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BIOLOGICAL CONTROL OF *CUCUMBER MOSAIC VIRUS* BY CERTAIN LOCAL *STREPTOMYCES* ISOLATES

1- Inhibitory Effects of Selected five Egyptian Isolates

[23]

El-Dougdoug[2], Kh.A; Ghaly[1], M.F. and Taha[1], M.A.

ABSTRACT

An antiviral producing *Streptomyces* species were isolated from soil rhizosphere in Zagazig province of Egypt. In order to identify the *Streptomyces* strains, morphological, physiological, biochemical and antagonism testes were performed. The Egyptian isolates of *Streptomyces* were found to be a species of *calvus, canarius, vinaceusdrappus, nogalater* and *viridosporus*. The *Streptomyces* spp were grown in glycerol asparagine broth medium and the culture supernatants obtained were filtered through 0.45 µl filter. These isolates were tested in two experiments for their ability to control a *Cucumber mosaic virus* (CMV). In the 1st experiment, One half of leaves of *Chenopodium amaranticolor* were treated with culture filtrate (CF) followed by CMV inoculation on both halves. In the 2nd experiment, The first pair of *Cucumis sativus* leaves were treated CF with CMV mechanically inoculated onto one leaf, the other non-treated leaf was CMV inoculated after 7 days of treatment. In 1st experiment, CF treatment was able to considerably reduced the number of local lesion and in 2nd experiment, plants treated with CF showed variable visible viral symptoms compared with the broth media treated control 15 days post inoculation and remained symptom less throughout the study period. Such five *Streptomyces* species identified were able to produce an antiviral component in the culture filtrate, non phytotoxic and effective in local as well as systematically control of CMV infection.

Keywords: Culture filtrate, CMV, local lesions, *Streptomyces* spp, acquired resistance.

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[1]Botany Dept., Fac. Sci., Zagazig University.
[2]Microbiology Dept., Fac. Agric., Ain Shams University, Egypt.

INTRODUCTION

Cucumber mosaic virus (CMV), belonging to the genus *Cucumovirus*, family *Bromoviridae* is one of the economically important viruses which causes enormous losses by infecting more than 1,000 species of plants, shrubs and trees world-wide. It is transmitted non-persistently into healthy plants by aphids which acquire the virus during their brief probes on infected hosts or the symptom less carrier weeds in the field **(Zehnder *et al.*, 2000)**. Various strategies based on the avoidance of sources of infection, control of vectors, modification of cultural practices, use of resistant varieties and transgenic plants have been conventionally employed to minimize the losses caused by CMV. These strategies; however, have not been effective as control measures. Many screening studies have been conducted on antiviral agents from different sources. Most of these come from plants sources with some showing systemic control ability against range of viruses that infect plants **(Kubo *et al.*, 1990)**. Comparatively, antivirals from microbial sources have been little studied. Recently, **Raupach *et al.* (1996)** showed the systemic control of CMV in cucumbers and tomatoes employing rhizosphere colorization of some bacteria by an induced systemic infection mechanism. **Kim *et al.* (2004)** used culture filtrate from *Acinetobacter* sp. KTB3 to systematically control some viruses in Korea.

This investigation was primarily concerned with the identification of five Streptomycetal isolates based on cultural growth, morphological, physiological, biochemical, tolerance of salinity, melanin pigment and antibiosis methods. Also, study the effect of heat stable culture filtrate of some *Streptomyces* spp isolates as antiviral substance against CMV, which produce local lesions in the hypersensitive host and systematically infects many important plants.

MATERIALS & METHODS

Collection of soil samples:

Rhizosphere soil samples (silty clay soil) under cultivated different crops were collected from different locations Zagazig, Hehia, Abu-Kabir and Fakous belong to El-Sharkia Governorate according to the procedures described by **Johnson *et al.* (1960)**. In this method, soil samples were collected by sterilized hand corer at depth of 15 cm from different regions in clean plastic bags. The collected samples were transferred

to the laboratory and kept in refrigerator till used.

Isolation and purification of *Streptomyces* spp.:

The collected soil samples were air-dried, ground in a mortar and then mixed with calcium carbonate ($CaCo_3$) and followed by sieving in 4 mm mesh screen. One gram of prepared soil was stirred in 100 ml sterile distilled water for about 5 min in a 250 ml Erlemeyer flask and the suspension allowed to stand for 30 min. Serial dilution (10^{-1}, 10^{-5}) of each obtained suspension were prepared in sterile saline solution (0.85% NaCl) and 1ml of each dilution was spread on Petri-dish containing 15 ml of starch nitrate agar medium according to **Tadashi (1975).** The dishes were rotated by hand to insure homogenous distribution of soil suspension dilution and then incubated for 7 days at 28 °C till *Streptomyces* colonies appearance.

The purification was achieved according to **Kuster and Williams (1964)** by picking up of unique single identical morphological *Streptomyces* colony based on cultural morphological characters and repeated streaking on starch nitrate agar plates. Each of these was assayed for antiviral activity using the half- leaf method as described by **Kubo *et al.* (1990).**

Five selected Streptomycetal isolates out of 30 actinomycetal isolates were used for identification and comparative study of the physiological, morphological, biochemical, tolerance of salinity, melanin pigment and antibiosis analyses, according to **Bergey's Mannual (1989).**

Morphological and physiological tests:

In order to identify the Streptomycetal isolates 48 physiological and biochemical tests were carried out. These tests included growth in different cultural media such as starch nitrate agar, glycerol nitrate agar, inorganic starch agar, Nutrient agar, glucose asparagines agar, yeast malt agar, glucose nitrate agar, glycerol asparagines agar, sucrose nitrate agar and oat meal. Morphological characters, presence or absence of sporangium, length of sporangiophore, spore mass, spore surface, spore chain, spore shape. Physiological characters, melanin pigment, proteolytic activity, lipolytic activity, lethicine activity, cellulytic activity, gelatin liquefaction, growth in different carbon sources, growth at different temperature, tolerance to NaCl and antibiosis were determined.

