Materials and Methods

3. Materials and Methods

3.1 Materials:

3.1.1Soil :

Soil was provided from 10 th of Ramadan area (east of Cairo). It's chemical and physical characteristics are presented in Table (1).

3.1.2 Organic manure:

Organic manure (sheep manure) was provided from Desert Research Center Station (Maruit), Alexandria. It has an organic carbon of 17.23%, total nitrogen 1.32%, C/N ratio 13.0, organic matter 29.82%, moisture content 29% and pH 7.8 as presented in Table (2).

3.1.3 Inorganic fertilizars :

Calcium ammonium nitrate (33.3% N), calcium-superphosphate (15.5% P_2O5) and potasium sulphate (48% K2O) were used as N, P and K mineral fertilizers, respectively.

3.1.4 Cultivars:

The seeds of Cucumber *(Cucumis sativus* —Pathandra f, Hybrid-Japan) were provided from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Soil texture				Coarse sand			
Sandy	Elf 0	ts'o	00'017	00 6C		St O	

Chemical analysis													
Anion meq				Cation meq j									
[illegible]		[illegible]	[illegible]		Z	to	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	E.C mmohs/cm	
						1.8					0.09	1.7	7.6

.52

Sheep manure

3.1.5. Media used

Different media were used throughout this investigation. The composition of each medium is given in gram per liter distilled water or otherwise stated, as follows:

3.1.5.1. Modified Bunt and Rovira medium (Abd El-Hafez, 1966):

Peptone	1.00
Yeast extract	1.00
Glucose	5.00
K_2HPO_4	0.40
(NH4)2SO4	0.50
KC1	0.40
MgSO4.7H20	0.50
$MgC1_2$	0.10
$FeC1_2$	0.01
$CaC1_2$	0.10
Soil extract	250 ml
Agar-agar	20.00
Tap water up to	1000 ml
pH adjusted to	6.8

This medium was used for determination of total microbial counts. The inoculated plates were incubated at 28+2°C for 7 days.

3.1.5.2. Modified Ashby's medium (Abd El-Malek and Ishac, 1968).

Mannitol	10.00
Sucrose	10.00
K_2HPO_4	0.50
$MgSO_4$-$7H_2O$	0.20
NaCl	0.20
CaS 04	0.10
CaCO3	5.00
$MnSO_4.4H_2O$	traces
$FeC1_3.6H_2O$	traces
$Na_2MoO_4.2H_2O$	traces
Agar-agar	1.5
Distilled water up to	1000 ml
pH adjusted to	7.0

It was used for isolation and determination of the most probable number (MPN) of azotobacters. The inoculated tubes were incubated at 28+2°C for 10 days.

3.1.5.3. Dobereiner's semi-solid malate medium (Dobereiner, 1978).

Malic acid	5.00
KOH	4.00
K_2HPO_4	0.50
$Mg50_4.7H_2O$	0.20
NaC1	0.10
$CaC1_2$	0.02

FeSO4.7H20	0.50
$MnSO_4$.4H20	0.01
Na_2Mo04.2H20	0.002
Alcoholic solution of-	
bromothymol-blue (0.5%)	2.00m1
Agar-agar	1.75
Distilled water up to	1000 ml
pH adjusted to	6.8

This medium was used for isolation and determination of the most probable number of azospirilla. The inoculated tubes were incubated at 28+2 °C for 7 days.

3.1.5.4. Potato dextrose agar medium (PDA) (Riker and Riker, 1936).

Potatoes extract	200.00
Dextrose	20.00
Agar-agar	15.00
Distilled water up to	1000m1

This medium was used for isolation, maintenance and purification of *Rhizoctonia solani.*

3.1.5.5. Czabek's Dox agar (Oxoid, 1982).

Sucrose	30.0
NaNO3	2.0
KH2PO4	1.0
MgSO4.7H20	0.5
KC1	0.5g

FeSO4.7H20	Traces
Agar-agar	20.0
Distilled water	1000 ml

The medium was used for isolation, maintenance and purification of *Rhizoctonia solani* and for antagonistic tests.

3.1.5.6. Starch nitrate medium (Waksman and Lechevalier 1962):

Soluble starch	10.0
KNO_3	2.0
K_2HPO_4	1.0
$MgSO_4$	0.5
NaC1	0.5
$CaCO_3$	3.0
$FeSO_4.7H_2O$	0.01
** Trace salt solution	1.0m1
Agar-agar	
Distilled water	1000m1.

Trace salt solution as adopted by **Pridham** *et aL,* (1958) is composed of 0.1 mg/L of each of the following salts: $Fe50_4$, MgC12, $Cu50_4$ and $ZnSO_4$.

The medium was used for isolation, maintenance and purification of *streptomyces* sp.

3.1.5.7. Inorganic salts - starch agar medium (Koster , 1959a):

Soluble starch	10.0 g
K2 HPo_4 (anhydrous)	1.0 g
$MgSo_4 . 7 H_2O$	1.0 g

NaCI	1.0 g
(NH4)2 Soo	2.0 g
CaCo3	2.0 g
Trace salts solution (T.S.S.)	1.0 ml
Agar agar	20.0 g
Distilled water	1.0 liter
pH	7.0-7.4

This medium used to determine the cultural characterestics of *Streptomyces* sp.

3.1.5.8. Oat meal agar medium (Koster, 1959, b):

Oat meal	20.0 g
Trace salt solution	1.0 ml
Agar- agar	20.0 g
Distilled water	1.0 Liter
pH	7.0-7.2

This medium used to determine the cultural characterestics of *Streptomyces* sp.

3.1.5.9. Glycerol asparagine agar medium (Pridham and Lyons,1961):

Glycerol	10.0 g
L-asparagine (anhydrous)	1.0 g
K_2HPO4 (anhydrous)	1.0 g
Trace salt solution	1.0 ml
Agar-agar	20.0 g

Distilled water	1.0 liter
PH	7.0-7.4

This medium used to determine the cultural characterestics of *Streptomyces* sp.

3.1.5.10.Yeast extract-malt extract agar medium (Pridham *et al*, 1956-1957):

Bacto-yeast extract	4.0 g
Bacto —malt extract	10.0 g
Dextrose	4.0 g
Agar — agar	20.0
Distilled water	1.0 liter
PH	7.3

This medium used to determine the cultural characterestics of *Streptomyces* sp.

3.1.5.11. Tyrosine agar (Shirting & Gottlieb, 1966):

Glycerol	15.0
L.tyrosine	0.5
L.asparagine	1.0
Dipotassium hydrogen phosphate	0.5
Magnesium sulphate	0.5

Sodium chloride	0.5
Ferrous sulphate	0.01
Trace salts solution	1.0 ml
Agar — Agar	20.0

This medium used to determine the ability of the *Streptomyces* sp. to produce melanoid pigment.

3.1.5.12. Peptone-yeast extract iron agar (Shirling & Gottlieb, 1966):

peptone	15.0
proteose peptone	5.0
ferric ammonium citrate	0.05
dipotassium hydrogen phosphate	1.0
Sodium thiosulphate	1.0
sodium chloride	0.5
yeast extract	1.0
Agar — agar	20.0

This medium used to determine the ability of the *Streptomyces* sp. to produce melanoid pigment.

3.1.6. Inoculants

3.1.6.1. Pathogenic fungus

R. solani isolated from rotted cucumber roots grown in Noubaria city. *R. solani* was kept on potato dextrose medium at 4°C.

3.1.6.2. Micobial fertilizers as biocontrol agents:

The most active N_2-fixing diazotrophs *Azotobacter chroococcum (Rf), Azospirillum lipoferum (ICE); Streptomyces lydicus* (N_{ew}') were isolated from 10th of Ramadan from soil under Foeniculum plants, El Khatatba from soil under cuminum plants and Noubaria from soil under cucumber, respectively. They were grown on nitrogen deficient of modified Ashby's medium **Abd El Malek and Ishac (1968)** for *Azotobacter*, **Dobereiner (1978)** for *Azospirillum* and starch-nitrate agar, **Waksman and Lechevalier (1962)** for streptomyces for 7 days and then kept them at 4-5°C for each until used.

3.2. Methods:

3.2.1:Identification of pathogenic fungus causing cucumber root rot:

* Samples of rotted cucumber roots were collected from Noubaria city.
* The infected roots were washed with tap water, surface sterilized by immersion in 1% sodium hypochloride for 2-3 minutes, then washed with a series of sterile water and dried between two pieces of filter paper.
* The infected roots were then cut into pieces under aseptic conditions and plated onto Petri-plates containting Czapek's agar medium supplemented with streptomycin to eliminate bacterial growth and incubated at 28°C for 3 days.
* Hyphae that grew from the root cuts were purified and kept on potato dextrose medium at 4 °C for identification.
* The identification of the pathogenic fungus were carried out according to **Parameter and Whitney (1970).**

3.2.2 Identification of biofertilizers inoculants:

3.2.2.1. Isolation and Purification of *Azotobacter, Azospirillum* and actinomycetes isolates

Fifteen *Azotobacter,* 10 *Azospirillum* and 11 actinomycetes isolates were picked up from the suitable three media used for *Azotobacter, Azospirillum* and actinomycetes isolation.

These 36 isolates were subjected to purification trials. All *Azotobacter, Azospirillum* and actinomycetes were purified by successive streaking on nitrogen deficient modified Ashby,

Dobereiner and starch nitrate agar media, respectively using the techniques adopted by **Abd El Malek and Ishac (1968)** for *Azotobacter*, **Dobereiner and day (1976),** for *Azospirillum* and starch-nitrate agar, **Waksman and Lechevalier (1962)** for actinomycetes.

* The Purified isolates were maintained on the same media mentioned before at 4-5°C for each until used.
* Subculturing of the purified isolates was usually done every month for *Azotobacter* and actinomycetes and 15 days for *Azospirillum.*
* Microscopical examination was carried out to make sure of the purity of cultures.

3.2.2.2. Selection and identification of the most efficient *Azotobacter, Azospirillum* and actinomycetes isolates.

- The purified twenty five *Azotobacter* and *Azospirillum* isolates were tested for their N_2-fixation activity according to the micro Kjeldahl method described by **Jackson (1958).**
- The 36 purified isolates were also tested for the antagonistic activity against the tested pathogenic fungus according to the method described by (**Waksman 1959, Hasegawa** ***et al.*** **1990),** antagonistic activity was determined by measuring the inhibition zone (mm)
- The most active isolates of *Azotobacter, Azospirillum* (2 isolates for each) in N_2-fixation as well as antagonistic activity and also 2 isolates of the most active actinomycetes in antagonistic activity were tested for their root colonizing ability

by the plate test method according to Kortemma *et al* (1994). The root colonization was calculated as the percentage of colonized root length of the total root length.

The most active isolates of *Azotobacter, Azospirillum* (one strain of each) in N_2-fixation, antagonistic activity and root colonized ability were subjected to complete identification according to their morphological and physiological characteristics using the methods described in Bergey's Manual of determinative bacteriology, 1984.

- The most active isolate of *Streptomyces* sp. in antagonistic activity as well as root-colonization ability was completely identified according to Bergey's Manual, 1974.

The Identification was based on cultural, morphological and physiological characteristics.

Media as well as methods used in this identification were described by Shirling and Gottlieb (1966).

Cultural characteristics

Cultural characteristics of the streptomyces sp. was determined as described by Shirting and Gottlieb (1966) using four media, i.e., inorganic salt —strarch agar medium (Koster 1959,a), Oat meal agar medium (Koster 1959, b), glycerol asparagine agar medium (Pridham and Lyons 1961) and yeast extract- malt extract agar medium (Pridham *et al* 1956 , 1957).

Morphological characteristics

The morphology of spore chain was determined by direct microscopic method (Kawato and Shinobu 1959) using starch nitrate agar medium (Walisman and lechevalier 1962) and the micromorphology of spore surface was determined using scanning electron microscope by the technique described by Tresner *et* a/(1961). This was carried out at Electron Microscope center, Faculty of Science, Ain Shams University.

The colour of mature aerial mycelium, the reverse side of substrate mycelium and the diffusible soluble pigments other than melanin were observed after 7.14 and 21 days. The standard media as described before in the part of cultural characteristics were also used in these tests.

were recorded as positive reaction, while the absence of these colours was recorded as negative reaction.

Physiological characteristics :

a) The ability to produce melanoid pigment:

This was carried out using two media, i.e. , Tyrosine agar medium Shirling and Gottlieb (1966). Peptone — yeast extract iron agar medium Shirling and Gottlieb (1966). Observation were made after two and four days of incubation.

The formation of diffusible greyish brown to brown black pigments or a distinct brown pigment modified by other colours were recorded as positive reaction, while the absence of these colours was recorded as negative reaction.

b) Ability to utilize different carbon sources

Basal mineral salts agar medium **(Pridham and Gottlieb 1948)** as described by **Shirling and Gottlieb (1966)** was used in this test.

Ten carbon compounds, namely, D-glucose, D-xylose, L-arabinose, L-rhamnose, D-fructosde,, raffinose, D-mannitol, i-inositol galactose and sucrose were separately added at the rate of 10 $g1^{-1}$ Medium containing D-glucose served as positive control, while medium without carbon source served as negative control.

The carbon sources were separately sterilized as follows: an appropriate weight of the carbon source was spread as a shallow layer in pre-sterilized flasks. Ethyl ether (acetone- fress) was added so as to cover the carbon source. The ether was allowed to evaporate at room temperature over night or longer. After the complete evaporation of the ether, sterile distilled water was added to make 10% w/v solution of the carbon source. The sterilized carbon source was added to the sterilized mineral salts agar medium after cooling the medium to 60°c to give a final concentration of 1%. The inoculum of streptomyces isolate was streaked on the surface of the petri dish (containing 25 ml of solid medium) After incubation for 14 days at 30°c + 2 the growth was observed and compared with the positive and negative controls.

C) Growth on Czapek –Dox agar medium

The ability of isolate to grow on Czapek — Dox agar medium **(Waksman, 1967)** was tested. The slants were inoculated and incubated at 30°c + w up to 15 days.

d) Antimicrobial activity.

- Antimicrobial activity of *Streptomyces* sp. against test organisms was carried out as Hollowing .

- G-ve Bacteria:- i.e., *Bacillus subtilis and B. cereus.*
- G+ ye Bacteria:- i.e., *E.coli and Pseudomonas flourescence.*
- Yeasts: i.e., *Candida albicans* and *Sacharomyces cerevisiae*
- Fungi: i.e., *Rhizoctonia solani* and *Aspiragillus niger.*

The antagonistic test were carried out on nutrient agar medium **(Jacobs and Gestein, 1960)** for bacteria and on potato dextrose agar medium **(Riker and Riker 1936)** for yeast and fungi

e) Sensitivity to streptomycin:

The streptomycin suplphate was added in a concentration of 4 mg ml^{-1} medium on strach nitrate agar medium **(Waksman and lechevalier, 1962).**

f) Nacl tolerance:

NaCI was added at different concentrations 4%, 7%, 10% and 13% on the starch nitrate agar medium.

- The 3 active strains of *Azotobacter*, *Azospirillum* and *Streptomyces.* were used for further inoculation studies.

3.2.3. Preparation of *Rhizoctonia solani* inoculum:

The inoculum was prepared by growing the pathogenic fungus on sterilized sorghum — sand — water (3:1:3 w/w/v) medium in 500 ml bottles, and incubated at 28 ± 2 °C for 2 weeks. The fungul inoculum was added to soil at the rate of 5% (500 g Pot^{-1})

3.2.4. Preparation of inocula:

Heavy cell suspension of the selected strains of *Azotobacter*, *Azospirillum* or *Streptomyces* was obtained by growing separately on Ashby's, Dobereiner's and starch nitrate media, respectively for 7 days at 28 ± 2 °C. Suspension of cells of *Azotobacter*, *Azospirillum* or *Streptomyces* strains containing about 10^8 cells $m1^{-1}$ was used as standard inocula.

3.2.5 Pot experiment:

- A greenhouse pot experiment was carried out at Desert Research Center (DRC) , Cairo, Egypt to study the effect of inoculation with the most efficient strains of *Azotobacter*, *Azospirillum* or *Streptomyces* individuals or as mixture of di or tri on the growth and yield of cucumber plants grown in sandy soil and on the controlling of root-rot disease causing by *Rhizoctonia solani* .
- This pot experiment was carried out using sandy soil collected from the 10^{th} of Ramadan district.
- This soil was collected from the top layer (20cm depth) air dried, ground to pass through 2 mm sieve and mixed thoroughly.
- Sheep manure was air dried, ground and milled using 2 mm sieve, to be analyzed for carbon and nitrogen contents. Sheep manure was thoroughly mixed with the experimental sandy soil for month before cultivation at the rate of 1.0% (100g Pot^{1}).

• For each treatment 12 pottery pots (40 cm in diameter) were filled with 10 Kg air dried sheep manured soil each.

• Calcium super-phosphate (15.5% P_2O_5) was added to all treatments at the rate of 150 Kg feddan^{-1} (1.5g Pot^{-1}) and mixed with the soil.

• The fungul inoculum was added to the soil at the rate of 5% (500 g Pot^{-1}). The infested soil was watered and mixed thoroughly for two weeks before cultivation to ensure growth and distribution of the inoculated fungus. Control pots were treated with the same amounts of sterilized sorghum-sandy medium without fungul inoculation.

• Seeds of cucumber were washed and immersed for 30 minutes in liquid culture of *Azotobacter chroococcum, Azospirillum lipoferm, Streptomyces* sp. strains individual or as mixture di or tri. Carboxy methyl cellulose (CMC) 0.5% was used as an adhesive agent. Seeds were then dried at room temperature for 2 hours.

• A number of ten cucumber seeds treated with tested bacteria were sown in each pot and also untreated seeds were sown by the same manner and used as control. After seedling, each pot was thinned later into 5 seedlings. Pots were directly irrigated to provide a suitable moisture for inocula.

