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Biological Control of Cucumber Mosaic Virus by Certain Local *Streptomyces* Isolates: Inhibitory Effects of Selected Five Egyptian Isolates

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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 tests 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 0.45-µm 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.

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

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, antiviral 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 and 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 AND 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 transfer 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 Erlenmeyer 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 1 mL of each dilution was spread on Petri-dish containing 15 mL of starch nitrate agar medium. 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 Kuester 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.

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, cellulolytic 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 Streptomyces species was grown in glycerol asparagines broth medium and the culture supernatants obtained were filtered through 0.45 µm filter. 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: $I = (1 - T/C) \times 100$, 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 Streptomyces 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 (Fig. 2) of the five isolates showed that no sporangium formation and different in sporangiophore where as, 6 µm, 4.5, 7.5, 4 and 3 for *St. calvus*, *St. canarius*, *St. vinaceus-drappus*, *St. nogalater* and *St. viridosporus*, respectively (Fig. 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 (Fig. 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*, respectively. The spore surface of *St. calvus* was hairy, *St. viridosporus* was spiny while *St. canarius*, *St. vinaceusdrappus* and *St. nogalater* were smooth.

Conidiospore morphology were differed among 5 isolates whereas *St. calvus* and *St. viridosporus* were revealed oval shape with diameter 12×17 and 10×13 µm, respectively while, *St. canarius*, *St. vinaceusdrappus* and *St. nogalater* were revealed barrel shape with 7×20, 9×11 and 8×10 µm, respectively (Table 2). Data represented in Table 3 recorded that *St. canarius* and

Table 1: Cultural characteristics of *Streptomyces* spp.

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

Growth: +++++: Very strong growth, +++: Strong growth, ++: Moderate growth, +: Weak strong, -ve: negative, +ve: Positive, V: Variable growth

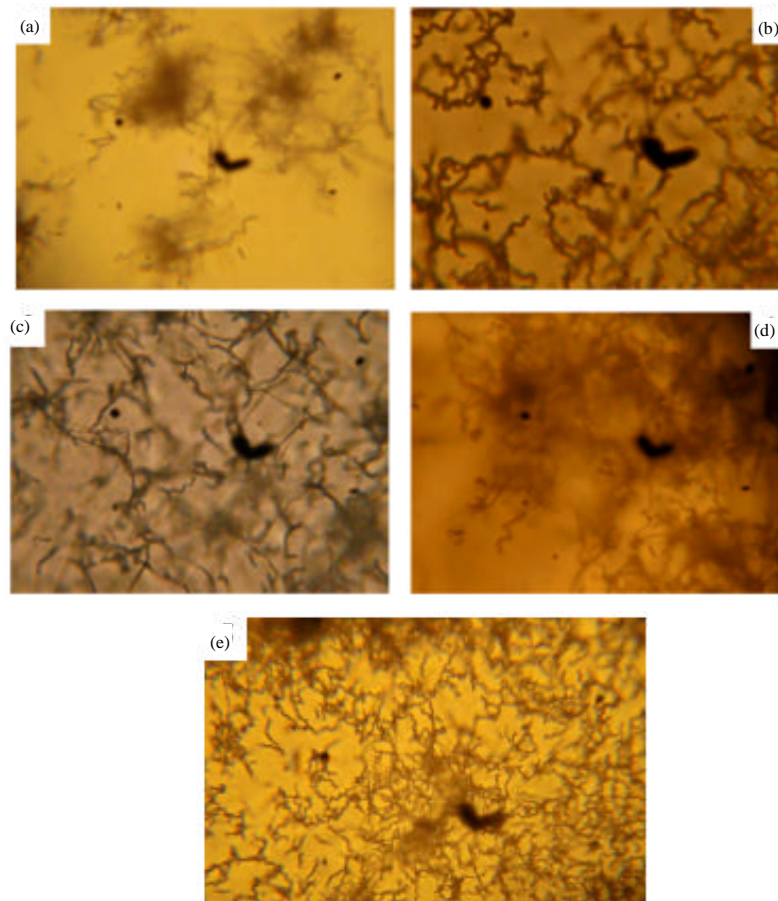


Fig. 1(a-e): Light micrograph of spore chains for *Streptomyces* spp. (X 200); (a) *St. calvus*, (b) *St. canaries*, (c) *St. vinaceusdrappus*, (d) *St. nogalater* and (e) *St. viridosporus*

Table 2: Morphological characteristics of *Streptomyces* spp.