Maintenance of virus:

CMV was kindly obtained from Virology Lab. Microbiology Dept., Fac. of Agric., Ain Shams University and maintained in *N. glutinosa* as CMV- propagation host. The inoculum of CMV was prepared from systematically infected *N. glutinosa* leaves ground in 0.1 M phosphate buffer, pH 7.2.

Antiviral bioassay:

The Streptomycetal species was grown in glycerol asparagine broth medium and the culture supernatants obtained were filtered through 0.45 µl litter. The antiviral activity of the CF from five selected *Streptomyces* spp was estimated in two experiments. In the first experiment, CF was assayed on a hypersensitive host for CMV, i.e. *C. amaranticolor* using the half leaf method, as previously described by **Kubo *et al.* (1990)**. The upper right halves of the leaves were treated with CF using paintbrush, and the upper left halves were treated with sterilized water as a control treatment. After one hour, the virus was inoculated onto both halves of the leaves. The experiment was performed in duplicate. The plants were kept in a greenhouse, at 12-14 h daylight and a temperature of 30°C. The number of local lesions were counted after seven days post inoculation. The inhibitory effect was calculated according to the formula: I = (1-T/C) x 100, where, T the number of local lesions on the treated half of the leaves and C is the number of local lesions on the control half of the leaves. In the second experiment, the 1st pair of cucumber (*Cucumis sativus*) cv Barakoda leaves were used under each treatment. One leaf was treated with CF, the other non-treated leaf was CMV inoculated after 7 days of treatment. As a control treatment, one leaf was treated with broth media, another untreated leaf was CMV inoculated after 7 days. Each experiment was replicated three times.

RESULTS

Identification of Streptomycetal isolates:

The five *Streptomyces* spp were isolated from soil rhizosphere according to variation in growth rate on differential media, morphological and biochemical tests. Five isolates were appeared variation in growth rate on differential media **(Table 1).** *St. viridosporus* was revealed variable growth on nutrient agar medium while *St. calvus* revealed weak rate growth on glycerol nitrate agar, nutrient agar, glucose asparagines agar and sucrose nitrate agar medium compared with other isolates. On the other hand, *St.*

vinaceusdrappus and *St. nogalater* were revealed strong growth rate on experimental media **(Table 1).** All five isolates showed diffusible pigment in all experimental media under study. Visual observations by light and electron micrographs of the five isolates showed that no sporangium formation and different in sporangiophore where as, 6µm, 4.5, 7.5, 4 and 3 for *St. calvus*, *St. canarius*, *St. vinaceus-drappus*, *St. nogalater* and *St. viridosporus*, respectively **(Figure 1).** In related to the spore chain were different in among five isolates, spiral short, spiral long, spiral open long, spiral open long and spiral long with *St. calvus*, *St. canarius*, *St. vinaceus-drappus*, *St. nogalater* and *St. viridosporus*, respectively. **(Figure 1)** In addition, the spore mass also differed between five isolates such as dark grayish, yellowish white, pale brownish, yellow brownish and green for *St. calvus*, *St. canarius*, *St. vinaceusdrappus*, *St. nogalater* and *St. viridosporus*, respectviely. The spore surface of *St. calvus* was hairy, *St. viridosporus* was spiny while *St. canarius*, *St. vinaceus-drappus* and *St. nogalater* were smooth. Conidiospore morphology were differed among 5 isolates whereas *St. calvus* and *St. viridosporus* were revealed oval shape with diameter 12x17 and 10 x 13mm respectively while, *St. canarius*, *St. vinaceusdrappus* and *St. nogalater* were revealed barrel shape with 7 x 20, 9 x 11 and 8 x 10 mm respectively **(Table 2).** Data represented in **Table (3)** recorded that *St. canarius & St. viridosporus* were able to secrete melanin pigment on tyrosine agar medium while *St. calvus*, *St. vinaceus-drappus* and *St. nogalater* not able. On the other hand five isolates were not able to secrete on tryptone broth and peptone yeast iron agar media.

Data also showed that 5 isolates have proteolytic and amylolytic activity while only *St. viridosporus*, had cellulytic activity. On other hand, *St. calvus*, *St. vinacousdrappus* and *St. nogalater* have pectinolytic activity while *St. canarius* and *St. viridosporus* not have. The result showed that only *St. canarius* able to lethicine degradation while the others not able. On the other hand 5 isolates able to make gelatin liquefaction and H_2S production as well as *St. vinaceus-drappus* and *St. nogalater* can reduce nitrate but other isolates can't reduce **(Table 3).**

Data showed that *St. calvus, St. vinaceusdrappus, St. nogalater* and *St. viridosporus* gave variable growth on medium without carbon source while *St. canarius* can't grows. On other hand 5 isolates gave

different growth rates on media with different carbon sources except *St. calvus* can't grow in medium with maltose as carbon source.

All 5 isolates showed antimicrobial potentialities against tested organisms except *St. vinacousdrappus* , *St. nogalater* and *St. viridosporus* not showed against *E. coli*. On the other hand, *Helminthosporium solani* appeared the most sensitive one for 5 isolates followed by *Fusarium* sp followed by *Staph. aureus* and finally *E. coli*. The data showed that fungal isolates were more sensitive to 5 isolates than bacterial isolates. On the other hand, *St. canaries* showed the higher antimicrobial potentialities against tested organisms due to increasing in the diameter of inhibition zone **(Table 4).**

Antiviral effects of Streptomycetal isolates.

The antiviral culture filtrate from five selected Streptomycetal isolates showed high inhibitory activity against CMV. The CF treated part of the hypersensitive host; *C. amaranticolor* leaves showed 70.2%, 71.4%, 74.4%, 80% and 82.6% inhibition of the production of local lesions compared to the untreated part of the leaves for *Streptomyces calvus, Streptomyces canarius, Strepotomyces vinaceusdrappus, Streptomyces nogalater* and *Streptomyces viridosporus* respectively. The control plants, treated with sterilized water were unable to show inhibition of CMV induced lesions **(Table 5).** The average number of local lesions in the case of the CF treated half leaves were much lower 36.2, 21.75, 32, 37,25 and 25 respectively than those of the sterilized water treated half leaves (125).

