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Inducing Systemic Resistance against Cucumber Mosaic Cucumovirus using *Streptomyces* spp.

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Seven *Streptomyces* spp. were screened for their potential to protect cucumber plants against Cucumber mosaic virus (CMV). Foliage treatment with the *Streptomyces* culture filtrates resulted in high reduction of the level of disease severity of CMV infection. In contrast, soil treatments were less effective in virus inhibition. Concerning foliage treatment, culture filtrates of *S. griseorebens* and *S. cavourensis* showed the highest inhibitory effect (90% and 85%, respectively) when applied 48 hrs prior to virus inoculation. On the other hand, soaking of cucumber seeds for 2hrs in the *Streptomyces* culture filtrates resulted in the highest viral inhibition. Generally, *S. griseorebens* recorded the highest percentage of viral inhibition (65%). Direct ELISA was carried-out as a diagnostic tool at the beginning and throughout this study. A number of de-novo synthesized proteins (induced proteins) were detected by polyacrylamide-gel electrophoretic analysis in cucumber leaves treated with culture filtrates of *Streptomyces* spp. relative to the control treatment. Considerable increase in total phenols as well as peroxidase and polyphenol oxidase activity levels in treated plants before virus inoculation were recorded comparing with the healthy and infected control plants. The highest values were observed in treated cucumber plants with *S. griseorebens* collected 14 days post virus inoculation.

Keywords: Cucumber, Cucumber mosaic virus (CMV), Induced systemic resistance and *Streptomyces* spp.

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops in Egypt and worldwide grown for local human consumption and exportation. Many viruses affect cucumber and cause mosaic diseases (Khereba *et al*., 2009). Cucumber mosaic Cucumo virus is a widespread and troublesome virus infecting cultivated plants. The diseases caused by CMV present a variety of global management problems in a wide range of agricultural and ecological settings. Management of a viral disease can be accomplished through the induction of a plant's natural defences, *e. g.,* systemic acquired resistance (SAR) (Falcioni *et al*., 2014).

Systemic acquired resistance (SAR) for virus infections can be induced in plants treated with certain *Streptomyces* strains (Galal, 2006). *Streptomyces* spp. were the source of many useful and consequently profitable antiviral agents (Sonya and Galal, 2005). Many antiviral substances were isolated from *Streptomyces* spp., *i.e.* borrelidin, clindamycin and fattiviracins (Ghaly *et al*., 2005, Bhikshapathi *et al*.,

2010 and Chaudhary *et al*., 2013). Cell-free suspension of *Streptomyces rochei* succeeded to inhibit Tobacco mosaic virus (TMV) in leaves on *Datura metel* (Mansour *et al*., 1988). Galal and El-Shirbiny (1995) enhanced the resistance of *Datura stramonium* plants against *Potato virus X* (PVX) using caeseorhodomycin (produced by *S. caeseorhodomyces*). However, Galal (2006) mentioned that treatment of cucumber plants with the filtrate of five *Streptomyces* strains, *i.e. S. violatus, S. violaceuisniger, S. aureofaciens, S. nasri* and *Streptomyces* sp., resulted in induced systemic resistance against CMV when applied before virus inoculation than after viral inoculation. He also found that soaking of cucumber seeds for 24hrs in the *S. violaceuisniger* filtrate resulted in the highest percentage of viral inhibition also the cell-free filtrate of *S. chibaensis* having antagonistic agent as a secondary product.

Culture filtrates of *S. canaries* and *S. viridosporus* showed high reduction in percentage of local lesion number produced by CMV on *Chenopodium amaranticolor* plants. Moreover *S. nogalater* was found to have a low percentage of disease severity on cucumber plants (El-Dougdoug *et al*., 2012).

The present study was conducted to evaluate: (1) the potential use of certain *Streptomyces* culture filtrates (either foliar, soil or seed treatment) for induction of systemic acquired resistance in cucumber against CMV infection, (2) study the enhancement of phenolic compounds, peroxidase and polyphenol oxidase activity as an defences enzymes, (3) and study the induction of pathogensis – related proteins (PR-Ps) in the observed resistance against CMV infection.

Materials and Methods

This study was conducted in greenhouse (22±3°C) at Virus and Phytoplasma Res. Dept.