• The amount of nitrogen fertilizer (calcium ammonium nitrate 33.3% N) was added to whole pot experiment in 3 equal parts after 15, 30, 50 days of sowing at a rate equal to 300 Kg feddan^{-1} (3 g Pot^{-1}).

• Potassium sulphate (48% K2O) was added to all pots before flowering stage at a rate equal to 100 Kg feddan (1 g Pot $^{-1}$).

• The same treatments were made in plastic cups (10 cm diameter) to record the percentage of pre and post emergence-damping off and number of survival plants 30 days after planting.

• The morphological characteristics of cucumber plants and microbiological analysis of soil were determined at intervals of 30, 50 , 70 , 90 days of planting, which represented vegetating flowering, fruiting and harvesting stages of plant growth respectively .

• Thus, all treatments used can be summarized as follows:

1- Uninoculated and uninfested soil (control).

2- Inoculated with *Azospirillum lipoferum* strains and uninfested soil.

3- Inoculated with *Azotobacter chroococcum* strains and uninfested soil.

4- Inoculated with a mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum* strains and uninfested soil.

5- Inoculated with *Streptomyces lydicus* and uninfested soil.

6- Inoculated with *Azospirillum lipoferum* and *Streptomyces lydicus* and uninfested soil.

7- Inoculated with *Azotobacter chroococcum* and *Streptomyces lydicus* and uninfested soil.

8- Inoculated with a mixture of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Streptomyces lydicus* and uninfected soil.

9- Uninoculated and infested soil with *Rhizoctonia solani.*

10- Inoculated with *Azospirillum lipoferum* and infested soil with *R. solani*

11- Inoculated with *Azotobacter chroococcum* and infested soil with *R. solani.*

12- Inoculated with a mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum* and infested soil with R. *solani.*

13- Inoculated with *Streptomyces lydicus* and infested soil with *R. solani.*

14- Inoculated with *Azospirillum lipoferum* and *Streptomyces lydicus* and infested soil with *R. solani.*

15- Inoculated with *Azotobacter chroococcum* and *Streptomyces lydicus* and infested soil with *R. solani.*

16- Inoculated with a mixture of *Azospirillum lipoferum*, *Azotobacter chroococcum* and *Streptomyces lydicus* and infested soil with *R. solani.*

3.2.6. Sampling and determinations

3.2.6.1. Physical analysis:

Soil samples were mechanically analyzed according to the methods described by Piper (1950). The electrical conductivity (EC) was measured in saturated soil paste according to method described by Jackson (1958).

3.2.6.2 Chemical analysis:

3.2.6.2.1: Soluble anions , cations and soil pH.

They were determined in saturated soil paste according to the method described by Richards (1954).

3.2.6.2.2: Organic Carbon:

It was determined in soil by the rapid titration method described by Jackson (1958).

3.2.6.2.3. Total nitrogen:

It was determined in cucumber plants by modified Kjeldahl method as described by Jackson (1958).

3.2.7. Microbiological determinations:

- Microbiological analysis of soil included the determination of total microbial counts by plating on modified Bunt and Rovira medium (Abd-El-Ralez, 1966) using the decimal plate count technique (Timinon, 1940).
- The most probable number (MPN) of azotobacters and azospirilla were determined after incubating the tubes at 28 ± 2 °C for 10 and 7 days, respectively on modified Ashby' s medium (Abd-El Malek and Ishac , 1968) and semi-solid malate medium (Dobereiner, 1978), respectively using Cochran' s Tables (Cochran, 1950).
- Determination of actinomycetes counts in soil samples was carried out by plating on starch-nitrate agar (Wakeman and Lechevalier, 1962).

3.2.8. Parameters of cucumber plants:

a) Shoots height and Roots length (cm plant $^{-1}$)

b) Fresh weight of both shoots and roots (gm/ plant

c) Dry weight of both shoots and roots: were recorded after oven drying at 70°C until reaching a constant weight (Black *et al.* 1965).

d) Chlorophyll content: was measured by using Minolta Chlorophyll meter (SPAD-502) to determine the total chlorophyll in fresh leaves.

e) Number of flowers, fruits per plant.

f) Fruit fresh weight (g plant $^{-1}$)

h) Total nitrogen and total protein percentage of cucumber fruit were determined by Kjeldahl method as described by Jackson (1958).

3.2.9. Disease Severity Index (DSI)

It was determined one month after sowing using the method described by Bull *et al.* (1991) as following scale:

1. Healthy plants, no symptoms.
2. Browning of primary and secondary roots.
3. Brown lesions of coleopotile; primary and secondary roots crown and leaves.
4. Seedlings weak; stunted and chlorotic.
5. Seedlings killed.

The disease severity index (DSI) was determined according to the following formula (**Meshram and Jager, 1983):**

$$DSI = \frac{\text{E rating}}{\text{N X5}} \times 100$$

which

DSI = Disease severity index

E rating = Summation of all ratings.

N = number of sprouts observed.

5 = maximum rating.

3.2.10. Pre and post-emergence-damping off:

Pre and post-emergence-damping off were determined after 15 and 30 days of sowing, respectively. The number of survival plants and seeds-decay also determined after 30 days of sowing.

3.2.11. Statistical analysis:

Data were subjected to statistical analysis using the method described by **Snedecor (1966).** The least significant difference (L.S.D.) was used to differentiate means (**Waller and Duncan, 1969).**

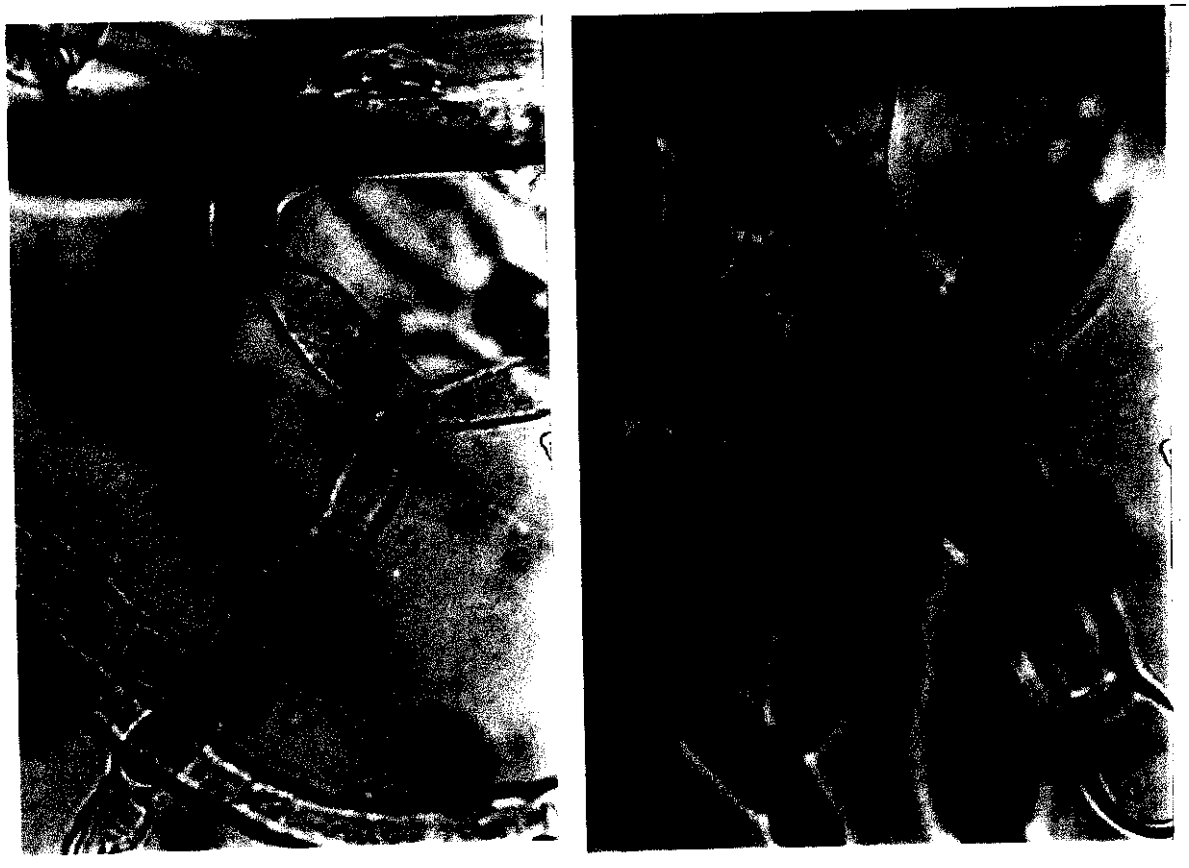


Fig. (1): Vegetative cells of *Rhizoctonia solani (x1000)*

Table (3) : N_2-fixation and antagonistic activities of azotobacters isolated from different localities cultivated with different crops against *R. solani* .

Azotobacter isolates	Locality	Crop	Total N (ppm)	Inhibition Zone diameter (mm)
R_1	10^{th} of Ramadan	Foeniculum	210	15
R.	10^{th} of Ramadan	Wheat	100	3
Re0	10^{th} of Ramadan	Corn	90	10
R,	10^{th} of Ramadan	Uncultivated	20	0
Rb	10^{th} of Ramadan	Barley	115	0
K,	El Khatatba	Cuminum	202	10
K_0	El Khatatba	Ocimum	202	0
Nc.1	Noubaria	Cucumber	150	5
N_{cu2}	Noubaria	Cucumber	110	5
N.	Noubaria	Wheat	40	0
Arco	Noubaria	Corn	190	0
F_a,	Fayoum	Cucumber	200	0
Fo	Fayoum	Barley	100	0
M_w	Maruit	Wheat	45	8
Bco	Behira	Corn	95	7

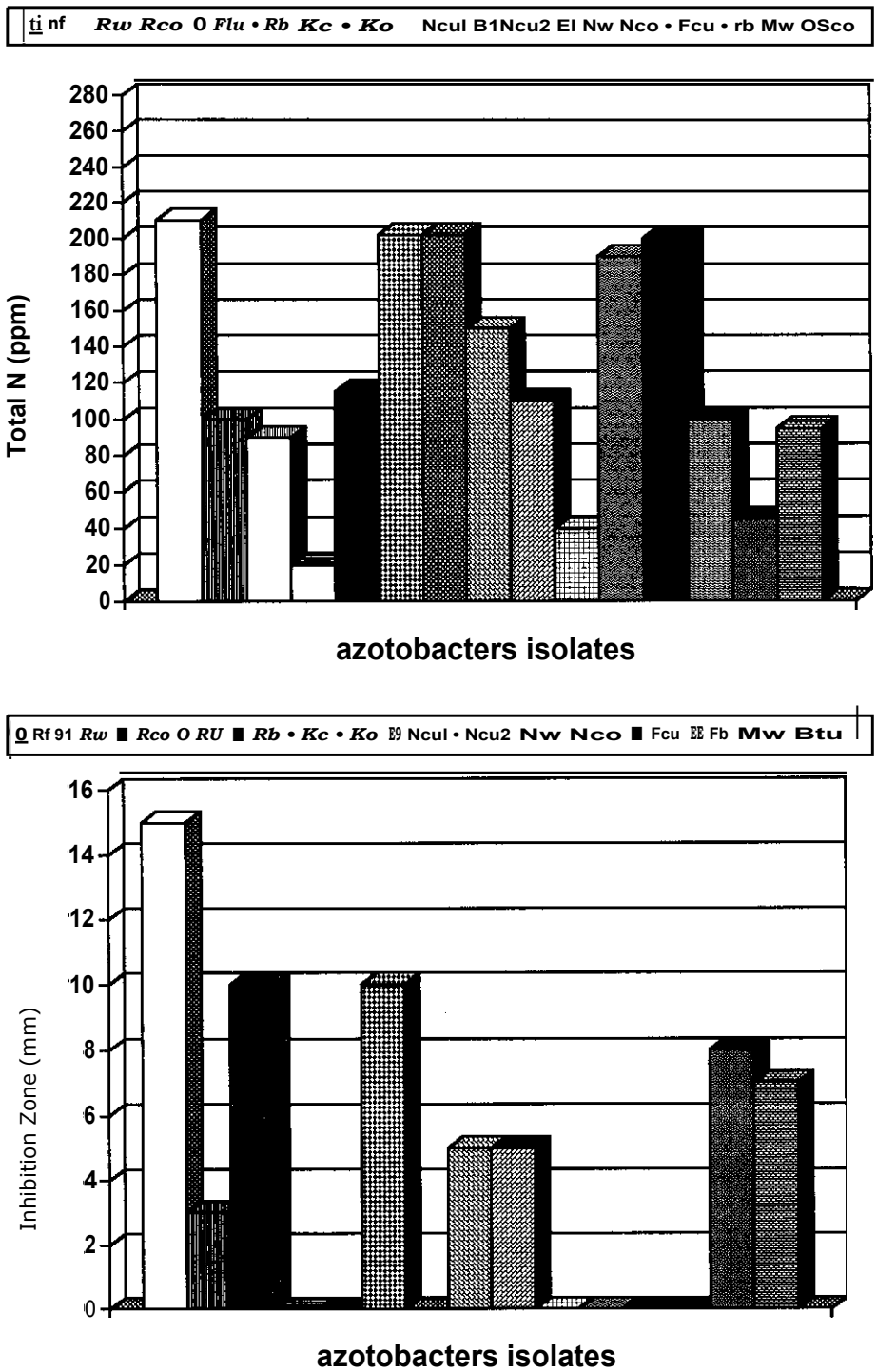


Fig (2):N_2-fixation and antagonistic activities of azotobacters isolated from different localities cultivated with different crops.

Table (4) : N_2-fixation and antagonistic activities of azospirilla isolated from different localities cultivated with different crops against *R. solani* .

Azospirillum isolates	Locality	Crop	Total N (PPm)	Inhibition Zone diameter (mm)
Rf	10th of Ramadan	Foeniculum	91	5
Rb	10th of Ramadan	Barley	93	0
K,	El Khatatba	Cuminum	133	*17*
K,	El Khatatba	Ocimum	133	14
N_na	Noubaria	Cucumber	100	9
N_{o42}	Noubaria	Cucumber	95	7
$N_{„,}$	Noubaria	Wheat	110	3
F.	Fayoum	Cucumber	101	0
M„	Maruit	Wheat	115	0
Bco	Behira	Corn	115	4

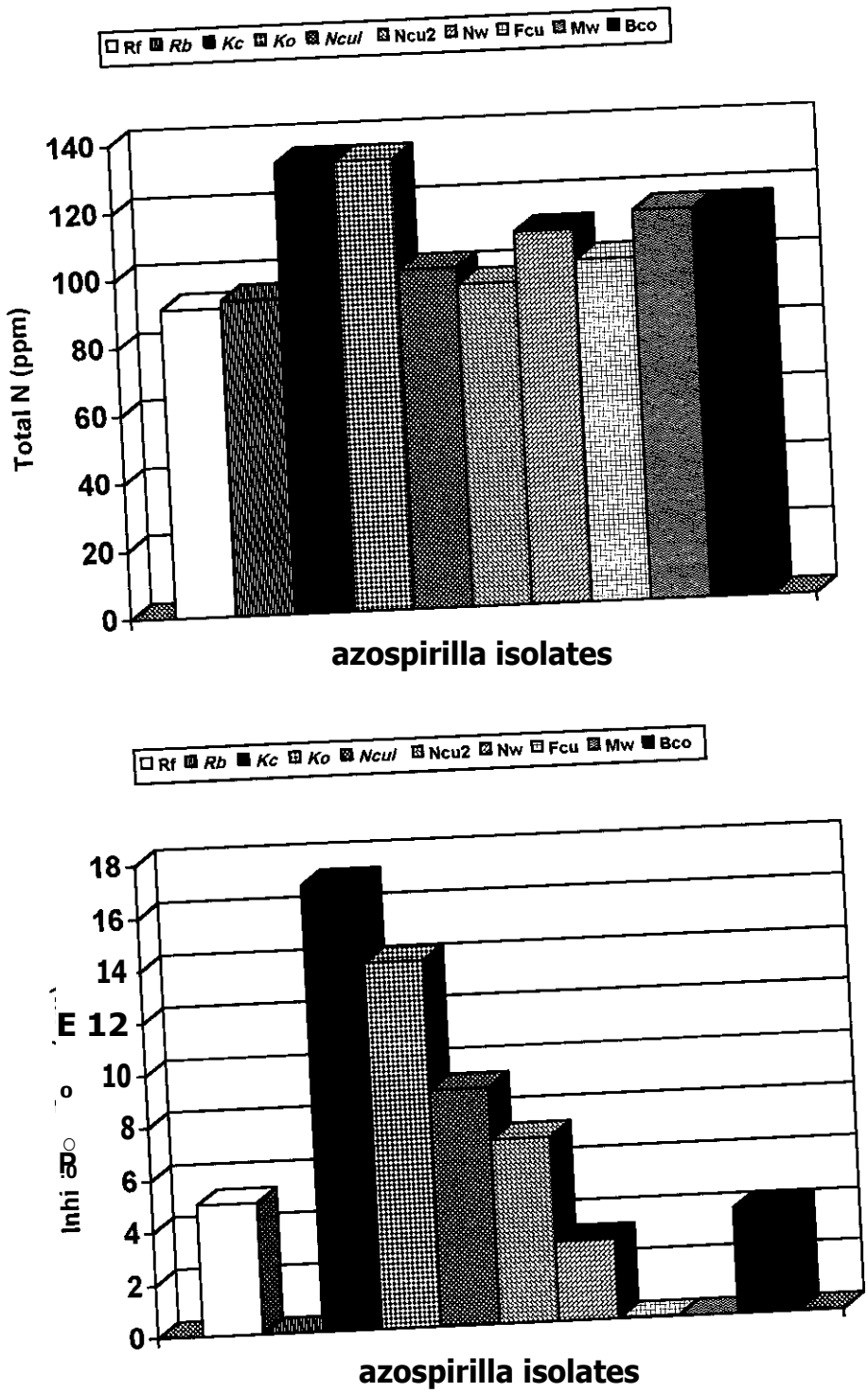


Fig (3) N_2-fixation and antagonistic activities of azospirilla isolated from different localities cultivated with different crops.