The five Streptomycetal isolates appeared CMV variability based on the variation of single L.L. *St. calvus* induced heterologous chlorotic L.L, 1 and 3 mm in diameter with reduction percentage 71.4%, **Figure (3-1).** *St. canarius* induced homologous chlorotic L.L. surrounding with brown halo, 2 mm in diameter with reduction percentage 82.6%, **Figure (3-10).** *St. vinaceusdrappus* produced homologous necrotic L.L, without halo, 1.5 mm in diameter with reduction percentage 74.4%, **Figure (3 -19).** *St. nogalater* induced ring necrotic L.L surrounded with yellow and brown halo, 3 mm in diameter with reduction percentage 70.2%, **Figure (3-28).** *St. viridosporus* produced ring necrotic L.L surrounded with yellow and brown halo, 3 mm in diameter with reduction percentage 80%, **Figure (3-29)** and the positive control produced necrotic L.L, 1.5 mm in a

diameter. The obtained previous screening Streptomycetal isolates results for induction systemic acquired resistance in cucumber plants against CMV infection revealed that, the most effective individual isolates were five *Streptomyces elicitors, Streptomyces calvus, Streptomyces canarius, Streptomyces vinaceusdrappus, Streptomyces nogalater and Streptomyces viridosporus* with no.1,10,19,28 and 29 respectively. These isolates were completely identified and used for next further studies. Five *Streptomyces* inducers out of 30 actinomycetal isolates (1) *St. calvus*, (10) *St. canarius*, (19) *St. vinaceusdrappus*, (28) *St. nogalater* and (29) *St. viridosporus* out of twenty three Streptomycetal isolates were used for induction systemic acquired resistance (SAR) in cucumber plants against CMV infection. The induced systemic resistance was detected by different methods, biologically (percentage of infection, disease severity (DS), virus variability). The five *Streptomyces* treatments have different percentage of DS, *St. nogalater* has a low percentage of DS (4.4%), while *St. canarius* has a high percentage of DS (22.2%). The other isolates *St. calvus*, *St.vinaceusdrappus* and *St. viridosporus* have DS percentage 10, 20, 10%, respectively. Individual *Streptomyces* treatments in **Table (6)** showed that, the selected five *Streptomyces* isolates reduced the percentage of CMV infection as follow: 71.4, 82.6, 74.4, 70.2 and 80% for (St 1), (St 10), (St 19), (St 28) and (St 29), respectively. The highest percentage of reduction was 82.6% for St 10, while St 28 isolate has the lowest percentage of reduction 70.2%.

The obtained previous results showed that, the filtrate spraying of *Streptomyces* induced SAR in cucumber plants which played the major role to reduced CMV infection. On other hands showed significant variations in symptoms severity and vegetative growth of CMV infected cucumber plants **(Figures 4&5)** compared with untreated CMV infected Cucumber plants as infected control **(Figures 4, 5, IC).**

The CMV symptoms on treated cucumber plants after CMV inoculation were differed such as; vein clearing, mosaic, malformation and blisters **(Figures 4, 5).**

Table 1. Cultural characteristics of *Streptomyces* spp.

Media	Cultural	*Streptomyces* spp				
		St. calvus	*St. canarius*	*St. vinacensdrappus*	*St. nogalaten*	*St. viridosporus*
Starch nitrate agar	Growth	++++	+++	++++	++++	++++
	Aerial mycelium color	Dark black grayish	Yellowish white	Pale brownish	Dark brownish yellow	Dark greenish white
	Substrate mycelium color	Pale greenish	Brounich yellow	Pale greenish yellow	Dark greenish yellow	Dark greenish
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Glycerol nitrate agar	Growth	+	++	++++	++++	+++
	Aerial mycelium color	Dark greyish	Yellowish white	Pale brownish grey	Dark brownish yellow	Dark greenish white
	Substrate mycelium color	Pale greenish	Pale yellowish	Pale brownish	Dark greenish yellow	Dark green
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Inorganic starch agar	Growth	+++	++++	++++	++++	++++
	Aerial mycelium color	Dark grayish green	Pale yellowish white	Pale brownish	Brownish yellow	Dark greenish white
	Substrate mycelium color	Pale greenish	Pale brownish yellow	Pale greenish	Dark brownish yellow	Greenish
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Nutrient agar	Growth	+	++	++	++	V
	Aerial mycelium color	Dark grayish	Pale yellowish white	Pale brownish	Yellowish grey	White
	Substrate mycelium color	Pale greenish	Pale yellowish	Pale yellowish	Pale yellowish	Pale yellowish
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Glucose asparagines agar	Growth	+	++	+++	+++	++
	Aerial mycelium color	Dark grayish	Pale yellow	Pale brownish grey	Dark brownish grey	Dark greenish white
	Substrate mycelium color	Pale greenish	Pale yellowish	Pale greenish yellow	Yellowish brown	Dark green
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Yeast malt agar	Growth	++	++	+++	++	+++
	Aerial mycelium color	Greenish grey	Pale yellowish white	Pale brounish grey	Pale brownish yellow	Dark greenish white
	Substrate mycelium color	Yellowish green	Pale yellowish	Pale yellowish gren	Olive greenish yellow	Dark green
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Glucose nitrate agar	Growth	++	++	+++	+++	++++
	Aerial mycelium color	Dark olive grayish grey	Pale yellowish white	Pale brownish grey	Pale brownish yellow	Dark greenish
	Substrate mycelium color	Pale greenish	Pale yellowish	Yellowish grey	Dark yellowish	Dark green black
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Glycerol asparagines agar	Growth	++	+++	+++	+++	+++
	Aerial mycelium color	Dark olive greenish grey	Pale yellowish white	Pale brownish gray	Pale brownish yellow	Dark greenish
	Substrate mycelium color	Pale greenish	Pale yellowish	Yellowish gray	Pale yellowish	Dark green
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Sucrose nitrate agar	Growth	+	+++	++++	++++	++++
	Aerial mycelium color	Yellowish pale greenish	Pale yellowish white	Pale brownish	Pale brownish yellow	Dark green
	Substrate mycelium color	Pale yellowish	Pale yellowish	Pale yellowish green	Dark greenish yellow	Greenish
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve
Oat meal	Growth	+++	++	+++	++	++++
	Aerial mycelium color	Dark green olive	Pale yellowish white	Pale brownish grey	Pale greenish grey	Pale greenish
	Substrate mycelium color	Pale greenish	Pale yellowish	Pale yellowish green	Pale yellowish	Dark green
	Diffusible pigment	ˉve	ˉve	ˉve	ˉve	ˉve

Growth:

++++ : Very strong growth
+++ : Strong growth
++ : Moderate growth
V : Variable growth

ˉve: negative
⁺ve: positive
+ : Weak strong

Table 2. Morphological characteristics of *Streptomyces* spp.