Plant material:

Seeds of cucumber (*Cucumis sativus* L.) cv. Beta alpha were kindly provided by the Vegetable Dis. Res. Dept., ARC.

Virus source:

Infected cucumber plants were collected from Ismailia Governorate. Biological purification of CMV was submitted to single- local lesion serial passage in *Chenopodium amaranticolor* as a local lesion host (Kuhn, 1964) and propagated in *Nicotiana tabacum* cv. White Burley (Noordam, 1973). The virus identity was confirmed serologically by using DAS ELISA described by Clark and Adams (1977).

Source of Streptomyces isolates:

Streptomyces species *i.e., S. aureofaciens, S. canarius, S. griseorebens, S. cavourensis, S. violaceuisniger, S. Violatus* and *S. viridosporus* were obtained from Bacterial Dis. Res. Dept., Plant Pathol. Res. Inst., ARC. *Streptomyces* species were cultivated on yeast extract-malt extract broth medium at 28°C for 10 days according to Raugthaworn *et al*. (2007). Cultures of each *Streptomyces* species were separately homogenized and centrifuged at 3000 rpm for 20 min to separate the microbial growth then the supernatants were collected.

In vitro effect of culture filtrates of some Streptomyces spp. on CMV infection:

For testing the effect of culture filtrates of seven *Streptomyces* spp. on CMV infectivity *in vitro*, 1ml of the infectious sap was added to 1ml (1:1 v/v) of each of *Streptomyces* culture filtrate, mixed well and allowed to stand for 15 min. Twenty seedlings of cucumber for each treatment were used. Two groups of plants were used for comparison, the first were inoculated with the virus and the second were treated with buffer solution (pH 7.4). Mixtures of sap containing virus and *Streptomyces* culture filtrate were inoculated into cotyledonary cucumber leaves. The symptoms were observed and recorded four weeks after challenge- inoculation. Percentage of inhibition was calculated according to the following formula (Taha and Mousa, 2000).

$$\text{Inhibition (\%)} = (A\text{-}B/A)\times 100$$

Whereas, A is the number of infected plants in control and B is the number of treated plants. In all cases, Direct ELISA was carried-out to confirm the obtained results in this study.

Effect of culture filtrates of some Streptomyces spp*. on CMV infectionin vivo:*
Pre-inoculation treatment:

One ml of each *Streptomyces* culture filtrate was rubbed on the cotyledonary leaves of cucumber then they were mechanically inoculated with CMV inoculum (1ml/plant) at two time intervals; 24 and 48 hrs. In check treatments plants were inoculated with either CMV or treated with buffer solution (pH 7.4). Symptoms were observed and recorded after four weeks of virus inoculation. Percentage of inhibition was calculated.

Post-inoculation treatment:

The cotyledonary leaves of cucumber were inoculated with CMV. *Streptomyces* filtrates were applied on the inoculated plants after 24 and 48 hrs. In check treatments plants were inoculated with CMV or treated with buffer solution (pH 7.4). The symptoms were observed and recorded after four weeks post inoculation. Percentage of inhibition was calculated according to the above mentioned formula.

Effect of seed treatment:

Cucumber seeds cv. Beta alpha were soaked in 50 ml of each *Streptomyces* species filtrates for different periods 1, 2 and 3 hrs. before planting. Twenty seeds were cultivated for each treatment. In check treatments, seeds were soaked in yeast extract- malt extract broth medium for similar time intervals. After germination, the cotyledonary leaves were inoculated with CMV. The symptoms were observed and recorded after four weeks. The percentage of inhibition was calculated.

Effect of Soil treatment:

Cucumber seeds were planted in plastic pots (25cm diam.) each was packed with 3 Kg of sterilized clay: peat moss: vermiculite (1:1:1) Two weeks later, each of *Streptomyces* culture filtrates 10 days old (100ml) was poured into each pot immediately after seedlings immergence. In check treatments, yeast extract- malt extract broth medium was used. The cotyledonary leaves were inoculated with CMV. The symptoms were observed and recorded after four weeks. The percentage of inhibition was calculated.

Disease severity:

All plants in each treatment were examined weekly for virus symptoms using the following rating scale: 10 = 100% of leaves showing mosaic symptoms; 8 = 50% of leaves showing mosaic symptoms; 6 = mosaic symptoms just starting; 4 = 50% of leaves appear puckering or curling just starting; 0 = no symptoms. Disease severity was calculated using the formula (Raupach *et al*., 1996):

Disease severity = [Σ(Rating No.× No. plants in rating) ×100] /(Total No. plants× highest rating).