Table (5): Antagonistic activities of actinomycetes isolated from different localities cultivated with different crops against *R. solar,'* .

actinomycetes isolates	Locality	Crop	Inhibition Zone diameter (mm)
	10th of Ramadan	Foeniculum	0
	10th of Ramadan	Wheat	0
	10th of Ramadan	Uncultivated	0
	10th of Ramdan	Corn	0
	El Khatatba	Cuminum	5
	El Khatatba	Ocimum	0
٣	Noubaria	Cucumber	22
٣A	Noubaria	Cucumber	5
-	Fayoum	Cucumber	20
	Maruit	Wheat	0
٢	Maruit	Wheat	7

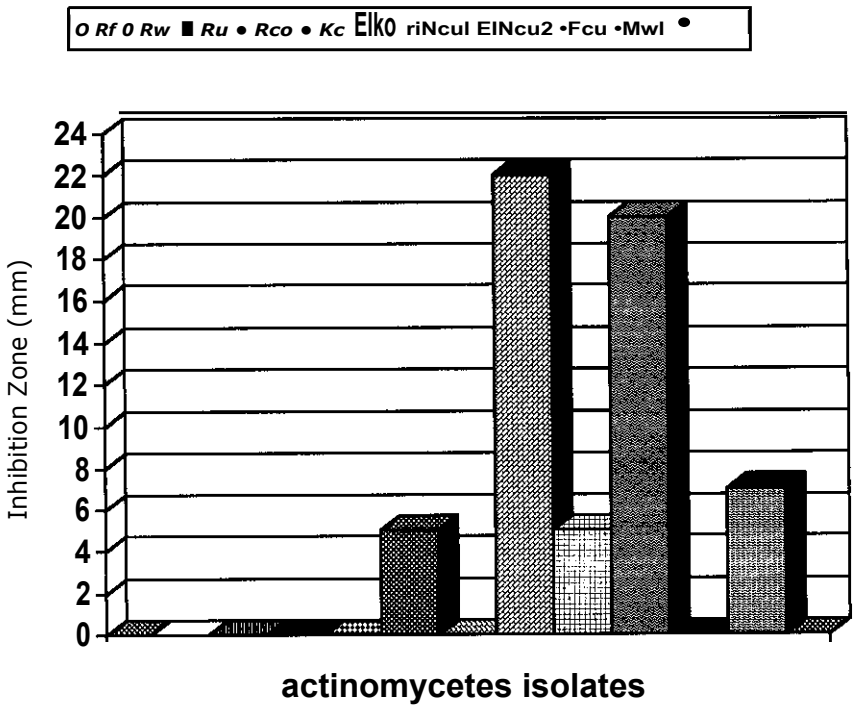
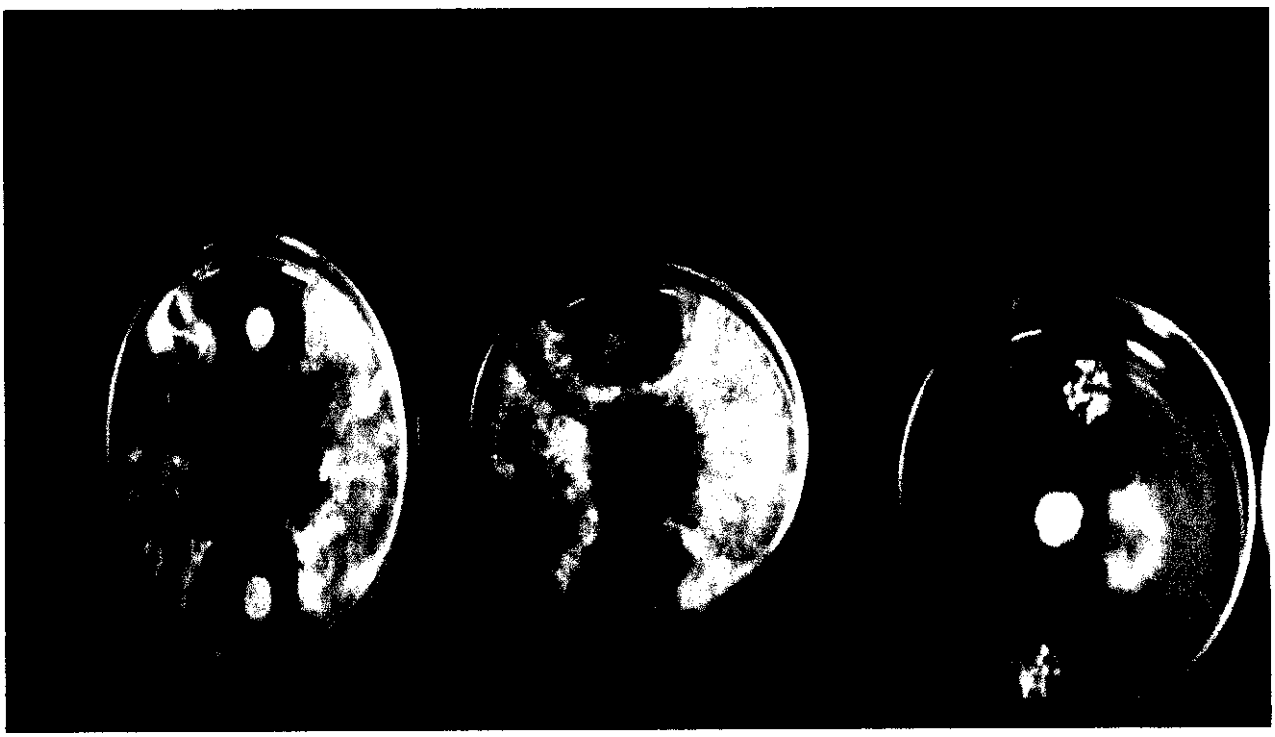


Fig (4):Antagonistic activities of actinomycetes isolated from different localities cultivated with different crops.



A B

Fig. (5): Antagoistic activity of
A) *Azospirillum lioferum*
B) *Azotobacter chroococcum*
C) *Streptomyces lychcus*
against *Rsolani*

the 25 N_2-fixing isolates were tested for their antagonistic against *R. solani* in vitro.

Data presented in Table (3) and Fig. (2) show that the most active *Azotobacter* isolate in fixing nitrogen Rf being (210 ppm) followed descendingly by the isolates IQ and K_o being (202 ppm). On the other hand the less active one Ru being (20 ppm).

With respect to azospirilla isolates , data in Table (4) and Fig (3) show that the highest *Azospirillum* isolates in fixing nitrogen K_o and lc being (133 ppm) followed by M_w and B. being (115 ppm) and the lowest *Azospirillum* isolate Rf being (91 ppm).

4.2.2. Antagonistic effect of isolates against *R. solani:*

Data presented in Tables (3 , 4 , 5) show inhibition zones of different diameter for N_2-fixing and actinomycetes isolates against *R. solani.* Fig(5) .

For *Azotobacter* isolates Table (3) show that the most active antagonistic isolate against *R. solani* Rf giving inhibition zone diameter of (15 mm), followed descendingly by isolates IQ and R. (10mm). Five azotobacter isolates showed less activities as they gave inhibition zones not exceed (8mm) in diameter. Seven *Azotobacter* isolates gave no inhibition zones indicating that they failed to antagonize *R. solani* growth.

With regard to azospirilla isolates the most active antagonistic isolate against *R. solani* was isolate IQ giving inhibition zone of (17 mm) in diameter followed by isolate K_o (14mm) The rest azospirilla

isolates proved to be weak against *R. solani* growth whereas they gave inhibition zones ranged from (0-9mm) in diameter.

For actinomycetes isolates Table (5) and Fig (4) show that the most active one against *R. solani* was actinomycete isolate Ncul which giving inhibition zone diameter of (22mm), followed by isolate F_{eu} (20mm) Three actinomycetes isolates showed less in activities against *R. solani* as they gave inhibition zones not exceeding (7mm) in diameter. Six actinomycetes isolates failed to antagonize *R. solani* growth in vitro, as they gave no inhibition zones.

4.2.3. Root colonization ability for N_2-fixing diazotrophs and actinomycetes isolates:

Data presented in Table (6) and Fig (6) show the root colonization ability by the most active isolates in fixing nitrogen and inhibiting the pathogenic fungus *R. solani* for N_2-fixing diazotrophs *(Azotobacter* and *Azospirillum)* isolates.

With respect to actinomycetes isolates, the most active were selected according to their highest inhibition zones against *R. solani.*

Root colonization for the isolates evaluated for cucumber seedlings found to be generally as percentage ranged between (26 and 63.5). For azotobacters isolates **Rf** (62%), K_e (51.3%), azospirilla isolates K_e (63.5%) and K_o (55.7%) but for actinomycetes isolates Ncui (41.4%) and F_u (26%).

Table (6) : Effect of inoculation with biofertilizer agents on root colonization of cucumber seedlings.

Bacterial isolates	Root length (cm)	Root colonization length (cm)	Root colonization (%)
Azotobacter Rf	10.1	6.2	62.0
Azotobacter IC,	7.4	3.8	51.3
Azospirillum K_o	10	6.5	63.5
Azospirillum K_o	9.5	5.3	55.7
Actinomycetes I \10u1	8.2	3.4	41.4
Actinomycetes F_o	7.5	2	26

Control • Uninoculated root length = 7.25 cm.

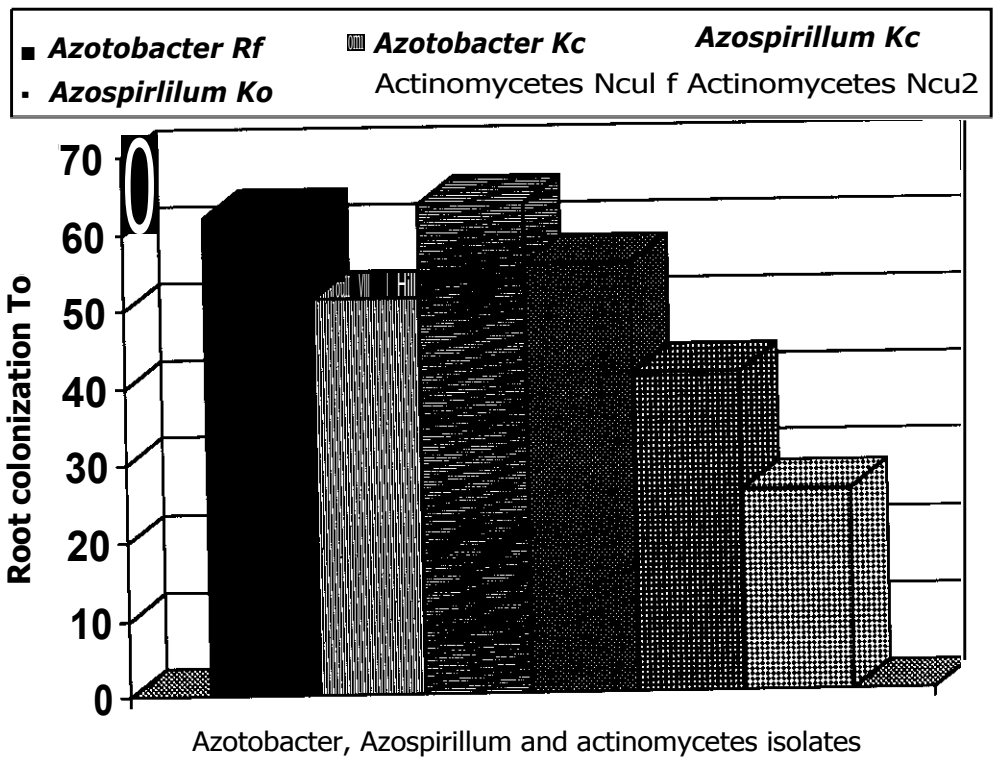


Fig (6):Effect of inoculation with biofertilizer agents on root colonization of cucumber seedlings.

4.2.4. Selection and identification of the most active isolates.

Data presented in Tables (7,8) and Figs. (7, 8) show the identification of the most active N_2-fixing diazotrophs (Rf, K_t) in fixing nitrogen, inhibiting pathogenic fungus *R. solani* and root colonization ability according to Bergey's Mannual (1984).

Azotobacter isolate Rf and *Azospirillum* isolate Kc were found to be *Azotobacter chroococcum* and *Azospirillum lipoferum,* respectively. With respect to the most active actinomycetes isolate Ncul in inhibiting *R. solani* and root colonization ability, data in Table (9) and Figs. (9) and (10) reveal that streptomyces isolate Ncul was given the name *Streptomyces lydicus* according to **Bergey's Manual (1974)** and the descr iption of streptomyces species given by **Shirting and Gottlieb (1968 a,b).**

These strains were selected for inoculation in a pot experiment as a single one or as a mixture in a rate (1:1) or (1:1:1) seven days old giving a final cell density of 10^8 cells $m1^{-1}$ to detect their effects as biofertilizers and biocontrol agents against *R. solani* in the same time.

4.2.5. Effect of inoculation with biofertilizer agents on disease severity index (DSI) of cucumber plants infected with *R. solani*

Data presented in Table (10) and Fig. (11) show that disease severity index (DSI) of cucumber plants infected with *R. solani* and treated with *Azotobacter chroococcum* Rf reduced the DSI to (63.8%), *Azospirillum lipoferm* K_e (61.1%). The highest reduction for DSI was giving by *Streptomyces lydicus* N_{eui} (55.5%).

Table (7) : Identification of *Azotobacter* isolate (Re)

Biochemical reaction	shapc
- Cell morphology	ovoid shaped
- Sucrose as sole carbon source	+
- Manitol as sole carbon source	+
- Benzoate as sole carbon source	+
- Motility	+
- Reduction of NO_3	+
- Catalase production	+
- Starch hydrolysis	+
- Production of non diffusible pigment	Brown pigment

Table (8) : Identification of *Azospirillum* isolate (KJ

Biochemical reaction	
- Cell morphology	shape slightly curved and straight rods
- Motility	
- Sucrose as sole carbon source	
- Glucose as sole carbon source	
- a- Ketoglutaric acid as sole c-source	
- Reduction of nitrate	
- Catalase production	
- Acidification of glucose	
- Biotin requirement	

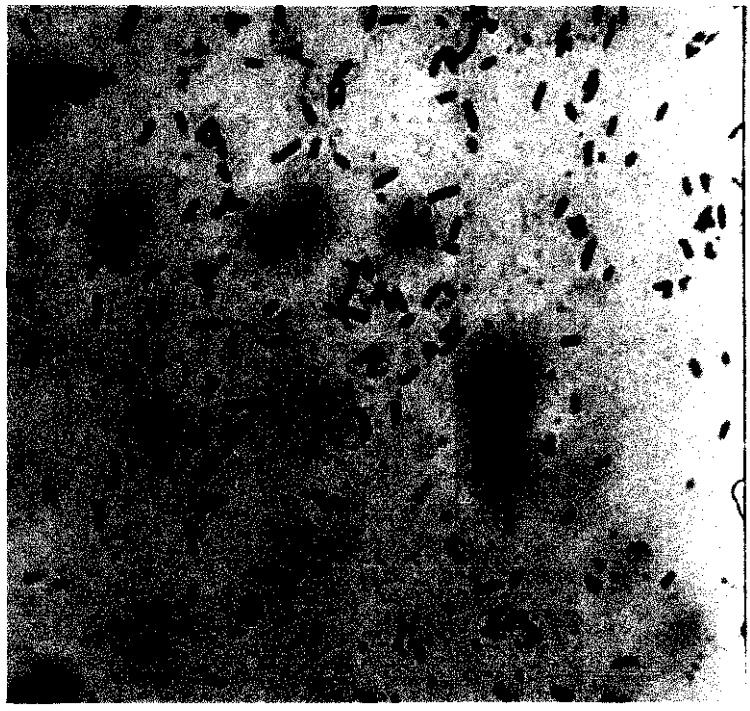


Fig. (7) : Cells of *Azospirillum lipoferum (x* 1000)

Fig. (8): Cells of *Azotobacter* chroococcum (x 000)

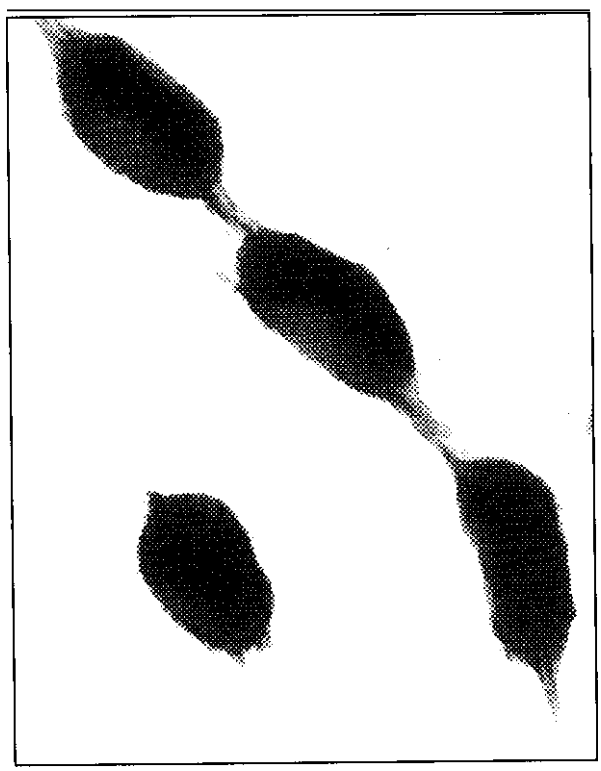


Fig (10): Electron micrograph of spore morphology of *Streptomyces*

Table (10) : Effect of inoculation with biofertilizer agents and infection with *R. solani* on the disease severity index (%) of cucumber plants.

