot disease have been isolated from infected faba bean leaves and it was identified as two species of fungi related to the genus *Botrytis*, namely *B.fabae* and *B.cinerea*.

(5) It was found that *Botrytis fabae* was the more pathogenic (virulent) fungus than *B.cinerea* on the three tested cultivars (Giza 40, G.3 and G.716) it was also observed that G.40 was the highly sensitive for the disease infection.

(6) It was found that some isolated microorganisms from soil have the ability to produce substances that have anti-microbial activity against some tested microorganisms (Gram positive and negative bacteria, fungi and yeast) but the isolate T_{118} of actinomycete was shown a wide anti-microbial effect against all the tested isolates.

(7) The isolate T_{118} of actinomycete also was the best one for reduction the mycelium growth of *B.fabae* which consider the most destructive agent on faba bean plants.

(8) Taxonimical (Biological & physiological) characters shown that, the isolate T_{118} of actinomycete related to the genus *Streptomyces* which called *S.violaceus* T_{118}.

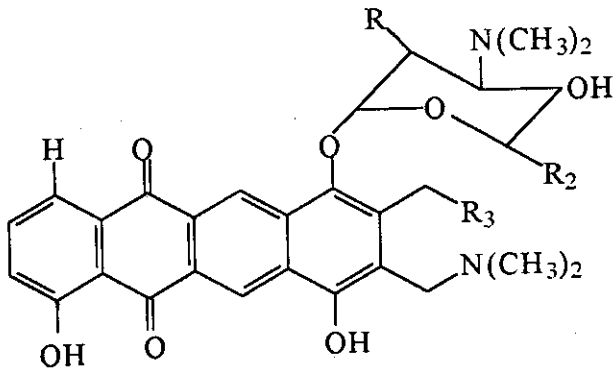
(9) The antibiotic was extracted from the culture filtrate of *S.violaceus* T_{118} after 6 days incubation, 30°C, pH 7.0 on starch nitrate medium. It was also found that, n. Butanol (immasbile water solvent) was the most effective one to extract the most part from the culture broth, it was also obvious from the bio-autograph that, the antibiotic is one compound and the highest Rf value appeared with ethyl acetate and NH_4Cl (3% in water).

(10) It was found that the extracted antibiotic from *S.violacues* T_{118} had a clear effect for controlling chocolate spot disease by concentration 85 and 90 mg/ 100 ml, it reduced both mycelium growth and disease severity (on detached leaves) and 100 mg /100 ml on plants in pots (under greenhouse conditions).

(11) The physicochemical properties of the antibiotic showed that, it was yellow colour, without characteristic odour, its melting point 195 ~ 198°C, suluble in chloroform, butanol and dimethyl sulfoxide, while sparingly soluble in water and insoluble in aceton, cyclohexan, petroleum ether and carbontetra-chloride. All of non reducing sugar (glucose), free NH_2 group and aromatic amines were present, while tyrosine, diketones or enolic group, free aldhyd, nitro group and amino acids containing sulphere were not present. The element and spectroscopic analysis (I.R., U.V. NMR and Mass spectrum) represented that, the compound may related to formula $C_{32}H_{38}N_2O_7$ with molecular weight 562 gm, which is related to microlide

compounds espically Antheracycline, so it identify as Antheracycline T_{118}.

According to both chemical and physical analysis it was classified as Anthracyclines compound .



(C_{32} $H_{38}N_2O_7$) M. wt 562 gm.

Anthracyclin T_{118}