Phytochemical analysis:

Samples were carefully collected at three time intervals (7, 14 and 21 days) after inoculation with CMV). Determination of phenolic compounds (mg/100g of plant fresh wt.) was carried out according to Maliak and Singh (1980). Ten grams fresh weight of the leaf samples were used for each replicate of each treatment in determination of phenolic compounds. Two grams fresh weight of the leaf samples were used for each replicate in determination of enzymes activity. Peroxidase activity was spectrophotometrically determined by measuring the oxidation of pyrogallol in the presence of H_2O_2 at 470 nm (Maxwell and Bateman, 1967). The activity of polyphenol oxidase enzyme was determined according to the method of Galeazzi *et al*. (1981).

Protein analysis:

CMV inoculated plants collected after five days from virus inoculation (30-day–old) and their corresponding healthy plants were used as a source of protein samples. Total soluble proteins were extracted from 1 g of each sample as described by Sambrook *et al*. (1989). The extracted proteins were suspended in loading buffer (Laemmli, 1970) and subjected to electrophoresis (SDS-PAGE) for 5 hours at 100 volt followed by staining the gel with silver nitrate according to Sammons *et al*. (1981). Molecular weight of the protein was estimated from molecular weight standard (MW from 10 to 116.0 KDa). Protein profiles was analysed using the Computer Protein Gel Image Analysis Software.

Statistical analysis:

Data were analysed with the statistical analysis system SAS. All multiple comparisons were first subjected to analysis of variance (ANOVA) comparisons among means was carried out according to Duncan's multiple range test (Duncan, 1995).

Results

In vitro effect of culture filtrates of seven Streptomyces spp. on CMV infection:

Data presented in Table (1) reveal that all tested Streptomyces culture filtrates had significant inhibitory effect against CMV infection when they mixed with the virus inoculum. *Streptomyces griseorebens* gave the highest percentage of inhibition (85%). Either *S. cavourensis* or *S. violaceuisniger* had an equal effect in this concern (80%). The seven *Streptomyces* spp. cause a different percentage of disease severity.

S. griseorebens gave the lowest percentage (8%), whereas *S. aureofaciens* gave the highest percentage (28%).

Table 1. *In vitro* effect of culture filtrates of seven different *Streptomyces* spp. on the percentages of inhibition of CMV

Treatment	Viral inhibition (%)	Disease severity (%)
S. aureofaciens	60.0	28.0
S. canarius	65.0	17.0
S. cavourensis	80.0	10.0
S. griseorebens	85.0	8.0
S. violaceuisniger	80.0	13.0
S. violatus	70.0	16.0
S. viridosporus	75.0	12.5
Inoculated control	00.0	65.5
Untreated uninoculated control	00.0	00.0
LSD at 0.05	13.6	2.3

In vivo effect of culture filtrates of seven Streptomyces spp. applied before and after virus inoculation:

Pre-inoculation treatment:

Data recorded in Table (2) show that all *Streptomyces* spp. gave encouraged results in inducing resistance against CMV infection when applied 24 or 48 hrs before virus inoculation. *S. griseorebens* significantly induced the highest systemic resistance against CMV infection (90%) as inhibitory percentage of systemic virus infection when applied 48 hrs before virus inoculation (Fig. 1) and reduction of 4% in disease severity. *S. cavourensis* and *S. viridosporus* which gave disease severity percentages of 7 and 8%, respectively. The inhibitory effect was higher at 48 than did 24 hrs before virus inoculation.