Morphological characters	Streptomycetal				
	<i>St. calvus</i>	<i>St. canarius</i>	<i>St. vinaceusdrappus</i>	<i>St. nogalater</i>	<i>St. viridosporus</i>
Sporangium	-	-	-	-	-
Sporangiophore	6 µm	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	12x17 mm	7x20 mm	9x11 mm	8x10 mm	10x13 mm

Magnification 10,000 X, -: Absence of sporangium

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.

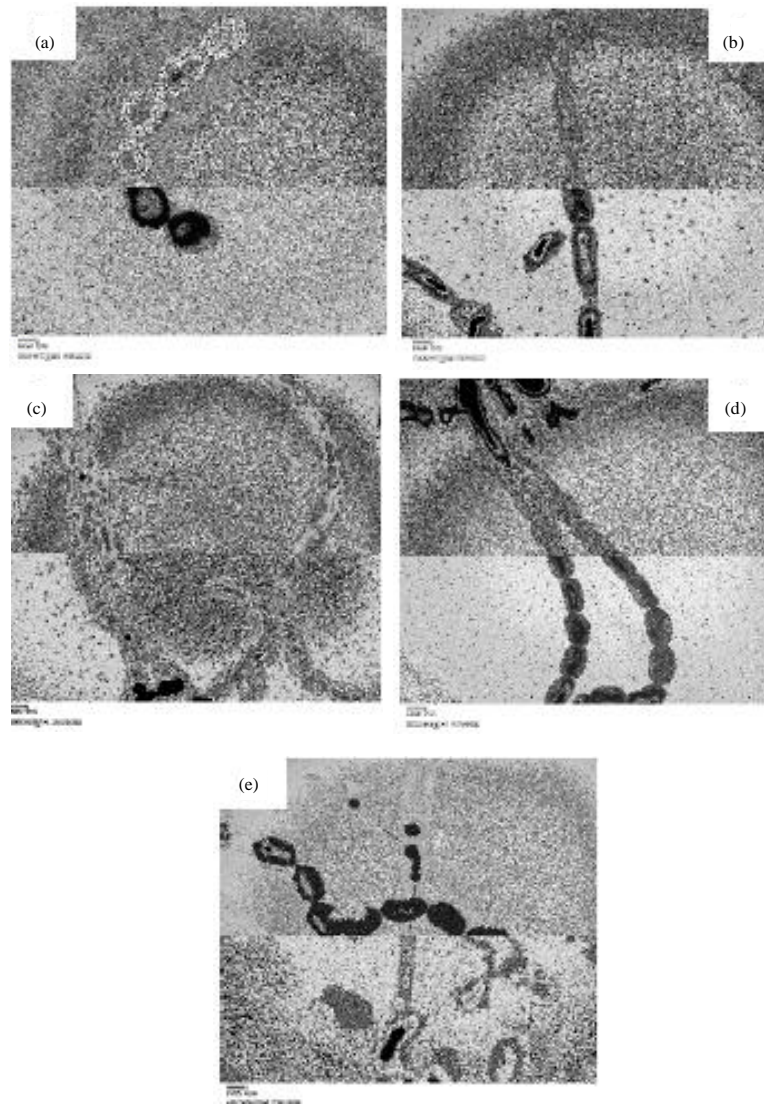


Fig. 2(a-e): Electron micrograph of spore surface for Streptomyces isolates (X 15000); (a) *St. calvus*, (b) *St. canarius*, (c) *St. vinaceusdrappus*, (d) *St. nogalater* and (e) *St. viridosporus*

Data also showed that 5 isolates have proteolytic and amylolytic activity while only *St. viridosporus*, had cellulytic activity. On other hand, *St. calvus*, *St. vinaceusdrappus* 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 H₂S production as well as *St. vinaceusdrappus* 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

Table 3: Physiological characteristics of *Streptomyces* spp.