Biofertilizer agents	DSI (%)	
	Value	% of control
Control	75	100
Azospirillum sp.	61.1	81.4
Azotobacter sp.	63.8	85
Azopirillum + *Azotobacter*	58.3	*77.7*
Streptomyces sp.	55.5	74
Azospirillum+ *Streptomyces*	50	66.6
Azotobacter + *Streptomyces*	52	69.3
Mixture	50	66.6

Control : Uninoculated
Mixture : *Azospirillum* + *Azotobacter* + *Streptomyces*
DSI : Disease severity index.

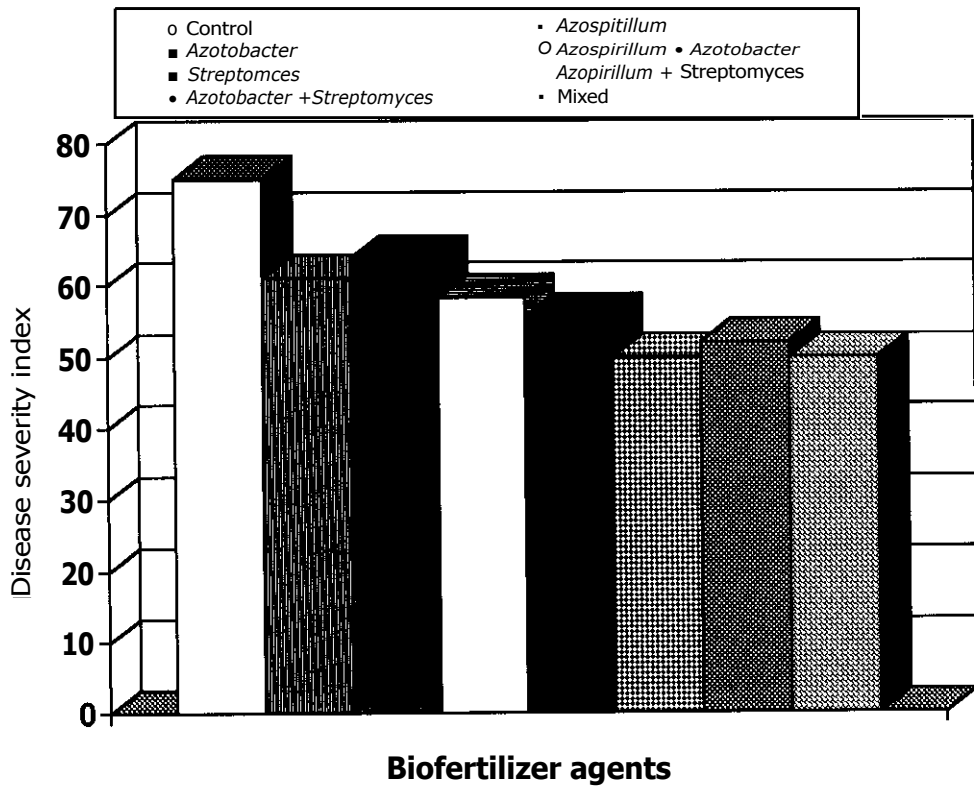


Fig (11): Effect of inoculation with biofertilizer agents and infection with *R. solani* on the disease severity index (%) of cucumber plants.

For *Azotobacter chroococcum + Azospirillum lipoferum* as a mixture, the reduction of DSI was (58.3%). Addition of *Streptomyces lydicus* strain to *Azotobacter chroococcum* or *Azospirillum lipoferum* singly or in a mixture of them decreased DSI between (52%-50%) Fig (12) .

4.2.6. Effect of inoculation with biofertilizer agents on the percentage of pre and post emergence damping off and survival of cucumber plants.

Data presented in Table (11) and Fig. (13)indicate that infection with *R.solani* gave the highest pre and post emergence damping off being 60 and 20% , respectively , and the lowest survival plants being 20% .

Inoculation lessened the harmful effect of *R. solani* ,decreased the pre and post emergence damping off and increased the survival plants Biofertilization with diazotrophs individual or as a mixture with *Streptomyces lydicus* decreased the pre and post emergence being 10-20 % and increased the survival plants being 60-70 % .

Also, inoculation with a trimixture of *Azotobacter, Azospirillum* and *Streptomyces lydicus* gave the highest number of survival plants.

4.3. Microbial densities

4.3.1. Total microbial counts

Data presented in Table (12) and Fig. (14) clearly indicate that microbial growth increased gradually towards fruiting then decreased towards harvesting stage of plant growth.

Table (11) : Effect of inoculation with biofertilizer agents and infection with *R. solani* on the percentage of pre and post emergence damping off and survival of cucumber plants.

Biofertilizer agents	Pre emergence damping off	post emergence damping off	Survival plants
Control	60	20	20
Azospirillum	20	30	50
Azotobacter	50	10	40
Azopirillum + Azotobacter	20	30	50
Streptomyces	40	5	55
Azospirillum+ Streptomyces	*10*	20	70
Azotobacter + Streptomyces	20	20	60
Mixture	20	10	70

Control : Uninoculated
Mixture : *Azospirillum + Azotobacter + Streptomyces*

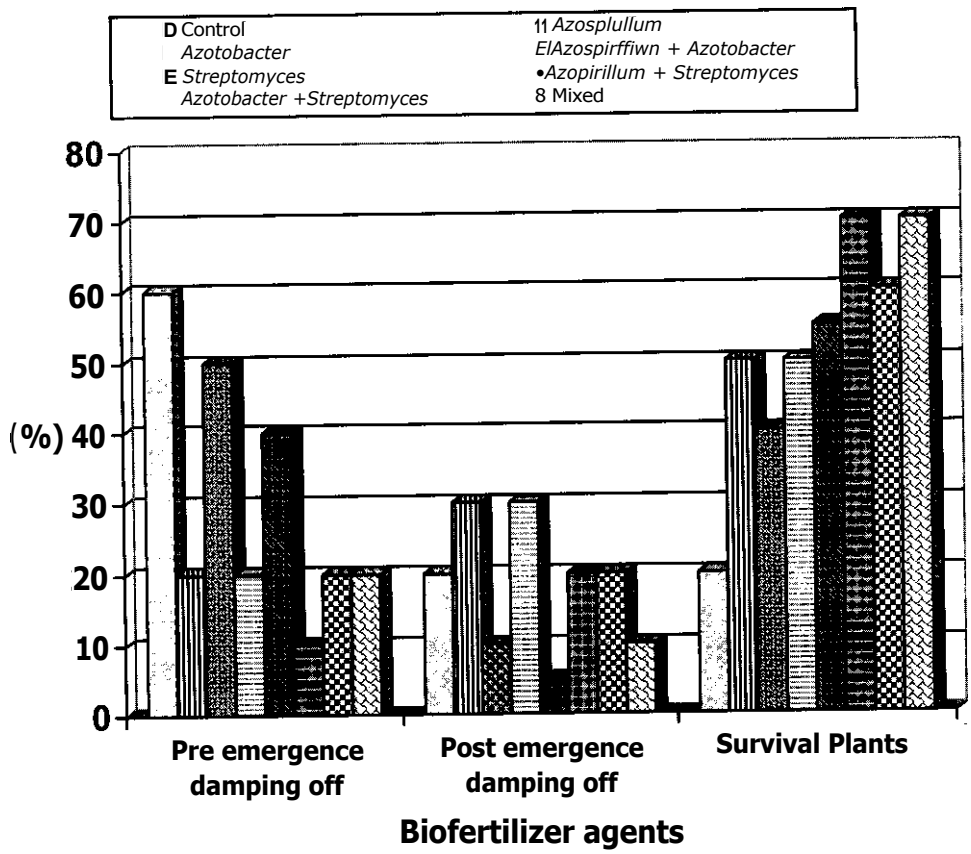


Fig (13): Effect of inoculation with biofertilizer agents and infection with *R. solani* on the percentage of pre and post emergence damping off and Survival of cucumber plants.

Inoculation of tested cucumber plants increased the growth and proliferation of microbial counts in cucumber rhizosphere.

In contrast, infection with *Rhizoctonia solani* reduced microbial counts in rhizosphere to some extent.

The maximal growth density being 276 x 10^4 cfue dry soil was obtained in uninfested cucumber rhizosphere inoculated with a mixture of *Azotobacter + Azospirillum + streptomyces or Azotobacter + Azospirillum* at fruiting stage.

In contrast, infested soil with *R. solani* and inoculated with a mixure of such organisms being 240 and 218 x 10^4 cfu g' dry soil at fruiting and harvesting stage of plant growth, respectively.

4.3.2. Total actinomycetes count:

The data presented in Table (13) and Fig. (15) showed that soil infested with *R. solani* harboured less densities of actinomycetes than uninfested one. A mixture of *Azotobacter+ Azospirillum + Streptomyces* recorded the highest effect of inoculation at vegetating, flowering, fruiting and harvesting being 45, 80, 99 and 89 x 10^3 cfu g^{-1} dry soil, respectively. Howevere the lowest increase of actinomycetes counts were 19, 40, 65 and 52 cfu g^{-1} dry soil under *Azospirillum* inoculation at vegetating, flowering, fruiting and harvesting stages of plant growth.

In contrast, infested soil with *R. solani* harboured less densities of such organisms being 38,65, 87 and 76 x 10^3 cfu g^{-1} dry soil and 16, 30, 51; 40x10^3 cfu g^{-1} dry soil using a mixture of *Azotobacter Azospirillun + streptomyces or Azospirillum* inoculation at vegetating,

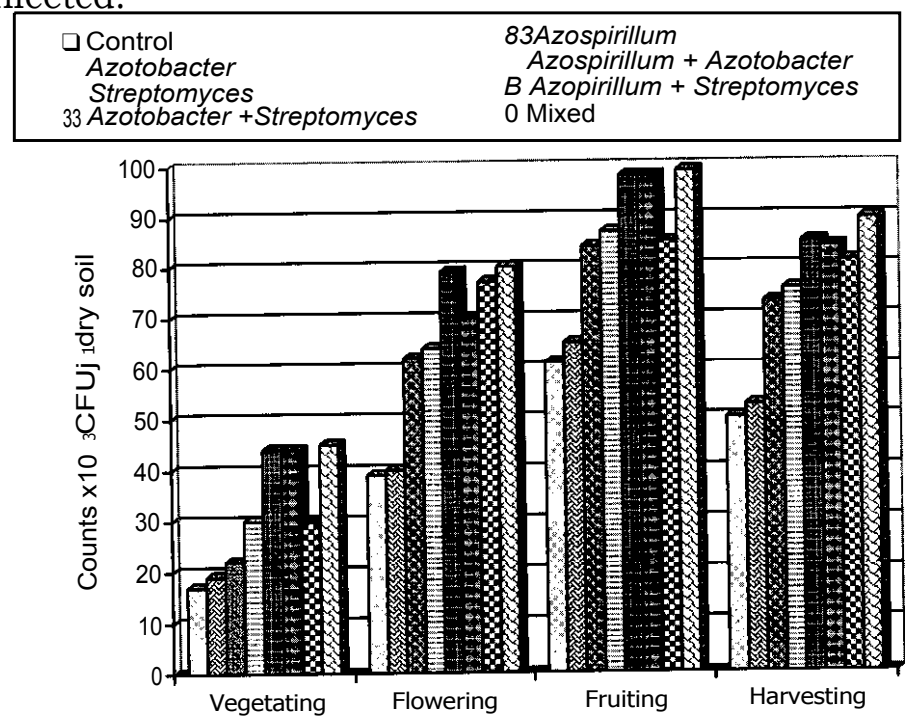
Biofertilizer agents	Stages of plant growth.							
	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	17	14	39	22	61	44	50	32
Azospirillum sp.	19	16	40	30	65	51	52.5	40.5
Azotobacter sp.	22	19	62	28	84	62	73	45
Azospirillum + *Azotobacter*	30	29	64	49	87	63	75.5	56
Streptomyces sp.	44	37	79	63	98	87	85	73
Azospirillum + *Streptomyces*	44	36	70	55	98	87	84	71
Azotobacter + *Streptomyces*	30	27	77	60	85	74	81	67
Mixture	45	38	80	65	99	87	89.5	76

Control : Uninoculated

Mixture : *Azotobacter* + *Azospirillum* + *Streptomyces.*

Initial count: 8x 10 CFU g dry soil

Uninfected:



Infected:

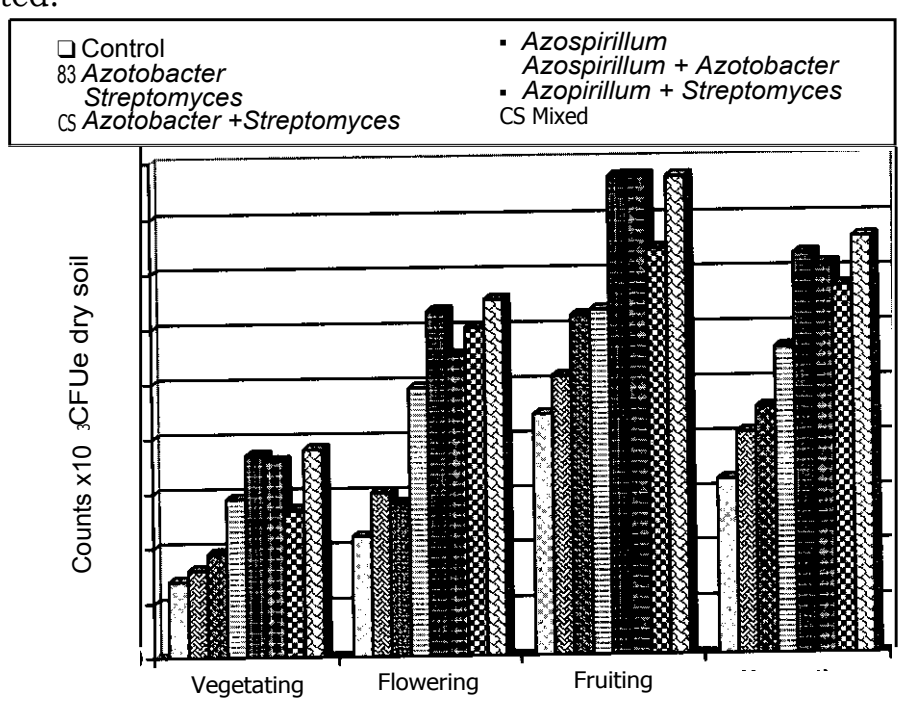


Fig (15): Effect of inoculation with biofertilizer agents and infection with *R. solani* on actinomycetes counts in the rhizosphere of cucumber plants.

flowering, fruitings and harvesting stage of cucumber plant growth, respectively.

Densities of asymbiotic N_2-fixers :

Azotobacters densities :

The data presented in Table (14) and Fig. (16) showed that soil infested with *R. solani* harboured less densities of azotobacters than uninfested one. Again inoculation with a mixture of *Azotobacter* + *Azospirillum* + *Streptomyces* increased azotobacters densities to its maximum being 1.1 x 10^4 cells g' dry soil reduced to 0.84 x 10^4 cells g'dry soil for infested soil at fruiting stage of plant growth.

Azospirilla densities :

It is clear from the data in Table (15) and Fig. (17) that inoculation increased the densities of azospirilla comparing with uninoculated Infested soil recorded less densities than uninfested soil.

The highest densities of azospirilla in the rhizosphere of cucumber plants was recorded at fruiting stage being 1.7 x 10^4 cells g^{i} dry soil decreased to 1.3 x 10^4 cells g^{-1} dry soil using *Azotobacter* + *Azospirillum* + *Streptomyces* strains for uninfested and infested soil, respectively.