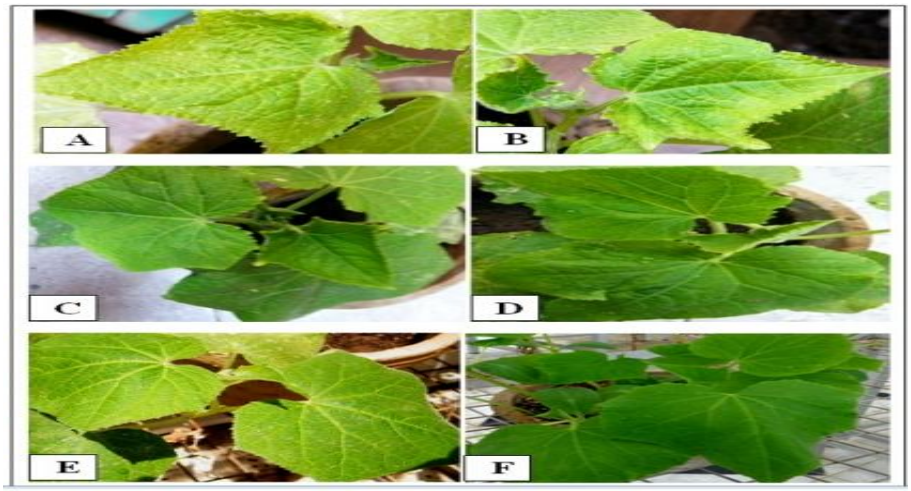

Fig. 1. Cucumber plant treated with different *Streptomyces* spp. A&B: Infected control C: *S. griseorebens*, D: *S. cavourensis*, E: *S. viridosporus*, F: Untreated uninoculated control.

Post-inoculation treatment:

In post-inoculation treatment, the highest effect against CMV infection was obtained with either *S. cavourensis* or *S. griseorebens* (70%) when applied 48hrs after viral inoculation (Table 2). Whereas, *S. aureofaciens* gave the lowest effect (45%). According of the recorded data pre-inoculation treatment was more effective in inducing systemic resistance against CMV infection than did post–inoculation treatment.

Table 2. Effect of pre- and post-inoculation of cucumber plants with seven culture filtrates of *Streptomyces* spp. on CMV infection at two time intervals *in vivo*

Treatment	Pre-inoculation				Post-inoculation			
	24 hrs.		48 hrs.		24 hrs.		48hrs.	
	Viral inhibition (%)	Dis. severity (%)	Viral inhibition (%)	Dis. severity (%)	Viral inhibition (%)	Dis. severity (%)	Viral inhibition (%)	Dis. severity (%)
S. aureofaciens	60.0	24.0	70.0	20.0	40.0	42.0	45.0	38.0
S. canarius	65.0	16.0	70.0	15.0	40.0	35.0	50.0	34.0
S. cavourensis	80.0	8.0	85.0	7.0	60.0	29.0	70.0	24.0
S. griseorebens	85.0	6.0	90.0	4.0	65.0	22.0	70.0	20.0
S. violaceuisniger	72.5	19.0	80.0	12.0	60.0	30.0	65.0	28.0
S. violatus	72.5	14.0	75.0	13.0	45.0	32.0	50.0	31.0
S. viridosporus	75.0	10.0	80.0	8.0	50.0	34.0	60.0	33.0
Inoculated control	00.0	65.5	00.0	65.5	00.0	65.5	00.0	65.5
Untreated uninoculated control	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
LSD at 0.05	2.9	1.6	2.9	2.6	3.1	2.6	2.7	1.3

Effect of seed treatment:

Data presented in Table (3) show the effect of soaking cucumber seeds for 1, 2 and 3 hrs in culture filtrates on induce systemic resistance against CMV infection. Variation was noticed in induce systemic resistance against CMV infection. Soaking of seeds for 2 hrs showed higher viral inhibition. Soaking cucumber seeds in culture filtrates of *Streptomyces* spp. for 3 hrs induce the lowest systemic resistance and the highest disease severity. *S. griseorebens* significantly induced the highest systemic resistance against CMV infection as inhibitory percentage for seeds soaked in culture filtrates for 2 and 1 hrs ; giving 85 and 75%, respectively and the lowest percentage of disease severity 5 and 6 %, respectively.

Effect of soil treatment:

Data in Table (4) reveal that among all treatments, soil treatment had the lowest effect in reducing CMV infection and disease severity. *S. griseorebens* gave the highest percentage of inhibition (65%). However, *S. aureofaciens* gave the lowest one (40%). Vice versa, *S. aureofaciens* showed higher disease severity (28%) than did *S. Griseorebens*(21%). Whereas, *S. canaries* had a lowest disease severity (17%), with contrast with *S. violatus* which gave the highest disease severity, being 33%.