Morphological characters	Streptomycetal				
	St. calvus	*St. canarius*	*St. vinaceusdrappus*	*St. nogalater*	*St. viridosporus*
Sporangium	-	-	-	-	-
Sporangiophore	6µm	4.5 µm	7.5 µm	4 µm	3 µm
Spore mass	Dark grayish	Yellowish white	Pale brownish	Yellow brownish	Green
Spore surface	Hairy	Smooth	Smooth	Smooth	Spiny
Spore chain	Spiral short	Spiral long	Spiral open long	Spiral open long	Spiral long
Spore shape	Oval	Barrel	Barrel	Barrel	Oval
*** Dimension**	12x17mm	7x20mm	9x11mm	8x10mm	10x13mm

- Magnification 10,000 X
- - Absence of sporangium

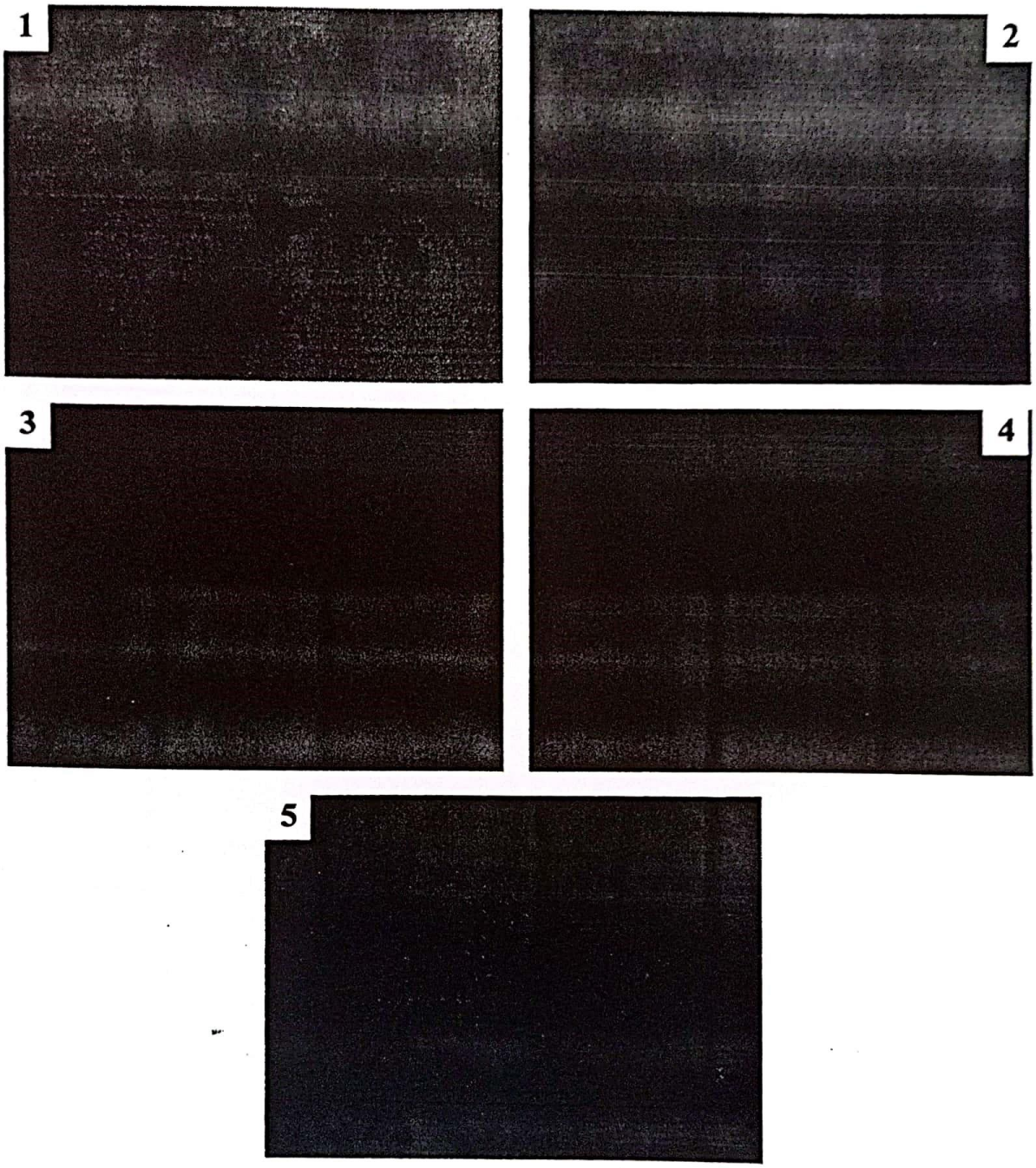

Figure 1. Light micrograph of spore chains for *Streptomyces* spp (X 200).
1: *St. calvus* 2: *St. canarius*
3: *St. vinaceusdrappus* 4: *St. nogalater*
5: *St. viridosporus*