Table (14) : Effect

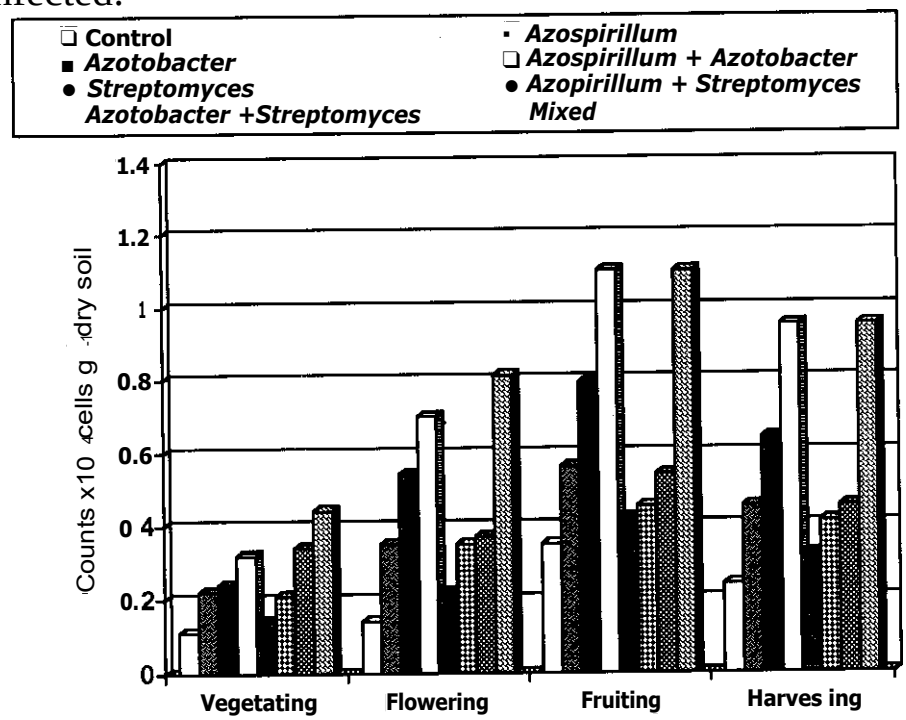
Biofertilizer agents	Stages of plant growth							
	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	0.11	0.061	0.14	0.11	0.35	0.27	0.24	0.19
Azospirillum sp.	0.22	0.12	0.35	0.22	0.56	0.44	0.45	0.33
Azotobacter sp.	0.24	0.21	0.54	0.52	0.79	0.62	0.64	0.56
Azospirillum + *Azotobacter*	0.32	0.24	0.7	0.56	1.1	0.81	0.95	0.80
Streptomyces sp.	0.14	0.12	0.22	0.17	0.42	0.34	0.32	0.25
Azospirillum + *Streptomyces*	0.21	0.14	0.35	0.27	0.45	0.34	0.41	0.31
Azotobacter + *Streptomyces*	0.34	0.33	0.37	0.36	0.54	0.41	0.45	0.38
Mixture	0.44	0.34	0.81	0.64	1.1	0.84	0.95	0.81

Control : Uninoculated

Mixture : *Azotobacter* + *Azospirillum* + *Streptomyces.*

Initial count: 0.03 x 10 cells e dry soil

Uninfected:



Infected:

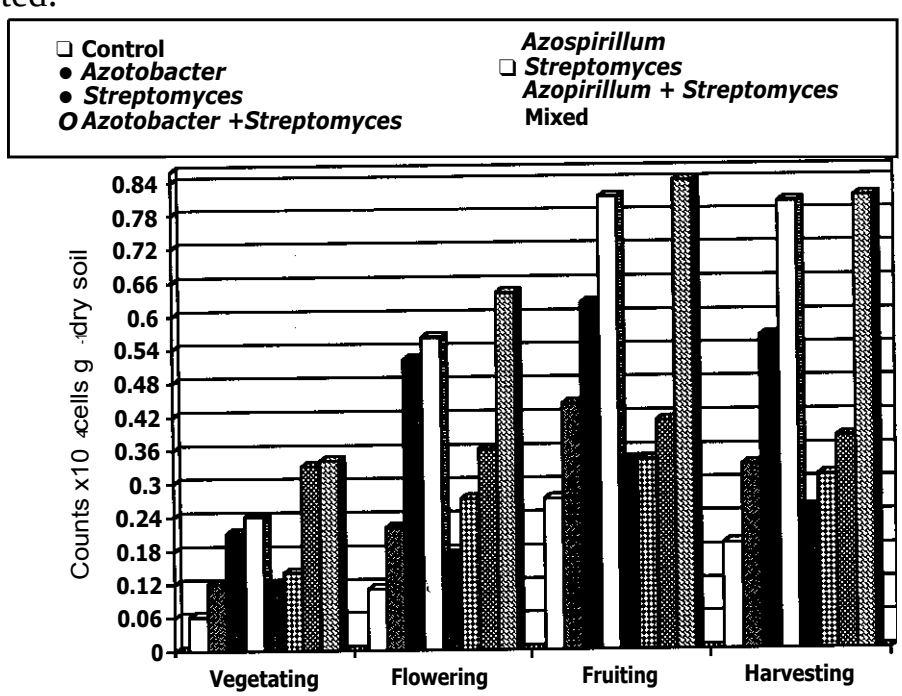


Fig (16): Effect of inoculation with biofertilizer agents and infection with *R. solani* on *Azotobacter* counts in the rhizosphere of cucumber plants.

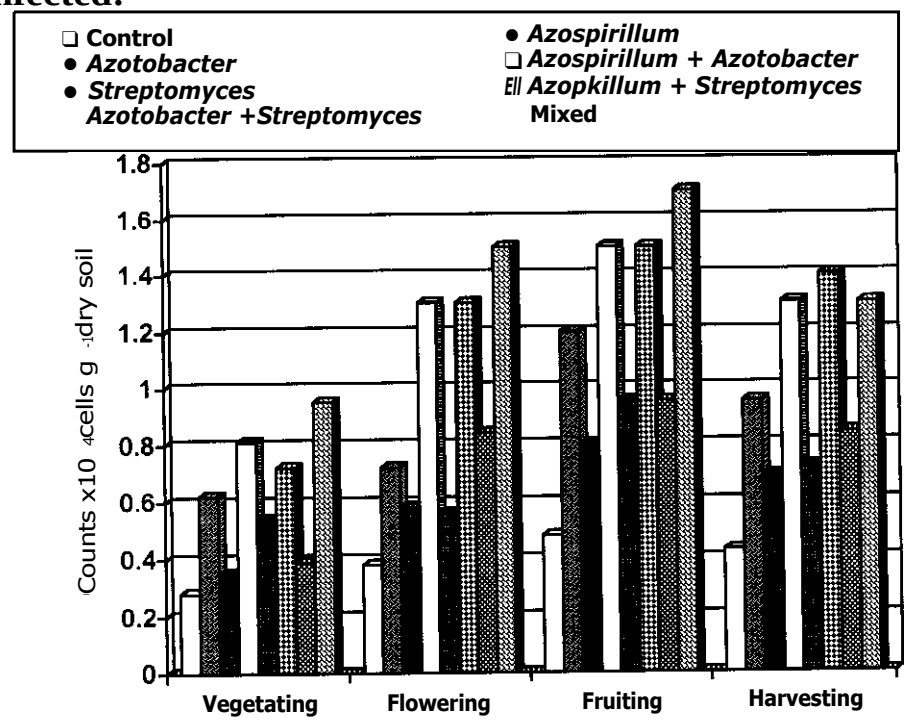
the rhizosphere of cucumber plants (count x 10^4 cells gm^{-1} dry soil).

Biofertilizer agents	Stages of plant growth							
	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	0.28	0.11	0.38	0.22	0.48	0.38	0.43	0.3
Azospirillum sp.	0.62	0.45	0.72	0.7	1.2	1.1	0.95	0.95
Azotobacter sp.	0.35	0.26	0.58	0.32	0.79	0.6	0.69	0.46
Azospirillum + *Azotobacter*	0.81	0.38	1.3	0.72	1.5	1.3	1.3	1.1
Streptomyces sp.	0.54	0.26	0.56	0.27	0.95	0.8	0.72	0.52
Azospirillum + *Streptomyces*	0.72	0.54	1.3	0.84	1.5	1.2	1.4	1.1
Azotobacter + *Streptomyces*	0.39	0.24	0.84	0.52	0.95	0.67	0.84	0.59
Mixture	0.95	0.64	1.5	1.1	1.7	1.3	1.3	1.2

Control : Uninoculated

Mixture :*Azotobacter* + *Azospirillum* + *Streptomyces.*

Uninfected:



Infected:

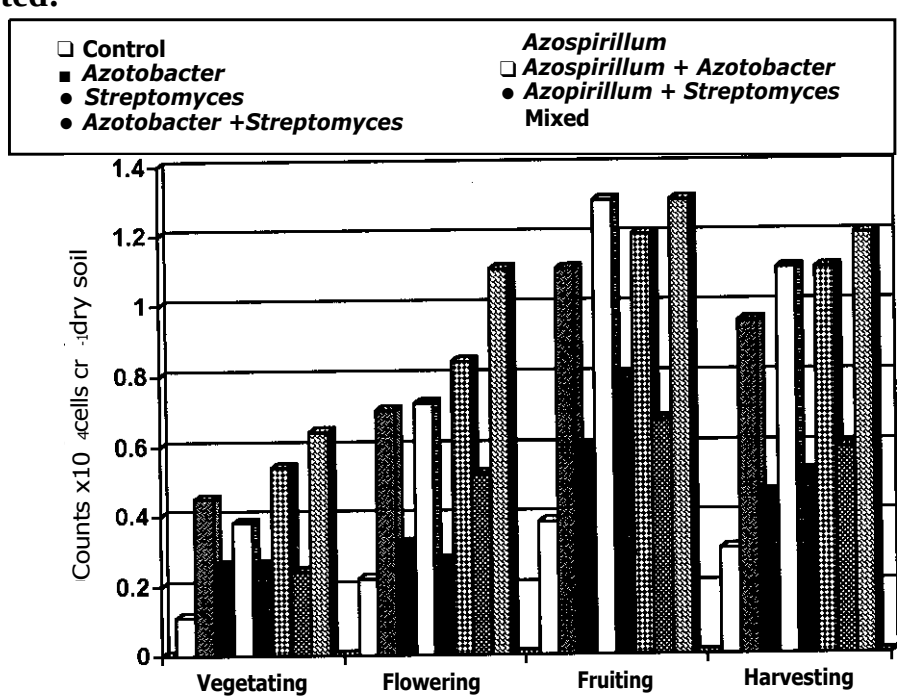


Fig (17): Effect of inoculation with biofertilizer agents and infection with *R. solani* on *Azospirillum* counts in the rhizosphere of cucumber plants.

4.4. Plant characteristics

4.4.1. Stem length .

Data in Table (16) and Fig. (18) show that infestation with *R. solani* reduced the growth of cucumber plants to different extents. It is clear from the data that inoculation with *Azotobacter, Azospirillum, Streptomyces* singly or in mixture di or tri reduced the harmful of such pathogenic fungus on cucumber plant heights. The plant heights in control infested soil were 22.8, 43.7, 65.7 and 87.8 cm. increased to 33.4, 74.5, 99.6 and 124.7 cm by inoculation with a mixture of *Azotobacter, Azospirillum* and *Streptomyces* strains for the 1st, 2nd 3rd and 4^{th} growth stages, respectively.

The obtained results in Table (18) indicated that effect of *R.Solani* can be significantly reduced by inoculation. The most effective inoculation treatment in such respect was the application of a mixture of *Azotobacter, Azospirillum* and *Streptomyces* followed by *Azotobacter* and *Azospirillum* and the lowest with *Streptomyces* inoculation.

4.4.2. Root length:

It is clear from the data presented in Table (17) and Fig.(19) that cucumber plant root length increased towards harvesting stage of plant growth.

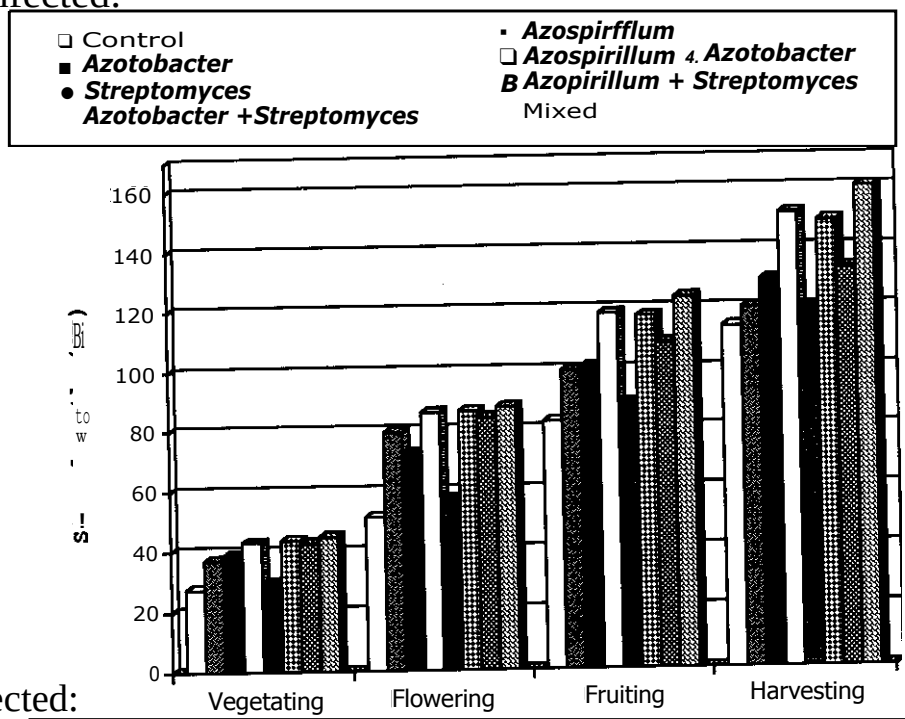
At harvesting stage, the cucumber plant root length was 22.1 cm in infested control soil compared with 28.1cm in uninfested one. Application with a mixture of *Azotobacter, Azospirillum* and *Streptomyces* increased root length to 38.1 cm and 43.7 in infested and uninfested soil, respectively.

Table (16) : Effect of inoculation with biofertilizer agents and infection with *R. solani* on stem length (cm) of cucumber plants.

Biofertilizer agents	Stages of plant growth							
	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	27.2	22.8	51.1	43.7	82.3	65.7	113.6	87.8
Azospirillum sp.	36.9	26.1	79	58.7	99.5	78.2	119.8	97.7
Azotobacter sp.	38.7	24.8	71.9	63	100.6	80.7	129.3	98.5
Azospirillum + *Azotobacter*	42.9	33	85.7	74.6	118.2	96.5	151.3	118.4
Streptomyces sp.	29.4	24	57	55	88.1	76.8	119.2	96.1
Azospirillum + *Streptomyces*	43.4	28.4	86	71.4	117.3	91.9	148.7	112.4
Azotobacter + *Streptomyces*	42.6	27.2	83.8	67.9	108.5	89.7	133.3	111.6
Mixture	44.7	33.4	87.4	74.5	123.7	99.6	160.1	124.7

* Control : Uninoculated
* Mixture : *Azotobacter* + *Azospirillum* + *Streptomyces*.
* LSD at 5% : Stages 1.668
  Biofertilizer agents 3.337
  Stages x Biofertilizer agents 6.676

Uninfected:



Infected:

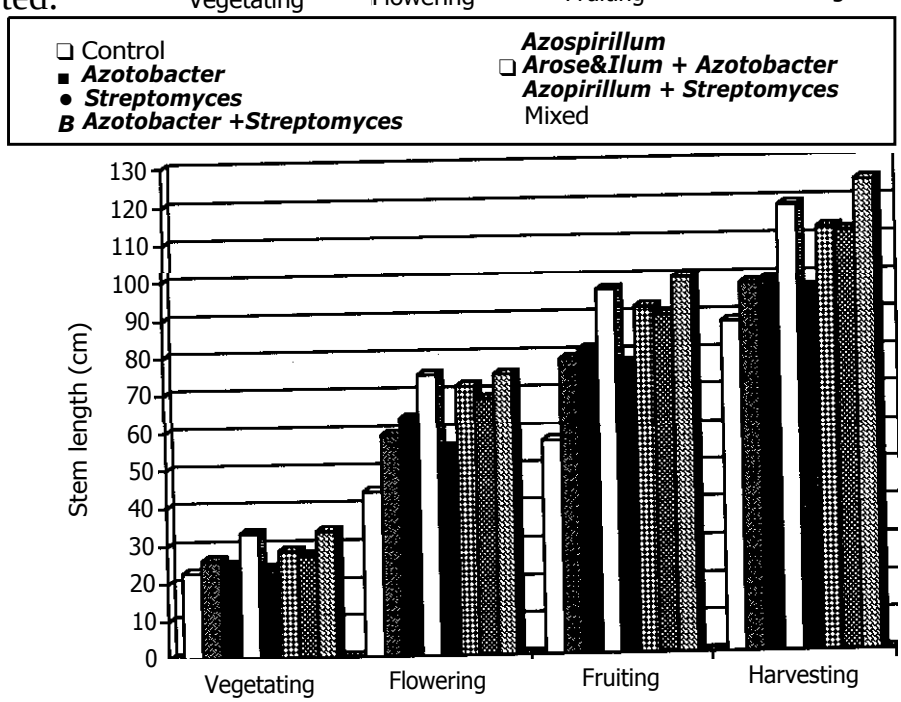


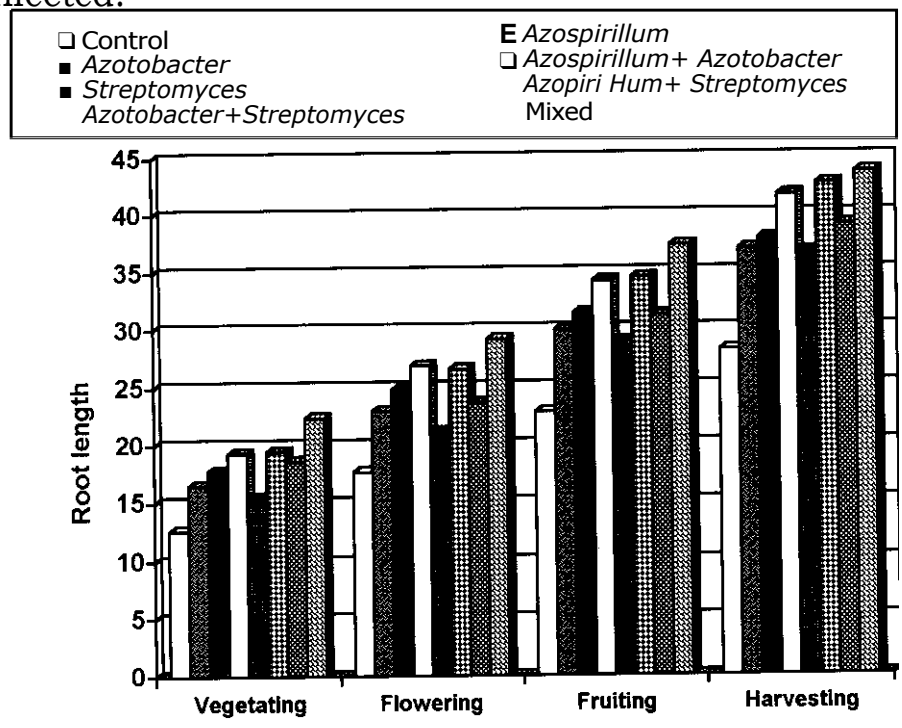
Fig (18): Effect of inoculation with biofertilizer agents and infection •vith *R. solani* on stem length of cucumber plants.

cucumber plants.