Table 3. Effect of soaking cucumber seeds in culture filtrates of seven *Streptomyces*spp. at three time intervals on CMV infection

Treatment	Time intervals					
	1 hr.		2 hrs.		3 hrs.	
	Viral inhibition (%)	Disease severity (%)	Viral inhibition (%)	Disease severity (%)	Viral inhibition (%)	Disease severity (%)
S. aureofaciens	40.0	40.0	50.0	18.0	40.0	30.0
S. canarius	50.0	33.0	55.0	14.0	45.0	29.0
S. cavourensis	70.0	9.0	80.0	8.0	65.0	11.0
S. griseorebens	75.0	6.0	85.0	5.0	70.0	8.0
S. violaceuisniger	65.0	32.0	80.0	9.0	60.0	27.0
S. violatus	60.0	26.0	75.0	10.0	50.0	20.0
S. viridosporus	65.0	14.0	70.0	11.0	60.0	12.0
Inoculated control	00.0	65.5	00.0	65.5	00.0	65.5
LSD at 0.05	2.8	2.9	2.8	3.2	3.1	2.5

Table 4. Effect of soil treatment with culture filtrates of seven *Streptomyces* spp. on CMV infection

Treatment	Viral inhibition (%)	Disease severity (%)
S. aureofaciens	40.0	28.0
S. canarius	45.0	17.0
S. cavourensis	60.0	27.0
S. griseorebens	65.0	21.0
S. violaceuisniger	60.0	28.0
S. violatus	45.0	33.0
S. viridosporus	50.0	30.0
Inoculated control	00.0	65.5
LSD at 0.05	15.2	2.1

Phytochemical analysis:

Data in Table (5) indicate that phenol contents and defence enzymes were significantly stimulated in treated plants compared with untreated ones. Accumulation of phenols was determined 7, 14 and 21 days post spraying with *Streptomyces* spp. The highest accumulation was observed in cucumber plants 14 days post inoculation spraying compared with untreated plants. Higher phenol levels (1.93 mg/100g fresh weight) were observed in plants treated with *S. griseorebens* in comparison with inoculated untreated plants (0.31 mg/100g fresh weight)**.** The peroxidase (POX) activity was significantly stimulated to reach the highest activity by the 14 days in all treatments, and then decreased by the 21 days. The highest peroxidase activity have been realized with *S. griseorebens* (2.93) compared with the untreated uninoculated control (0.38). The activity of polyphenoloxidase (PPO) gradually increased and gave the highest level, 14 days after spraying with all treatments. The highest activity of PPO was recorded from plants treated with culture filtrate of *S. griseorebens* after 14 days of inoculation with CMV, being 2.8.

Table 5. Effect of spraying cucumber plants with culture filtrates of seven Streptomyces spp. and inoculated with CMV on activity of peroxidase (POX), polyphenol oxidase (PPO) and total phenols

Treatment	Pox Activity (µg/g F.Wt) after			PPO activity (µg/g F.Wt) after			Total phenols (mg/100g F.Wt) after		
	7 day	14 day	21 day	7 day	14 day	21 day	7 day	14 day	21 day
S.aureofaciens	1.58	1.78	1.39	1.11	1.29	1.09	0.76	1.03	1.03
S.canarius	1.81	1.98	1.63	1.48	1.68	1.36	1.12	1.23	1.05
S.cavourensis	2.41	2.79	2.30	2.43	2.63	2.26	1.57	1.78	1.46
S.griseorebens	2.8	2.93	2.58	2.63	2.80	2.53	1.80	1.93	1.76
S.violaceuisniger	1.90	2.08	1.79	1.55	1.79	1.37	1.57	1.78	1.46
S.violatus	2.11	2.27	2.01	1.63	1.86	1.48	1.35	1.50	1.28
S.viridosporus	2.33	2.51	2.22	2.28	2.40	2.18	1.49	1.63	1.32
Inoculated control	0.53	0.69	0.48	0.21	0.31	0.11	0.20	0.31	0.01
Untreated uninoculated control	0.26	0.38	0.15	0.14	0.23	0.06	0.11	0.21	0.09
LSD at 0.05	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.1

Protein analysis:

The effect of spraying with *Streptomyces* spp. before 48hrs from virus inoculation was determined using markers as standard proteins. Fig. (2) reveals the induction of *de-novo*- synthesized proteins in treated leaves, such proteins were not detected in control treatment. Treatment with *S. griseorebens*, *S. cavourensis*, *S. violaceuisniger* and *S. viridosporus* resulted in the detection of similar induced proteins (with molecular weights of 16, 18, 20, 25, 27, 30 and 32 KDa) in approximately similar amounts. It was found that bands of 14, 35, 40 and 42 KDa were released with high density in treated cucumber plants but not identical to those electrophoretic in control and healthy plants.