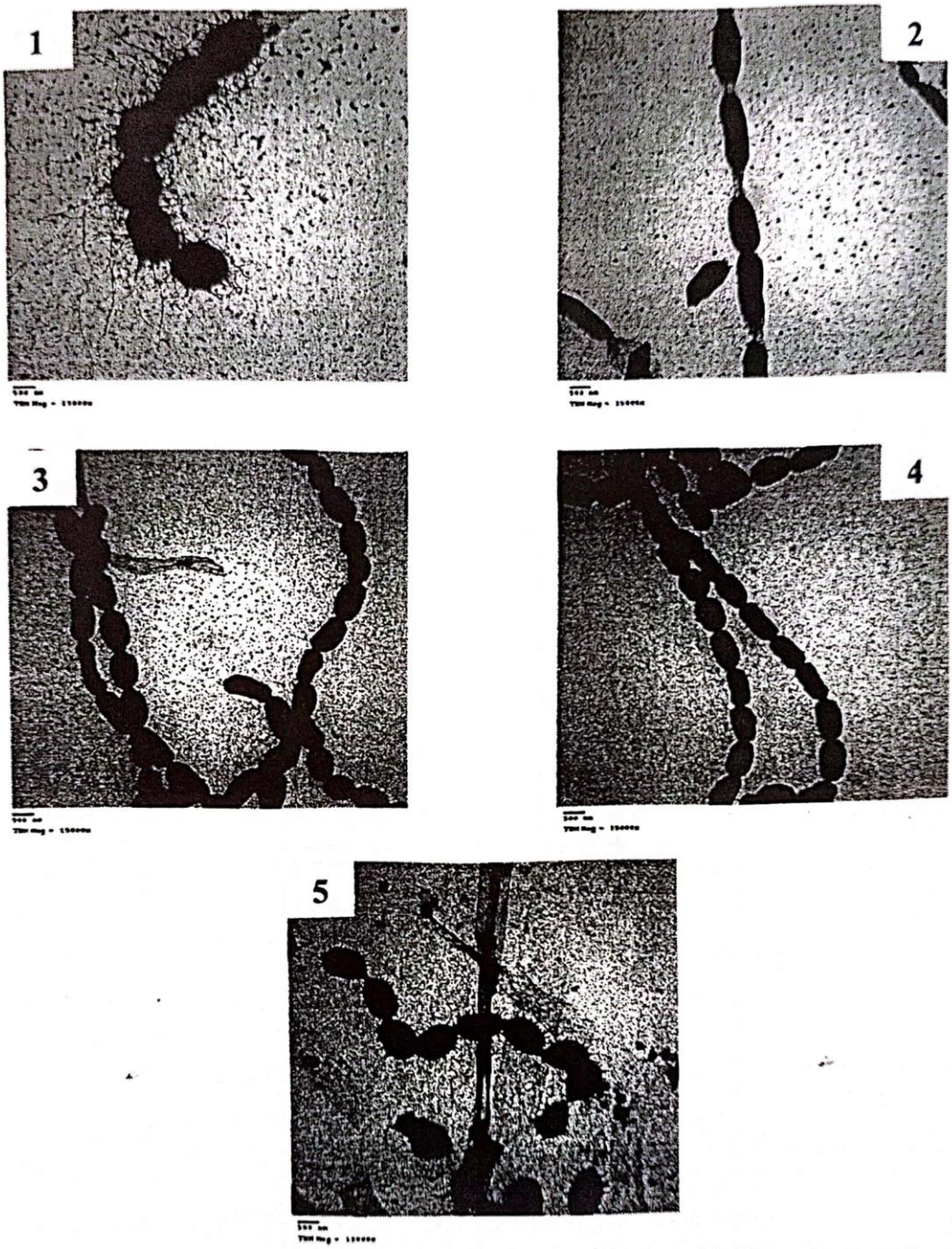

Figure 2. Electron micrograph of spore surface for Streptomycetal isolates (X 15000).

1: *St. calvus* 2: *St. canarius*
3: *St. vinaceusdrappus* 4: *St. nogalater*
5: *St. viridosporus*

Table 3. Physiological characteristics of *Streptomyces* spp.

Physiological characters	Streotomycetal isolates				
	St. calvus	*St. canarius*	*St. vinaceusdrappus*	*St. nogalater*	*St. viridosporus*
Melanin pigment					
Tyrosine-agar	-ve	+ve	-ve	-ve	+ve
Tryptone-broth	-ve	-ve	-ve	-ve	-ve
Peptone yeast					
Iron agar	-ve	-ve	-ve	-ve	-ve
Enzymatic activity					
Proteolytic activity	+ve	+ve	+ve	+ve	+ve
Lipolytic activity	-ve	+ve	+ve	-ve	+ve
Lethicine activity	-ve	+ve	-ve	-ve	-ve
Amylolytic activity	+ve	+ve	+ve	+ve	+ve
Pectinolytic activity	+ve	+ve	+ve	+ve	-ve
Celurlytic activity	-ve	-ve	-ve	-ve	+ve
Gelatin Liquefaction	+ve	+ve	+ve	+ve	+ve
H2S production	+ve	+ve	+ve	+ve	+ve
Nitrate reduction	-ve	-ve	+ve	+ve	-ve
C-source					
No carbon	v	-	v	v	v
D-glucose	++	++	+	+	+++
D-fructose	+	++	+	++	+++
Sucrose	+	++	+	+	++
D-mannitol	+++	++	++	++	++++
D-xylose	++	++	++	++	+++
L-Arabinose	+	++	++	+	+
L-Rhmnose	++++	+++	+++	++	++
Lactose	+	++	+	+	+++
I-inositol	+++	+++	++	++	+++
Maltose	-	+	++	++	++
Mannose	++	++	+	++	++++
Galactose	++	+++	+++	++	+++
Tolerance to NaCl					
2%	++++	++++	++++	++++	++++
4%	++	+++	+++	+++	++
6%	-	++	++	++	-
8%	-	-	-	-	-
10%	-	-	-	-	-
12%	-	-	-	-	-
Growth at different temperature					
20	+	+	+	+	+
30	++++	++++	++++	++++	++++
40	++	++	++	++	++
50	-	-	-	-	-

++++ : Very strong growth +++ : Strong growth ++ : Moderate growth

+ : Weak growth v : Variable growth - : No growth

Table 4. Antimicrobial activity of *Streptomyces* spp:

Streptomycetal Isolates / Tested organism	*St. calvus*		*St. canarius*		*St. vinaceusdrappus*		*St. nogalater*		*St. viridosporus*	
	Relative inhibition	Diameter of inhibition zone (mm)	Relative inhibition	Diameter of inhibition zone (mm)	I Relative inhibition	Diameter of inhibition zone (mm)	Relative inhibition	Diameter of inhibition zone (mm)	Relative inhibition	Diameter of inhibition zone (mm)
Fusarium Sp.	28.6	20	71.4	50	21.4	15	21.4	15	42.8	30
Helminthosporium solani	42.8	30	57.1	40	42.8	30	42.8	30	35.7	25
Staph. aureus	21.4	15	28.6	20	14.2	10	28.6	20	17.1	12
E. coli	18.6	13	18.6	13	0	-	-	-	-	-

Table 5. Single local lesion variability of CMV-inoculated plants treated with *Streptomyces* isolates.

Streptomyces isolates	Variability				% inhibition
	No of L.L	Similarity	Mean diameter of L.L	Morphology	
St. calvus	36.2	Heterologous	1and 3 mm	Chlorotic L.L	71.4
St. canarius	21.75	Homologous	2 mm	Chlorotic L.L surrounded with brown halo	82.6
St. vinaceusdrappus	32	Homologous	1.5 mm	Necrotic L.L without halo	74.4
St. nogalater	37.25	Homologous	3 mm	Ring necrotic L.L surrounded with yellow and brown halo	70.2
St. viridosporus	25	Homologous	3 mm	Ring necrotic L.L surrounded with yellow and brown halo	80
+ve control	125	Homologous	1.5 mm	Necrotic L.L	0.00

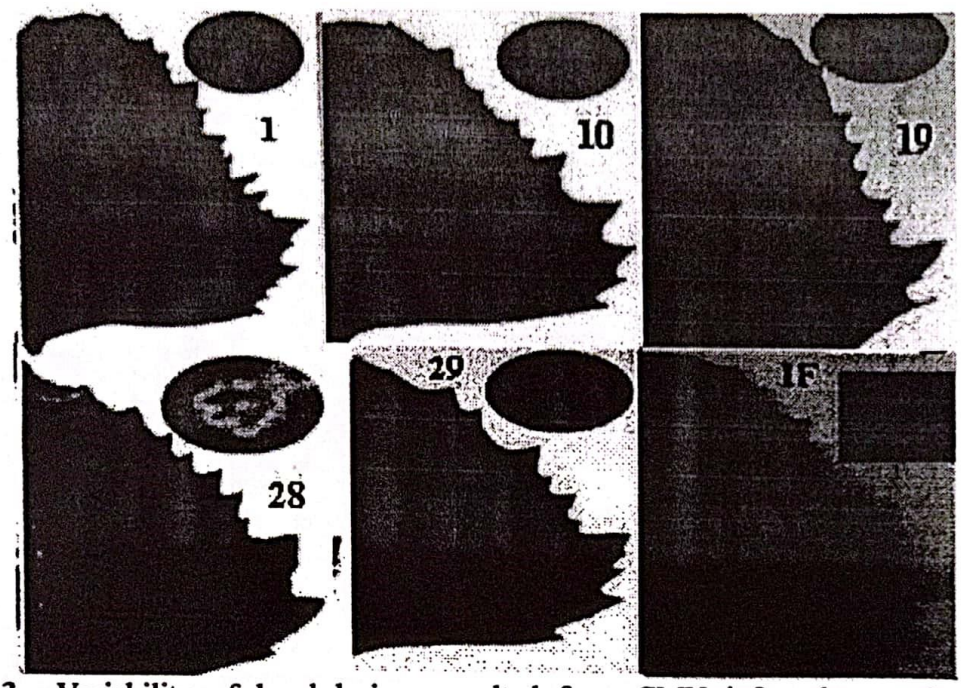

Figure 3. Variability of local lesions resulted from CMV infected cucumber plant treated with 5 Streptomycetal isolates using *C. amaranticolor* as CMV indicator host

* All photo numbers point to numbers of different isolated *Streptomyces* as used in this work.

Table 6. Effect of individual inducers on CMV infectivity in cucumber.

Treatments	% of infection	% of R.I	DS (%)	Virus concentration
Infected control	100	0	65.4	125
St. calvus	28.6	71.4	10	36.2
St. canarius	17.4	82.6	22.2	21.7
St. vinaceusdrappus	25.6	74.4	20	32
St. nogalater	29.8	70.2	4.4	37.25
St. viridosporus	20	80	10	25

R.I (Reduction of virus infection) = (1 - T/C) x 100
T = Treated plants C = Control DS = Disease severity
Conc.= Virus concentration (mean number of local lesion) calculated from 5 replicates