Physiological characters	Streptomycetal isolates				
	<i>St. calvus</i>	<i>St. canarius</i>	<i>St. vinaceusdrappus</i>	<i>St. nogalater</i>	<i>St. viridosporus</i>
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
H ₂ S 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

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. 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. canaries* showed the higher antimicrobial potentialities against tested organisms due to increasing in the diameter of inhibition zone (Table 4).

Table 4: Antimicrobial activity of *Streptomyces* spp.

Tested organism	Streptomycetal isolates									
	<i>St. calvus</i>		<i>St. canarius</i>		<i>St. vinaceusdrappus</i>		<i>St. nogalater</i>		<i>St. viridosporus</i>	
	RI	DI (mm)	RI	DI (mm)	RI	DI (mm)	RI	DI (mm)	RI	DI (mm)
<i>Fusarium</i> sp.	28.6	20	71.4	50	21.4	15	21.4	15	42.8	30
<i>Helminthosporium solani</i>	42.8	30	57.1	40	42.8	30	42.8	30	35.7	25
<i>Staph. aureus</i>	21.4	15	28.6	20	14.2	10	28.6	20	17.1	12
<i>E. coli</i>	18.6	13	18.6	13	0	-	-	-	-	-

RI : Relative inhibition, DI: Diameter of inhibition zone

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

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

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*, *Streptomyces 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%, Fig. 3-St. 1. *St. canarius* induced homologous chlorotic L.L. surrounding with brown halo, 2 mm in diameter with reduction percentage 82.6%, St. 10 (Fig. 3). *St. vinaceusdrappus* produced homologous necrotic L.L, without halo, 1.5 mm in diameter with reduction percentage 74.4%, Fig. 3-St. 19. *St. nogalater* induced ring necrotic L.L surrounded with yellow and brown halo, 3 mm in diameter with reduction percentage 70.2%, Fig. 3-St. 28. *St. viridosporus* produced ring necrotic L.L surrounded with yellow and brown halo, 3 mm in diameter with reduction percentage 80%, Fig. 3-St. 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

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

Treatments	% of infection	% of R.I	DS (%)	Virus concentration
Infected control	100.0	0	65.4	125
<i>St. calvus</i>	28.6	71.4	10	36.2
<i>St. canarius</i>	17.4	82.6	22.2	21.7
<i>St. vinaceusdrappus</i>	25.6	74.4	20	32
<i>St. nogalater</i>	29.8	70.2	4.4	37.25
<i>St. viridosporus</i>	20.0	80	10	25