Biofertilizer agents	Stages of plant growth							
	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	12.6	9.5	17.6	13.9	22.8	18	28.1	22.1
Azospirillum sp.	16.4	12.2	22.9	19.5	29.9	23.3	36.9	27
Azotobacter sp.	17.7	14.3	24.8	17.1	31.4	23.2	37.8	29.4
Azospirillum + *Azotobacter*	19.2	16.3	26.8	22.8	34.2	30.1	41.7	37.5
Streptomyces sp.	15.4	11	21.1	17.1	28.9	21.4	36.6	25.7
Azospirillum + *Streptomyces*	19.4	15.3	26.5	21.4	34.5	27.6	42.7	33.8
Azotobacter + *Streptomyces*	18.5	14.4	23.6	20.2	31.2	25.9	39	31.5
Mixture	22.3	17.5	29.1	24.6	37.3	31.3	43.7	38.1

* Control : Uninoculated
* Mixture : *Azotobacter* + *Azospirillum* + *Streptomyces*.
* LSD at 5% : Stages 0.761
  Biofertilizer agents 1.522
  Stages x Biofertilizer agents 3.04

Uninfected:



Infected:

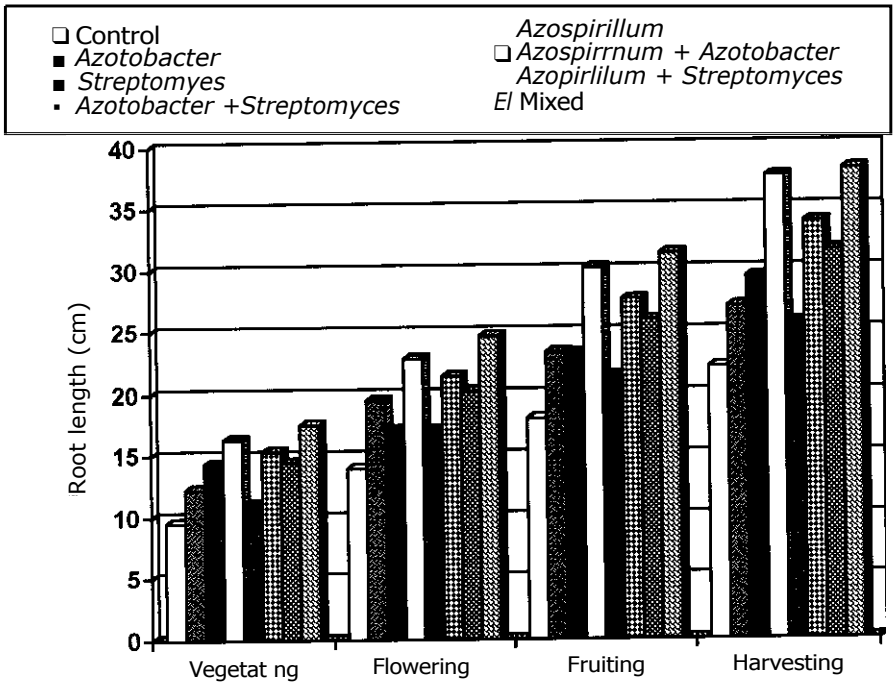


Fig (19): Effect of inoculation with biofertilizer agents and infection with *R. solani* on the root length of cucumber plants.

Table(18): Statistical main effects of stages, infection with *R.solant* and inoculation with biofertilizer agents on shoot and root length of cucumber plants.

Factors		Shoot length	Root length
Stages	Vegetative	32.939 d	15.764 d
	Flowering	69.604 c	21.818 c
	Fruiting	94.710 b	28.412 b
	Harvesting	119.995 a	34.268 a
Uninfected	Control	68.583 g	20.291 fg
	Azospirillum	83.925 de	26.533 cd
	Azotobacter	85.133d	27.916 cd
	Azopirillum + Azotobacter	99.633 b	30.133 b
	Streptomyces	73.425f	26.316 d
	Azospirillurn+Streptomyces	97.658 b	30.608 b
	Azotobacter +Streptomyces	92.091 c	28.141 c
	Mixture	103.99 a	33.308 a
Infected	Control	55.016 i	15.875 h
	Azospirillum	65.175 gh	20.5 f
	Azotobacter	66.575 gh	20.991 f
	Azopirillum + Azotobacter	80.716 e	26.708 cd
	Streptomyces	63.6 h	18.783 g
	Azospirillum+ Streptomyces	76.3 f	24.541e
	Azotobacter +Streptomyces	74.116 f	23.041 e
	Mixture	83.058 de	26.736 cd

Mean representing the effect of each factor on a particular and not followed by the same letter are significantly different by Duncan's multiple range test (P< 0.05)

Data presented in Table (18) show that infection with *R.solami,* in all cases, significantly decreased root length of cucumber plants, especially in control infested soil less than control uninfested one. The maximum root length was obtained in plants collected at harvesting stage of plant growth. Inoculated cucumber plants with mixed inoculatiun generally produces significantly higher length of roots followed by *Azotobacter* and *Azospirillum* or *Azospirillum* and *Streptomyces* and the lowest was with *Streptomyces.*

4.4.3. Stem weight :

Data presented in Tables (19,20) and Figs. (20,21) show that application of biofertilizer reduced the negative effect of *R. solani* on the fresh and dry weights of cucumber plants at different stages.

With respect to cucumber fresh weights data obviously reveal that inoculation with a mixture of *Azotobacter, Azospirillum and Streptomyces* strains gave remarkable increase in such parameter in soil whether uninfested or infested with *R. solani.* At harvesting stage cucumber fresh weight infected with *R. solani* (control) was 23.3 gm increased to 37.2 gm by application with a mixture of such organisms, and the lowest one was 27.7 gm by *Streptomyces* inoculating. The maximum fresh weight was 45 5 gm in uninfested soil at harvesting stage of cucumber plant growth. The same response was also clear in case of plant oven dry weights.

Data in Table (23) show that shoot fresh or dry weight significantly increased towards harvesting stage of plant growth. Application of biofertilizer agents (especially mixed or *Azotobacter*

and *Azospirillum)* significantly reduces the negative effect of *R.solani* on plant growth . These treatments recorded significantly the highest weights and the lowest with single inoculation.

4.4.4. Root weight:

It is obvious from the data presented in Tables (21,22) and Figs. (22,23) that biofertilizer agents singly or in a mixture significantly increased root fresh or dry weight of cucumber plants at different stages of plant growth comparing with control and reduced the negative effect of *R. solani* on fresh or dry weight of cucumber plants.

At harvesting stage of cucumber plant growth biofertilizer application with a mixture of such organisms increased root fresh weight from 4.85 to 5.9 gm and 0.91 to 1.2 gm for dry weight in infested and uninfested soil, respectively.

It is obvious from the data recorded in Table (23) that, inoculation significantly increased root fresh or dry weight in uninfested soil more than infested one. Hence the lowest records were derived from infested control soil. Mixed culture of *Azotobacter* and *Azospirillum* with or without *Streptomyces* significantly recorded the highest root fresh or dry weight followed either by *Streptomyces* and *Azotobacter* or *Azospirillum* and the lowest one was derived from single inoculation .

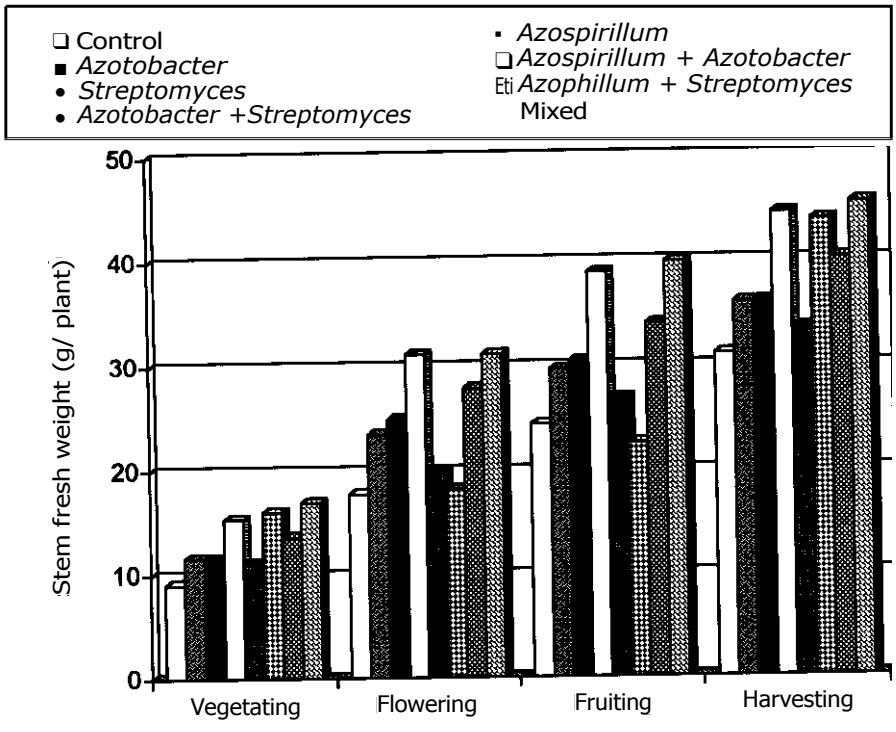
Inoculation significantly reduced the harmful effect of *R. solani* and increased shoot and root weight in compaison with infested soil .

(g/plant) of cucumber plants.

Biofertilizer agents	Stages of plant growth							
	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	9.1	6.8	17.7	15.9	24.3	19.6	31	23.3
Azospirillum sp.	11.6	7.5	23.4	20	29.6	23.9	35.9	27.8
Azotobacter sp.	11.5	7.9	24.8	21.7	30.4	25.6	36	29.5
Azospirillum + *Azotobacter*	15.3	11.7	31.1	26.2	38.8	29.9	44.6	33.7
Streptomyces sp.	11.1	7.5	20	19.7	26.7	23.7	33.4	27.7
Azospirillum + *Streptomyces*	16	9.2	18.2	22.8	22.5	27.3	43.9	31.9
Azotobacter + *Streptomyces*	13.6	9.1	27.8	20.8	33.9	25.4	40.1	30
Mixture	16.9	11.7	31.1	27.8	39.8	32.5	45.5	37.2

* Control : Uninoculated
* Mixture : *Azotobacter* + *Azospirillum* + *Streptomyces.*
* LSD at 5% : Stages 0.585
  Biofertilizer agents 1.170
  Stages x Biofertilizer agents 2.34

Uninfected:



Infected:

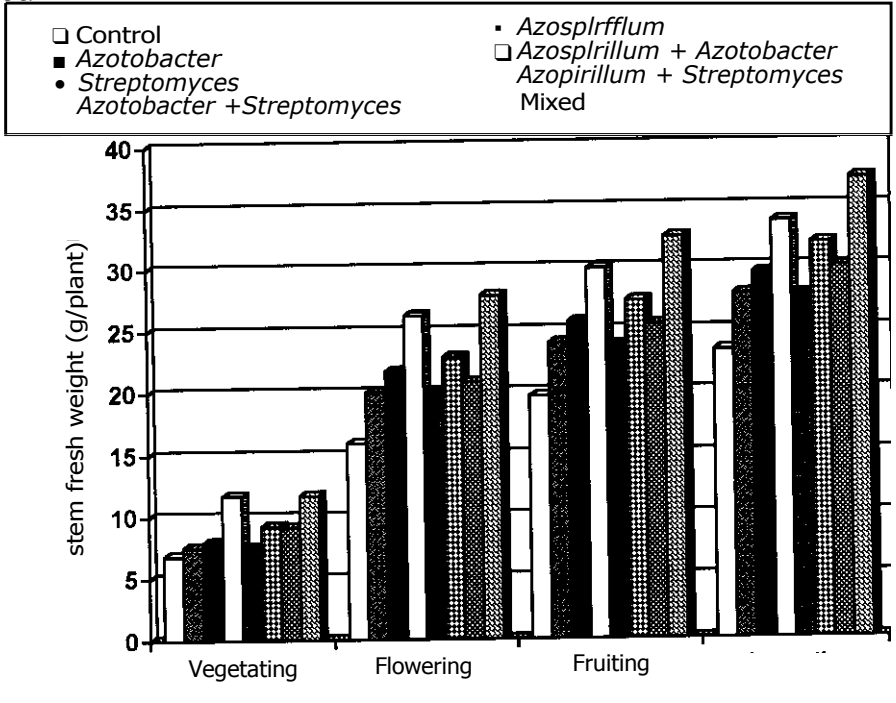


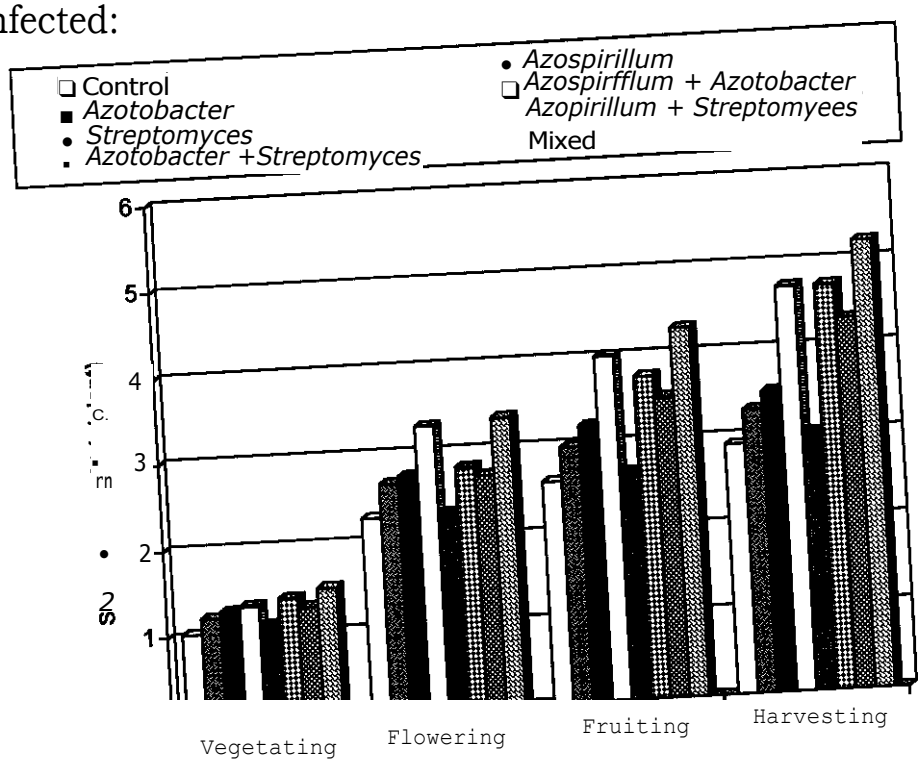
Fig (20): Effect of inoculation with biofertilizer agents and infection with *R. solani* on shoot stem fresh weight of cucumber plants.

Table (20) : Effect of inoculation with biofertilizer agents and infection with *R. solani* on the shoot dry weight (g/plant) of cucumber plants.

Biofertilizer agents	Stages of plant growth .							
	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	1.03	0.65	2.27	1.7	2.58	2.1	2.9	2.5
Azospirillum sp.	1.2	1.05	2.65	2.1	2.97	2.49	3.3	2.88
Azotobacter sp.	1.27	0.96	2.73	2.3	3.12	2.7	3.5	3.1
Azospirillum + *Azotobacter*	1.32	1.18	3.3	2.6	4	3.1	4.7	3.5
Streptomyces sp.	1.1	0.88	2.29	2.1	2.64	2.4	3	2.7
Azospirillum + *Streptomyces*	1.39	1.1	2.8	2.6	3.75	2.95	4.7	3.3
Azotobacter + *Streptomyces*	1.28	1.07	2.7	2.4	3.5	2.77	4.3	3.15
Mixture	1.49	1.17	3.36	2.85	4.28	3.17	5.2	3.5

* Control : Uninoculated
* Mixture : *Azotobacter* + *Azospirillum* + *Streptomyces.*
* LSD at 5% : Stages 0.084
  Biofertilizer agents 0.169
  Stages x Biofertilizer agents 0.339

Uninfected:



Infected:

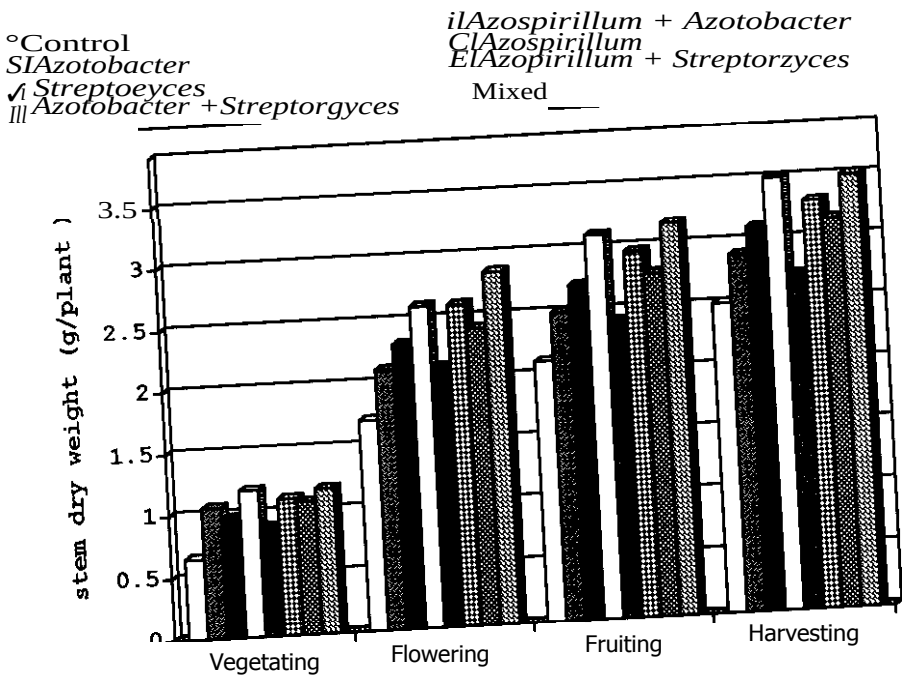
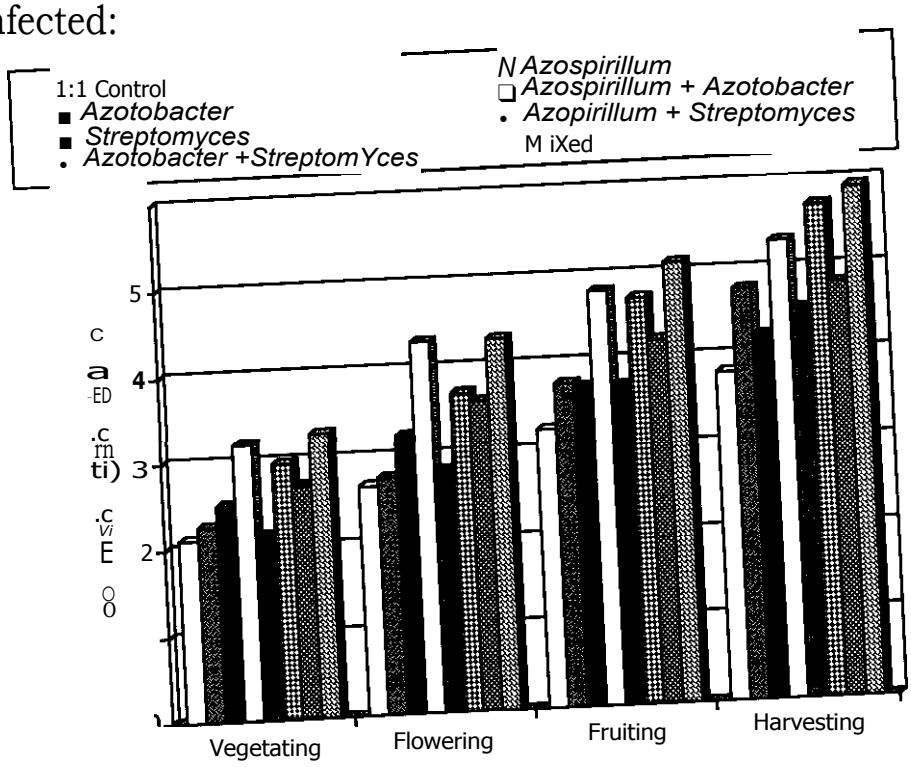


Fig (21): Effect of inoculation with biofertilizer agents and infection with *R. solani* on the stem dry weight of cucumbser plants.