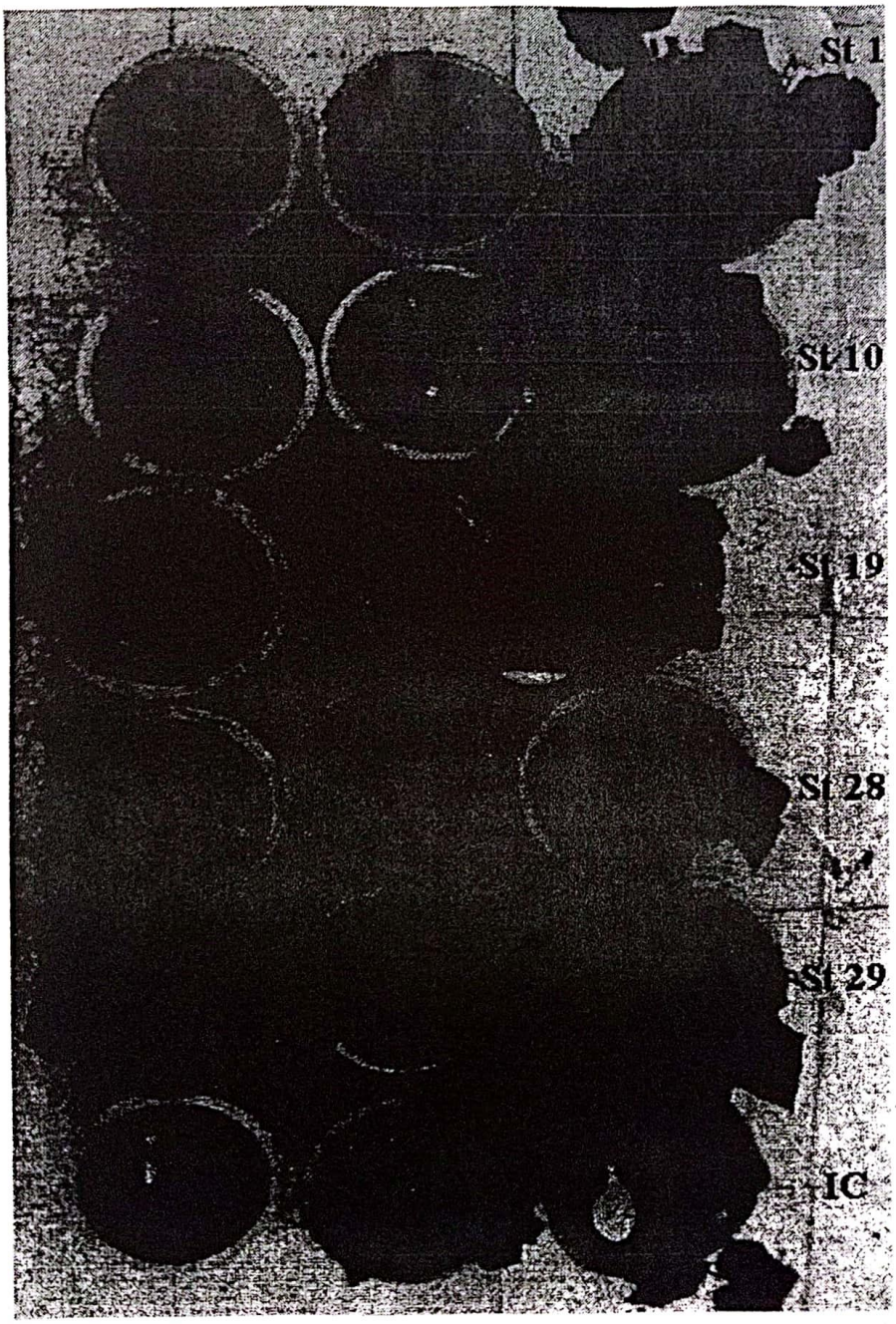

Figure 4. Illustrate the cucumber plant treated with different filtrate of Streptomycetal isolates.

St 1: *St. calvus* St 10: *St. canarius*
St 19: *St. vinaceusdrappus*
St 28: *St. nogalater* St29: *St. viridosporus*
IC: Infected control.