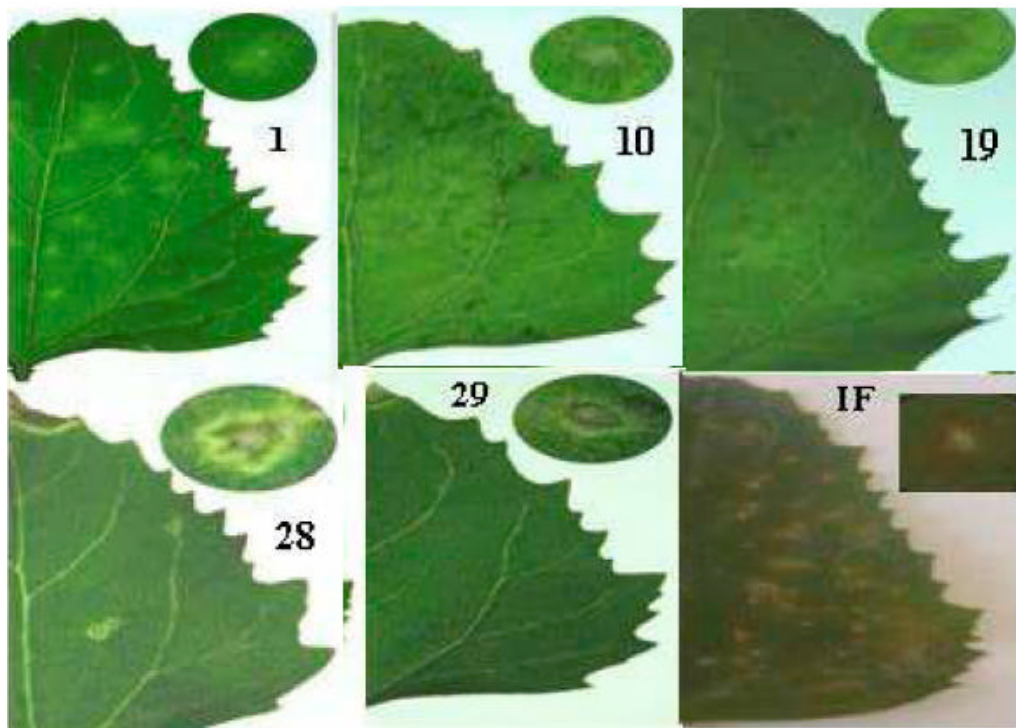


Fig. 3: Variability of local lesions resulted from CMV infected cucumber plant treated with 5 Streptomycetal isolates using *C. amaranticolor* as CMV indicator host. *All photo numbers point to numbers of different isolated *Streptomyces* as used in this work

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 (Fig. 4, 5) compared with untreated CMV infected Cucumber plants as infected control (Fig. 4, 5).

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

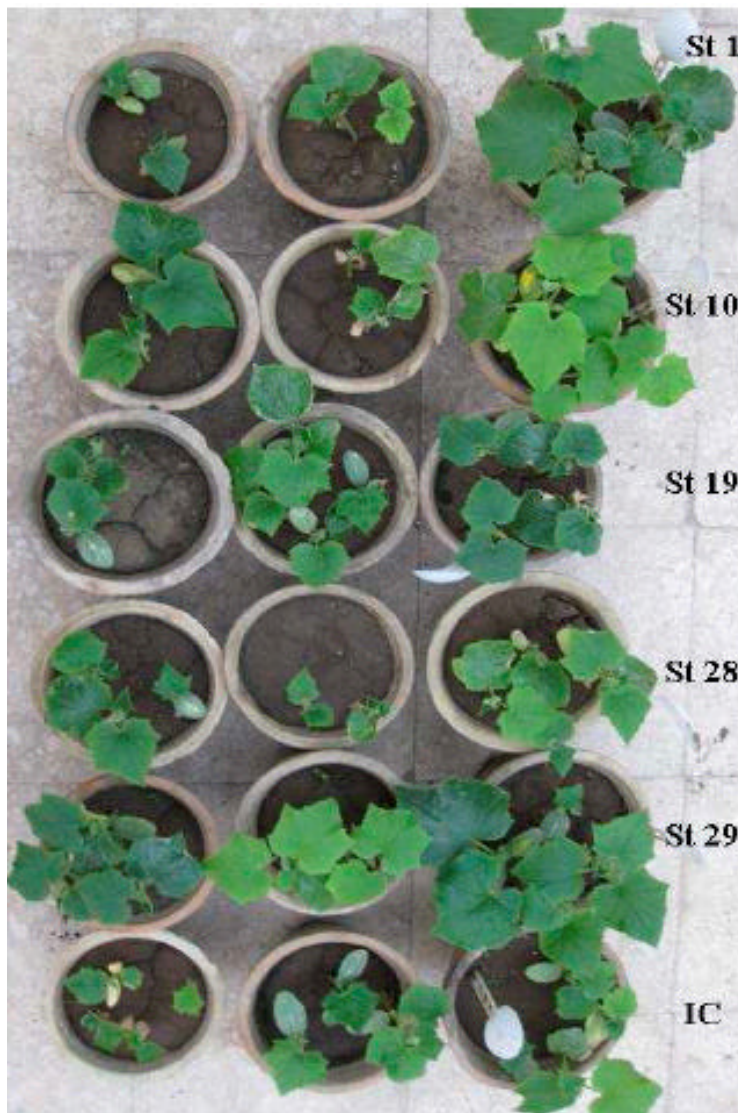


Fig. 4: Illustrate the cucumber plant treated with different filtrate of Streptomycetal isolates. St 1: *St. calvus*, St 10: *St. canarius*, St 19: *St. vinaceusdrappus*, St 28: *St. nogalater*, St29: *St. viridosporus*, IC: Infected control