	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
ontro	2.1	1.5	2.66	2.1	3.23	2.4	3.8	2.8
zospi	2.26	1.84	2.75	2.4	3.75	2.9	4.76	3.46
zotol	2.5	1.96	3.23	2.5	3.7	2.92	4.2	3.3
zosp	3.2	2.46	4.3	3.4	4.8	4	5.3	4.7
trept	2.14	1.83	2.8	2.53	3.7	2.9	4.5	3.3
tzosp	2.96	2.26	3.7	3.13	4.7	3.7	5.7	4.23
4zoto	2.7	2.2	3.6	3.1	4.2	3.6	4.8	4.16
Vixtu	3.3	2.5	4.3	3.5	5.1	4.2	5.9	4.85

Uninfected:



Infected:

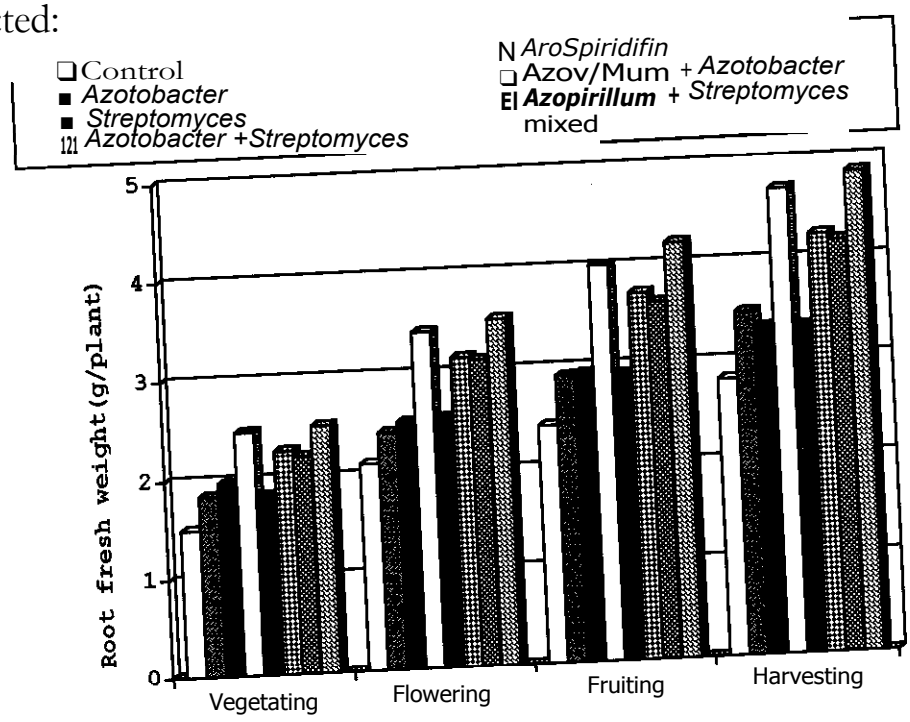


Fig (22): Effect of inoculation with biofertilizer agents and infection with *R. solani* on the root fresh weight of cucumber plants.

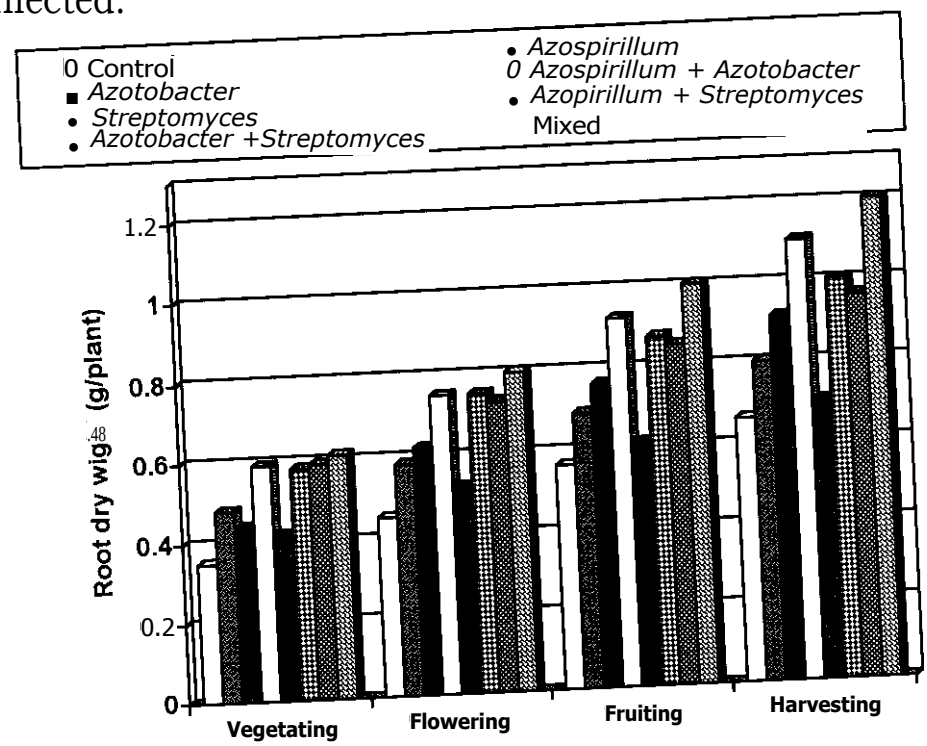
Biofertilizer agents	Root (cm) at different stages of plant growth							
	Vegetating		Flowering		Fruiting			
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	0.35	0.28	0.45	0.32	0.56	0.39	0.66	0.47
Azospirillum sp.	0.48	0.37	0.58	0.4	0.69	0.52	0.8	0.65
Azotobacter sp.	0.44	0.35	0.62	0.4	0.76	0.53	0.91	0.63
Azospirillum + *Azotobacter*	0.59	0.48	0.75	0.59	0.92	0.74	1.1	0.89
Streptomyces sp.	0.42	0.31	0.52	0.37	0.61	0.45	0.7	0.54
Azospirillum + *Streptomyces*	0.58	0.42	0.75	0.6	0.87	0.75	1	0.91
Azotobacter + *Streptomyces*	0.59	0.43	0.73	0.55	0.85	0.7	0.96	0.84
Mixture	0.61	0.5	0.8	0.59	1	0.75	1.2	0.91

Streptomyces.

0.0266
0.053
0.105

agents

Uninfected:



Infected:

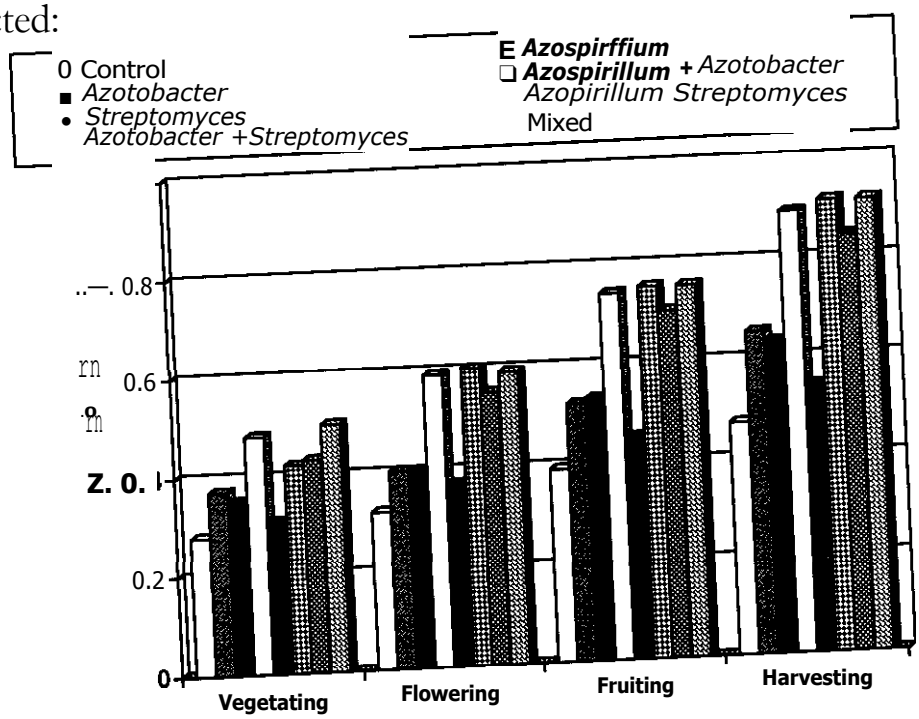


Fig (23): Effect of incoulation with bioferlilizer agents and infection with *R. solani* on the root dry weight of cucumber plants.

Table(23): Statistical main effects of stages, infection with *R.solain* and inoculation with biofertilizer agents on shoot fresh and dry weights and root fresh and dry weights of cucumber plants.

Factors	Shoot F.W.	Shoot Dry W.	Root fresh W.	Root D.W.
Vegetative	11.077d	1.140 d	2.344d	0.451d
Flowering	23.947c	2.564 c	3.129 c	0.563 c
Fruiting	29.258b	3.028 b	3.739 b	0.689 b
Harvestin:	34.802a	3.507 a	4.364 a	0.822a
Control	20.558 gh	2.205 ij	2.916 f	0.501 h
Azospirillum	25.63 3e	2.522 efg	3.466 de	0.628ef
Azotobacter	25.99 le	2.667 e	3.358 e	0.695 d
Azopirillum +Azotobacter	32.95 a	3.340 b	4.408 ab	0.843ab
Streptomyces	22.81 6f	2.246 ij	3.219 e	0.565 g
Azospirillum+ Streptomyces	31.35 8b	3.155 c	4.25 b	0.801 be
Azotobacter +Streptomyces	28.89 1c	2.951 d	3.808 c	0.780 c
Mixture	34.09 1a	3.599 a	4.641 a	0.895a
Control	16.4171	1.725 1	2.202 h	0.365 j
Azospirillum	19.791 h	2.12 jk	2.651 fg	0.487 h
Azotobacter	21.2 g	2.304 hij	2.675 fg	0.466 hi
Azopirillum + Azotobacter	25.391e	2.583 of	3.675 cd	0.674def
Streptomyces	19.725 h	1.993 k	3.275 e	0.420 I
Azospirillum+ Streptomyces	22.875 f	2.481 fgh	3.275 e	0.671def
Azotobacter +Streptomyces	21.358 g	2.372 ghi	2.608 g	0.625 f
Mixture	27.291 d	2.692 e	3.758 c	0.685 de

Me representing the effect of each factor on apart icularPar ameter
Mean
and not followed by the same letter are signcantly different by Duncan's multiple range test (P< 0.05)

4.4.5. Chlorophyll content:

Chlorophyll contents (model SPAD 502) in fresh leaves were determined at different stages of cucumber plant growth Table (24)and Fig. (24). The highest chlorophyll centent was at flowering stage in uninfested soil using a mixture inoculation

Biofertilizer application increased chlorophyll content. In uninfested soil, the maximum increase was 47.9% using a mixture of *Azotobacter* + *Azospirillum* + *Streptomyces*, but the lowest one was 44% with *Streptomyces* sp. inoculation comparing with control 35.6%

In contrast, in infested soil, chlorophyll content decreased to 42.1% in a mixture treatment comparing with control 33.3% and the lowest effect for biofertilizer was under *Azospirillum* inoculation 36.2%.

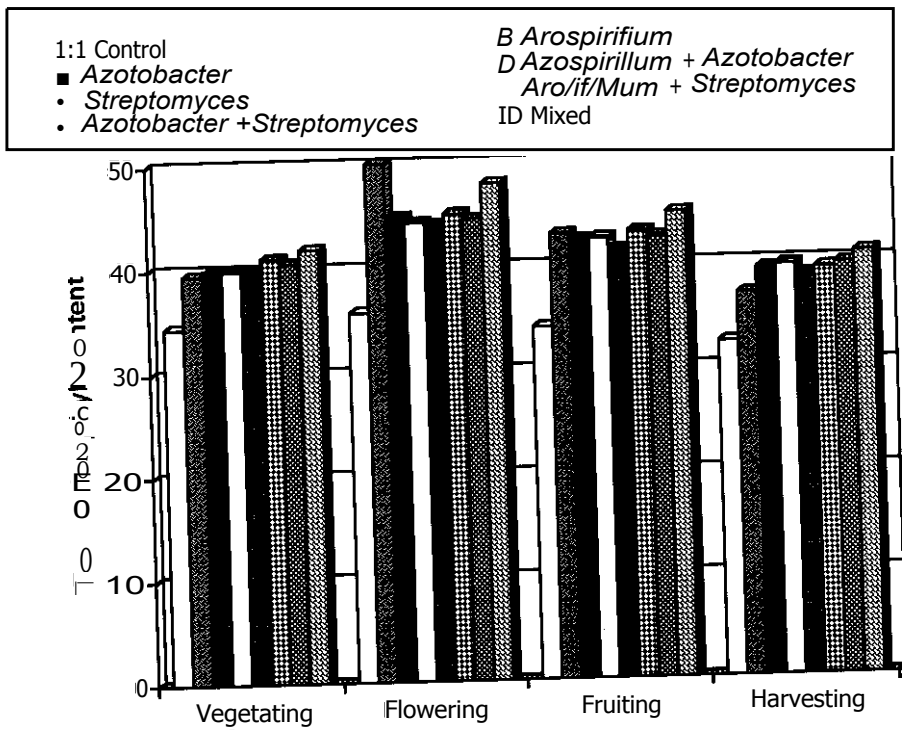
Data presented in Table (25) show that the maximum chlorophyll content was obtained in plants collected at flowering stage of plant growth . For inoculation the results showed significant differences between types of inoculation in infested or uninfested soil. Inoculated cucumber plants with diazotrophs with or without *Streptomyces* sp. produced significant higher chlorophyll content. The lowest records were derived from *Streptomyces* sp. . Incontrast , infested soil gave gradual and significant decreases in chlorophyll content. Hence, the lowest records were derived from infested soil and inoculated with *Azospirillum* with or without *Streptomyces* sp.

Table (24) : Effect of inoculation with biofertilizer agents and infection with *R. solani* on the total chlorophyll content in cucumber plants.