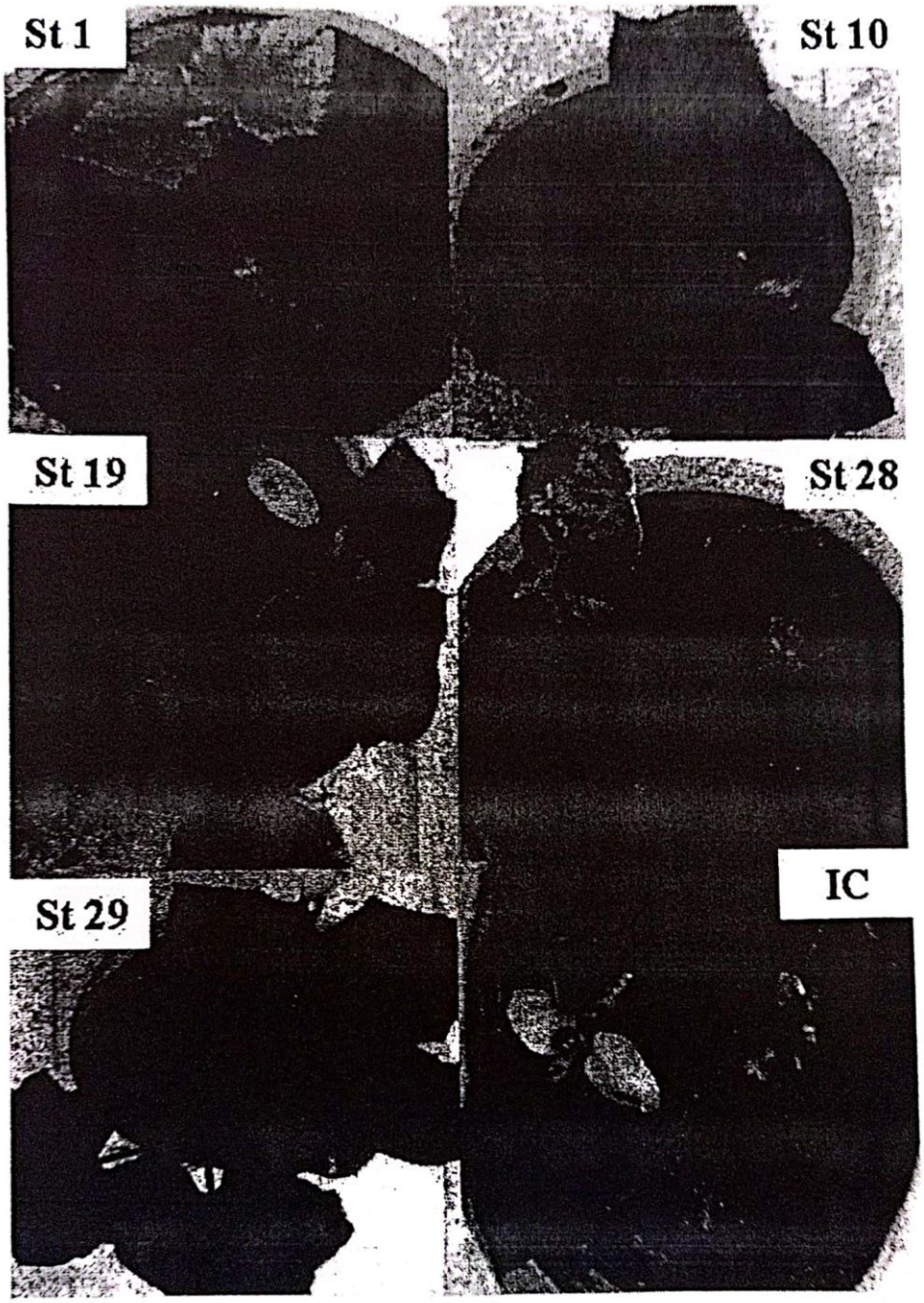

Figure 5. Development of different CMV symptoms in cucumber plants treated with different filtrate of Streptomycetal isolates.
St 1& St 10: vein clearing.
St 19, St28 & St 29: mosaic, malformation and blisters.
IC: infected control.

DISCUSSION

An antiviral producing Streptomycetal species were isolated from soil rhizosphere in Zagazig province of Egypt. In order to identify the Streptomycetal strains, morphological, physiological, biochemical and antagonism testes were performed. The Streptomycetal were found to be a species of *Streptomyces calvus, Streptomyces canarius, Strepotomyces vinaceusdrappus, Streptomyces nogalater* and *Streptomyces viridosporus* which were designated as Egyptian isolates. The collected actinomycete isolates were subjected for a process of purification using the specific nutrient growth medium of starch-nitrate agar. The purification of the actinomycete isolates was conducted by means of different purification media which included starch nitrate agar and starch-inorganic media **(Abo-Elanain, 2004)**. Many authors reported that, the isolation of one hundred and ten isolates of actinomycete cultures from eight soil samples was carried out using the conventional dilution plate method on Humic acid-vitamin agar, starch casein agar and sorenson's agar **(Tan *et al.*, 2001)**. The purified actinomycete isolates were subjected to screening against program of antimicrobial activities. Most of isolates exhibited antimicrobial activities against Gram-positive, Gram-negative, acid-fast bacteria, yeasts and filamentous fungi **(El-Abyad *et al.*, 1996)**. Actinomycetal isolates were tested for their antagonistic potentialities according to diffusion (Cork borer) method **(Betina, 1983)**. All 5 isolates showed antimicrobial potentialities against tested organisms except *St. vinaceusdrappus* , *St. nogalater* and *St. viridosporus* not showed against *E. coli*. On the other hand, *Helminthosporium solani* appeared the most sensitive one for 5 isolates followed by *Fusarium* sp followed by *Staph. aureus* and finally *E. coli*. The data showed that fungal isolates were more sensitive to 5 isolates than bacterial isolates. On the other hand, *St. canarius* showed the higher antimicrobial potentialities against tested organisms due to increasing in the relative inhibition. This percentage is agreed with those described by many authors studying the activity of soil actinomycetes **(Saadoun and ElMomani, 1997; Saadoun *et al.*, 1998; Ndonde and Semu, 2000)**. The highest antimicrobial

Streptomycetal isolates selected and inoculated in various liquid nutrient media for investigating its antiviral activity against CMV. Concerning of identification of the most active Streptomycetal isolates that have antiviral activities. The morphological and physiological properties of the actinomycete isolates no. 10 are consistent with assignment of *Streptomyces canarius*. This reveals that the antiviral activity of CF from Streptomycetal isolates were due to involvement of plant defense mechanism. In both the above experiments, no damage to the host plant was observed due to CF treatment. CF; thus, can be characterized as a non-toxic antiviral agent, which would give the necessary efficiency in combating CMV. The activity of the inhibitory agent present in the CF obtain from Streptomycetal isolates was non-toxic and induces protection against CMV in both local as well as systemic hosts. *Cucumber mosaic cucumovirus* (S. CMV. EG) was obtained from Virology Lab, Agric Microbiological Dept., Fac. of Agric., Ain Shams University. Data indicates that the translocation of the antiviral effect from the CF treated half-leaf to the untreated part of the same leaf. When CF was used to elucidate the systemic control effect of Streptomycetal isolates, it was found that the plants treated with CF showed no visible viral symptoms 25 dpi (days post inoculation), and remained symptom less throughout the study period. The plants treated with broth media showed symptom less. CMV was confirmed by single local lesion assay of *C. amaranticolor* as reported by many investigators **(Polak, 1999; El Baz, 2004; El-Afifi *et al.*, 2007 and Megahed, 2008)**. CMV was transmitted mechanically to healthy susceptible test plants **(Polak, 1999, Paradies *et al.*, 2000; El-Baz, 2004; Awasthi *et al.*; 2005; Hu and Chang, 2006. El-Afifi *et al.*, 2007 and Megahed, 2008)**. Accordingly, anti-infective activity may induce one or more of the following activities:

1- Direct inactivation virus by the extract without affecting cell receptors or intracellular targets i.e., virucidal effect. This may be achieved by blocking the virus receptor molecules, or by virolysis if the inactivant has an enzymatic activity, or physically by antagonizing the net electric charge that lead to virus attraction to the host cells,

or by increasing the size of the virion and preventation of the fitting into the receptor.

2- Induction of changes in cell membrane or cellular receptor of the virus.

3- Preventation the virus adsorption and / or uncoating. The last two activities are related to the protective function of a drug.

The protective activity of an extract may include one or more of the following activities:

1- Induction changes at the cell membrane leading to inhibition of the virus adsorption and / or penetration.

2- Induction of changes in the cell lysosomes inhibiting virus uncoating.

3- Setting the intracellular biochemical mechanisms in such a way, which resists the virus replication as in case of interferon **(Dimmock and Primrose, 1994).** Finally, the anti-replicative activity may include one or more of the following activities:

1- Inhibition of virus uncoating, or Inhibition of cellular and / or viral translation mechanisms.

2- Inhibition of cellular and / or viral transcription and replication function.

3- Inhibition of viral protein processing and / or Capsid assembly or maturation.

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