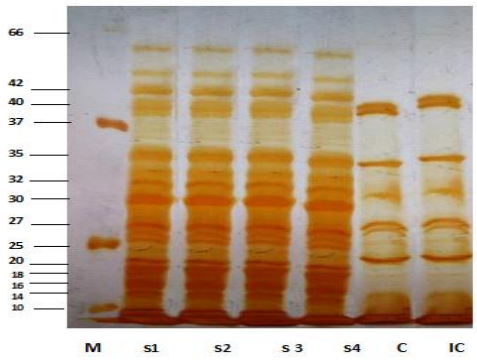

Fig. 2. SDS-PAGE analysis of total protein extracted from cucumber leaves pre-treated with *Streptomyces* spp. Whereas: Lane M contained molecular mass markers, Lane S1-S4 are cucumber plants sprayed with *S. griseorebens*, *S. cavourensis*, *S. violaceuisniger*, *S. viridosporus*, respectively, Lane C unsprayed cucumber leaves and IC: Lane infected unsprayed cucumber leaves.

Discussion

Streptomyces spp. are among the various groups of plant-associated microorganisms that can elicit a natural plant defence (Kurth *et al*., 2014). In the present study we evaluated the efficacy of seven *Streptomyces* spp. to induce resistance and biological suppress against CMV infection in cucumber plants. The obtained data show that treatment with culture filtrates of all the tested *Streptomyces* spp. resulted in a significant reduction in CMV infection when they individually applied before inoculation. Yassin and Galal (1998) reported that the filtrate of some *Actinomycetes* had an inhibitory effect against *Tobacco necrosis virus*. Mohamed and Galal (2005) found that mixing each isolate of *Streptomyces* spp. with *Potato virus Y* inoculum completely inhibited the inducing of necrotic local lesions produced on *Ch.quinoa*. Mixture of the *Streptomyces* isolates with the crude sap on infected source of TMV reduced the number of necrotic local lesions formed on *Datura metel* leaves (Ara *et al*., 2012 and Mohamed *et al*., 2012). The data showed that application of the *Streptomyces* spp. on cucumber plants at different periods before inoculation with CMV led to an inhibitory effect, the highest effect was obtained after 48hrs.of application. These results were in agreement with Galal (2006) who mentioned that culture filtrate of *Streptomyces* strains significantly inhibited CMV infection when applied 24 or 48hrs. before virus inoculation. Inhibition of *Banana bunchy top virus* infection was highly significant when culture filtrate of *Streptomyces chibaensis* was applied 10 days before virus inoculation (Hewedy *et al*., 2008). Similar results were reported by Shoman *et al*. (2003) and El-Dougdoug *et al*. (2012). The variation in viral inhibition percentages may reflect the variation in resistance against CMV which may be due to the presence of different metabolites in the microbial cultures which may have an inductive effect in the host plant to inhibit the infection of CMV (Ghaly *et al*., 2005).

Soaking of cucumber seeds in *Streptomyces* spp. culture filtrates for three time intervals, *i.e.* 1, 2 and 3 hrs reduced CMV infection. The highest inhibition of CMV was achieved after 2 hrs. These results were in harmony with Galal (2006) who mentioned that soaking cucumber seeds in culture filtrate of the *Streptomyces* strains for 2 hrs gave the highest inhibition of CMV infection. There was a consistent difference in resistance induced by application method. More resistance was induced when *Streptomyces* spp. were provided to plant leaves (foliage treatment) than those inoculated in soil (soil treatment). This suggests that a longer period of association between the microbial cultures and plant roots may be necessary for the induction of resistance (Leeman *et al*., 1995). It has been reported that the biologically active microbial metabolites are only weakly taken up by the root and transported to the site of infection (Wei *et al*., 1991). The results obtained show that *Streptomyces* spp. reduced the level of disease severity of CMV infection in either foliar or seed treatment. Spraying cucumber plants with culture filtrate of *Streptomyces* isolates reduced the percentage of CMV infection and disease severity (El-Dougdoug *et al*., 2012).