Biofertilizer agents	Stages of plant growth.							
	Vegetating		Flowering		Fruiting		Harvesting	
	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected	Uninf.	Infected
Control	34.3	30.5	35.6	33.3	33.9	31.9	32.2	23.8
Azospirillum sp.	39.4	33.2	46.5	36.2	42.9	35	36.8	33.5
Azotobacter sp.	39.7	35.2	44.8	38.4	42.3	36.8	39.2	34.5
Azospirillum + *Azotobacter*	39.8	37.4	44.2	38.3	42.4	37.8	39.5	35
Streptomyces sp.	39.8	37	44	39.2	41.3	38.1	38.5	33.5
Azospirillum + *Streptomyces*	41	33.5	45	36.4	43	35	39.2	33.2
Azotobacter + *Streptomyces*	40.5	35.5	44.5	38.7	42.5	37.1	39.6	33.4
Mixture	41.8	37.9	47.9	42.1	44.8	40	40.7	35.2

* Control : Uninoculated
* Mixture : *Azotobacter* + *Azospirillum* + *Streptomyces.*
* LSD at 5% : Stages 1.349
  Biofertilizer agents 2.7
  Stages x Biofertilizer agents 5.4

Uninfected:



Infected:

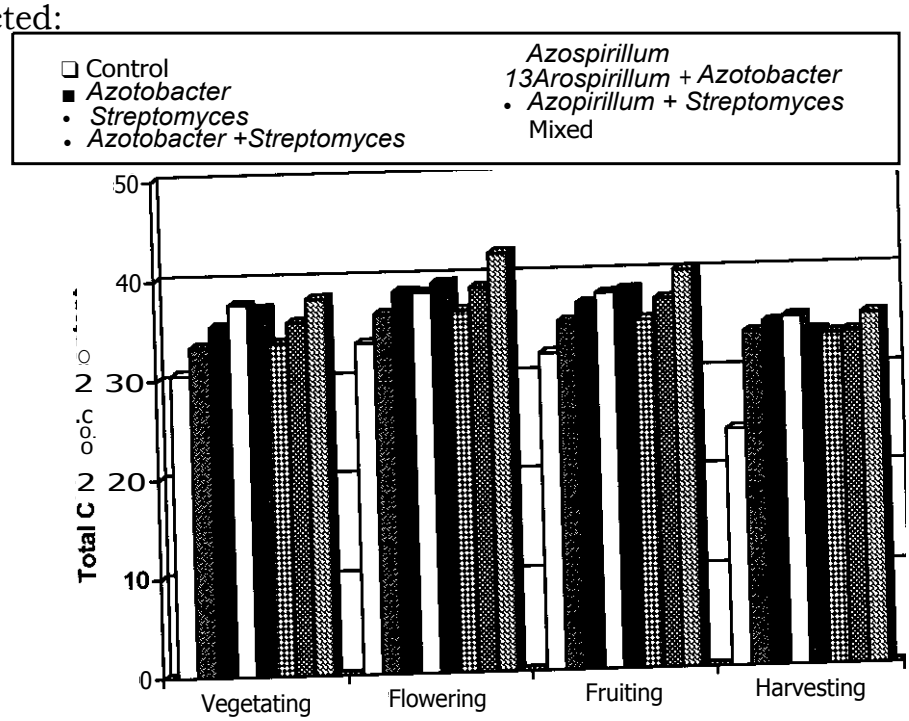


Fig (24):Effect of inoculation with biofertilizer agents and infection with *R.solani* on the total chlorophyll content at cucumbe plants.

Table(25) : Statistical main effects of stages, infection with *R.solain* and inoculation with biofertilizer agents on total chlorophyll content of cucumber plants.

Factors		Total Chlorophyll content
Stages	Vegetative	37.170 be
	Flowering	41.112 a
	Fruiting	38.2 b
	Harvesting	35.869 c
Uninfected	Control	34.016 f
	Azospirillum	41.925 ab
	Azotobacter	41.658 ab
	Azopirillum + Azotobacter	40.908 abc
	Streptomyces	38.616 cd
	Azospirillum +Streptomyces	41.791 ab
	Azotobacter +Streptomyces	41.794 ab
	Mixture	43.791 a
Infected	Control	29.875 g
	Azospirillum	35.075 ef
	Azotobacter	36.233 def
	Azopirillum + Azotobacter	37.141 de
	Streptomyces	36.975 def
	Azospirillum +Streptomyces	34.55 ef
	Azotobacter +Streptornyces	36.175 def
	Mixture	38.88 bcd

Mean representing the effect of each factor on a particular Parameter and not followed by the same letter are significantly different by Duncan's multiple range test ($P< 0.05$)

4.4.6. Flowering and Fruiting:

Data presented in Table (26) and Fig. (25) obviously reveal that number of flowers and fruits of cucumber plant in control infested soil were 25.2 and 7.3 respectively. Using a mixture of *Azotobacter Azospirillum* + *Streptomyces* significantly reduced the effect of pathogenic fungus and increased the number of flowers plant' to 39.3 and cucumber fruit numbers plant' to 16. Comparing with uninfested soil at the same treatment significantly increased to 54.6 flower plant and 22.7 fruit plant'. Data showed the positive effect of such organisms as biofertilizers on number of flowers and fruits of cucumber plants in the same time.

For cucumber fruit weight the lowest one was 83.9 g in control infested soil significantly increased to 201.6 g using a mixture of such organisms. For the uninfested soil the cucumber fruit weight significantly increased from 165.2 gm to 297 3 gm in control and using a mixture biofertilizer treatment, respectively. Weight of fruits significantly decreased under single inoculation as compared with other treatments, Table (26) and Fig. (26) .

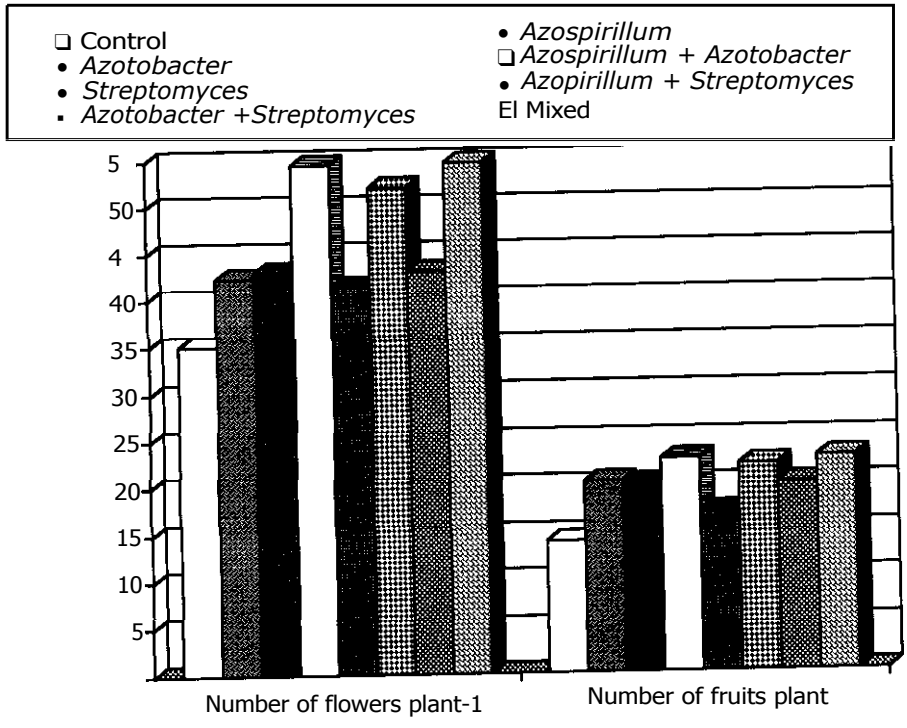
4.4.7. Fruits total nitrogen and total protein.

It is obvious from the data presented in Table (27) and Fig. (27) that biofertilizer agents singly or as a mixture significantly increased total N and total protein comparing with control and reduced the negative effect of *R. solani.*

Table(26):Effect of inoculation with biofertilizer agents and infection with *R. solani* on the number of flowers, fruits and weight of fruits of cucumber plants.

Biofertilizer agents	Number of flowers Plant'		Number of fruits Plant'		weight of fruits (g) plant'	
	uninfected	Infected	uninfected	Infected	uninfected	Infected
Control	35 ef	25.2 g	14 de	7.3 f	165.2 e	83.9 f
Azospirillum	42.3 be	33.3 f	20.3 b	13.3 e	243.6be	155.6 e
Azotobacter	43b	34 f	20 b	13.2 e	246 cd	153 e
Azospirillum+ Azotobacter	54.5 a	39 cde	22.6 a	15.7 cd	289.2 a	188.5 de
Streptomyces	41.3 be	33.3 f	17 c	13.2 e	212.5 cd	158.4 e
Azopirillum + Streptomyces	52 a	36.9 def	22 ab	14.6 de	286 a	185.4 de
Azotobacter + Streptomyces	43 be	35.3 ef	20 b	14.3 de	264 ab	180 de
Mixed	54.6 a	39.3 cd	22.7 a	16 cd	297.3 a	201.6 d
L.S.D. 0.05%	3.89		1.909		32.561	

Uninfected:



Infected:

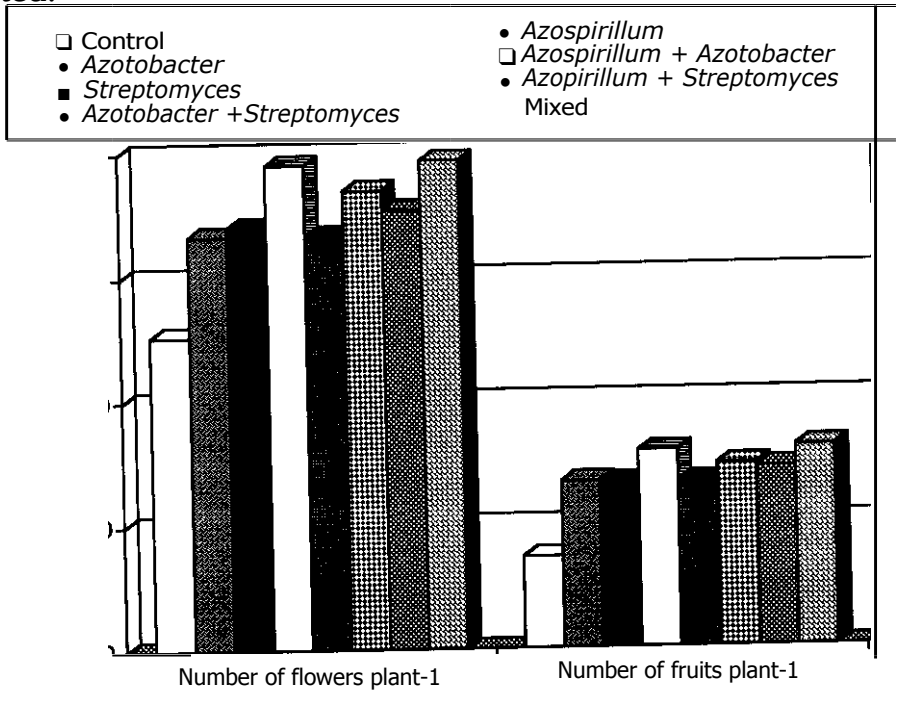


Fig (25): Effect of inoculation with biofertilizer agents and infection with *R.solani* on the number of flower and fruits of cucumber Plants.

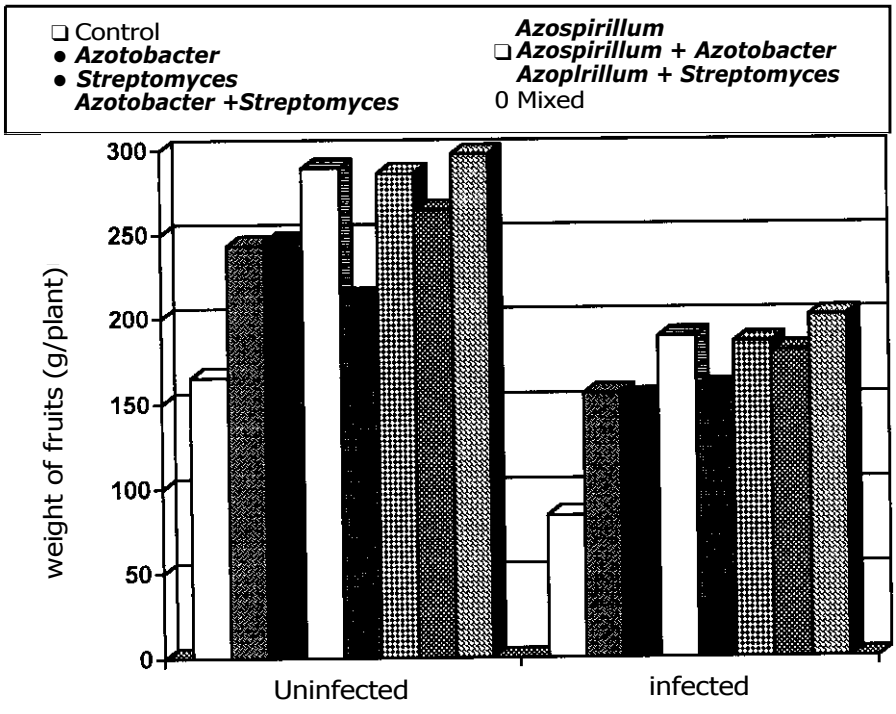
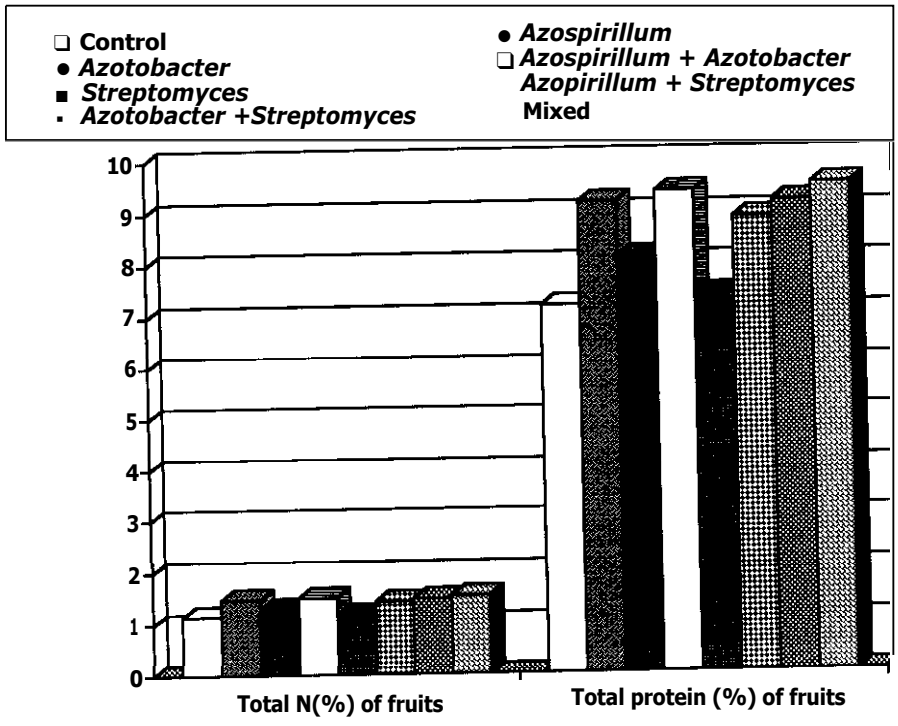


Fig (26): Effect of inoculation with biofertilizer agents and infection with *R. solani* on the weight of fruits of cucumber plants.

Table (27):Effect of inoculation with biofertilizer agents and infection with *R. solani* on the total nitrogen and total protein percentage of fruits of cucumber plants.

Biofertilizer agents	Total N% of fruits		Total protein % of fruits	
	uninfected	Infected	uninfected	Infected
Control	1.15 fgh	1 h	7.18 fgh	6.25 h
Azospirillum	1.47 ab	1.22 efg	9.18 ab	7.62 efg
Azotobacter	1.3 def	1.26 efg	8.12 def	7.87 efg
Azospirillum + *Azotobacter*	1.5 ab	1.4 cde	9.37 ab	8.75 cde
Streptomyces	1.18 fgh	1.1 gh	7.37 fgh	6.87 gh
Azopirillum + *Streptomyces*	1.42 bed	1.36 def	8.87 bcd	8.5 def
Azotobacter +*Streptomyces*	1.47ab	1.16 fgh	9.18 ab	7.25fgh
Mixed	1.52 a	1.4 cde	9.5 a	8.75cde
L.S.D. 0.05%		0.2113		1.312

Uninfected:



Infected:

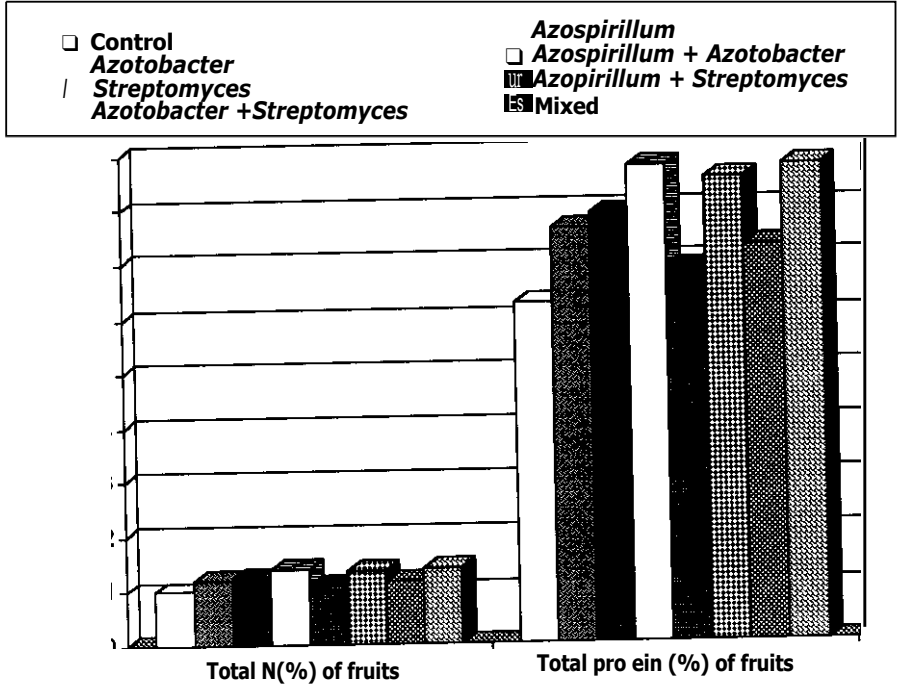


Fig (27):Effect of inoculation with biofertilizer agents and infection with *R. solani* on the total Nitrogen and total Protein percentage of fruits of cucumber Plants.

The maximum significant increase for biofertilizer agents in uninfested soil was 1.52% and 9.5% using a mixture of *Azotobacter Azospirillum* with or without *Streptomyces,* but the lowest one was1.18% and 7.37% under *Streptomyces* inoculation as compared with control 1.15% and 7.18% for total N and total protein, respectively.

In contrast, in infested soil total N and total protein percentage decreased to 1.4% and 8.75% in a mixture treatment comparing with control infested soil 1.0% and 6.25 % and the lowest effect was 1.1% and 6.87 % under *Streptomyces* inoculation, respectively.