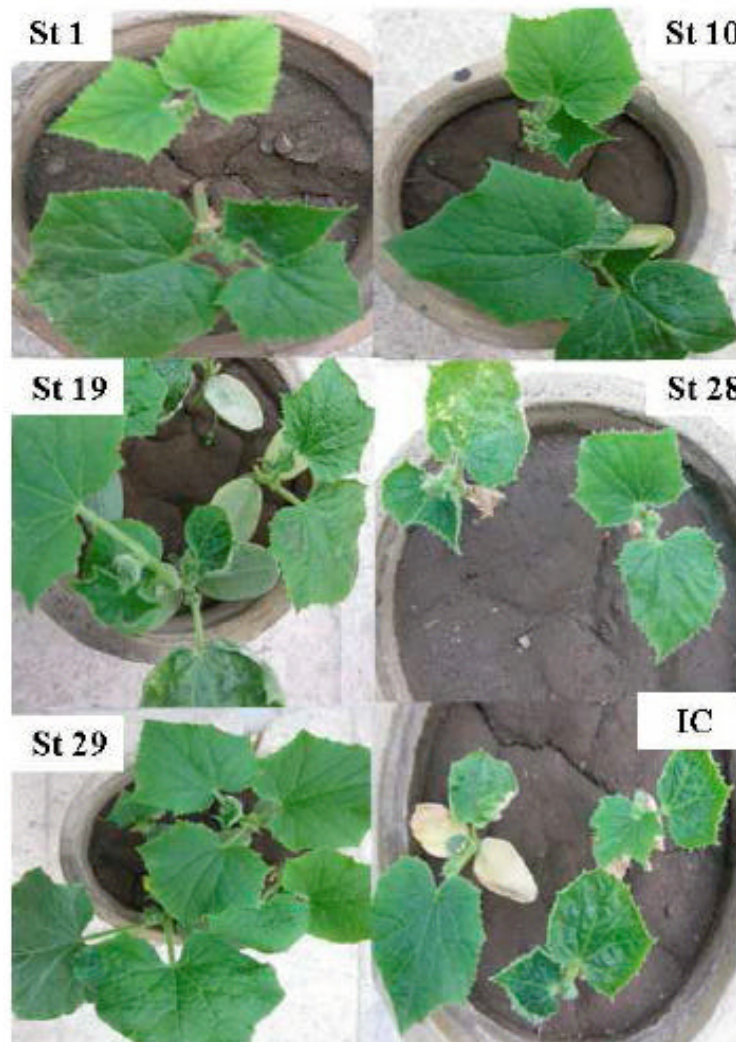


Fig. 5: Development of different CMV symptoms in cucumber plants treated with different filtrate of Streptomyces isolates. St 1 and St 10: vein clearing. St 19, St 28 and St 29: Mosaic, malformation and blisters. IC: infected control

DISCUSSION

An antiviral producing streptomycetal species were isolated from soil rhizosphere in Zagazig province of Egypt. In order to identify the Streptomyces strains, morphological, physiological, biochemical and antagonism testes were performed. The Streptomyces were found to be a species of *Streptomyces calvus*, *Streptomyces canarius*, *Streptomyces 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-Elanin, 2004). Many authors reported that, the isolation of 110 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 Al-Momani, 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; 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; Megahed, 2008). Accordingly, anti-infective activity may induce one or more of the following activities:

- 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 prevention of the fitting into the receptor
- Induction of changes in cell membrane or cellular receptor of the virus
- Prevention 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:

- Induction changes at the cell membrane leading to inhibition of the virus adsorption and/or penetration
- Induction of changes in the cell lysosomes inhibiting virus uncoating
- 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:
 - Inhibition of virus uncoating or Inhibition of cellular and/or viral translation mechanisms
 - Inhibition of cellular and/or viral transcription and replication function
 - Inhibition of viral protein processing and/or Capsid assembly or maturation

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