The application of *Streptomyces* spp. culture filtrates increased phenols and defence enzymes such as peroxidase and polyphenol oxidase activities. Phenolic compounds are known to play a major role in the defence mechanisms of plants

against various external infectious agents (Sofy *et al*., 2014). Systemic acquired resistance is characteristically associated with accumulation of salicylic acid, enhanced expression of pathogenesis-related proteins and activation of phenylpropanoid pathway, leading to the synthesis of higher phenolic compounds (Behuvaneshwari *et al*., 2015). Phenolics have been associated extensively with the defense of plants against viruses (Din Umar *et al*., 2016). Phenol metabolism and cell wall lignification are thus involved in, and have consequences for, a number of cellular, whole plant and ecological processes, that might even provide plants, the immunity against destructive agents. Phenolic acids are involved in phytoalexin accumulation, biosynthesis of lignin and formation of structural barriers, which play a major role in resistance against the pathogen (Sudhakar *et al*., 2007). Peroxidase and polyphenol oxidase activities were greater in the cucumber plants treated with *Streptomyces* spp. compared to control plants. This might be due to increase specific defence gene expression or one possibility is that the initial signal from the inoculated leaf leads to the activation of an enzyme or group of defence enzymes (Jeffery, 2002). Peroxidase is an important enzyme in the reinforcement of plant cell walls, and helps in protein extension to generate a firmer matrix material to be a part of the activated defence response (Jabs *et al*., 1996). Peroxidase has been found to play a major role in the phenolic compounds oxidation and mediate the final step in the biosynthesis of lignin and other oxidative phenols (Aldesuquy *et al*., 2015). Polyphenol oxidase can be induced through octadecanoid defence signal pathway and it oxidizes phenolic compounds to quinines, and the enzyme itself is inhibitory to viruses by inactivating the RNA of the virus (Constabel *et al*., 1995).

Regarding the results of SDS (PAGE), upon treatment of cucumber leaves with the four *Streptomyces* spp., new patterns of protein, were appeared. These patterns had calculated molecular weights (about 20, 25, 27, 30 and 32 KDa). It could be suggested that in resistance induced plants, the accumulated intercellular proteins forms the first line of defence to a challenging pathogen and they are implicated in plant defence because of their antiviral activities (Van-Loon *et al*., 1994). Shoman *et al*. (2003) declared that induction of plant resistance with *Streptomyces gibsonii* against *Tobacco necrosis virus* was associated with accumulation of pathogensis – related proteins with molecular weights of 24.6 and 33.5 KDa. It was found that a band of 30 KDa was observed. This protein is believed to be a protein that belonged to the chitinase family as assumed by Santos *et al*. (2004). So the disappearance of disease symptoms in cucumber plants might be due to the high level of chitinase enzyme as one of pathogensis related proteins. This assumption comes in agreement with Harish *et al*. (2004) who found that the plants treated with endophytic bacteria seem more resistance to *Banana bunchy top virus* more than the untreated ones and the activity of defence related proteins in plants, such as chitinase increase. Biotic inducers increased many PR-proteins such as isozymes of peroxidase and chitinase (Neetu *et al*., 2008).Van Loon *et al*. (1994) reported that, some of the pathogensis-related proteins (PRs) possess a potential antipathogenic activities. The time course of accumulation of novel proteins was very essential to accumulate pathogensis related proteins; such proteins had been found to play a key role in inducing strong systemic resistance against viruses (Devi *et al*., 2004). *Streptomyces* spp. produce a wide range of biologically active substances including antibiotics, siderophores,

Indol acetic acid, enzymes as chitinase, cellulase, pectinase and xylanase (Petinate *et al*., 1999 and Verma, 2011). These compounds may play an important role in induced resistance and disease suppression (Klopper *et al*., 1980; Pierson and Thomashow, 1992 and Botha *et al*., 1998).

The results of the present study demonstrated that application of *Streptomyces* spp. culture filtrates is a promising approach for the eco-friendly management of CMV in cucumber plants.

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استحثاث المقاومة الجهازية ضد فيروس موزيك الخيار باستخدام عزلات الأستربتوميسيز

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تم اختبار سبعة أنواع من الأستربتومايسيز لمعرفه قدرتهم على حماية نباتات الخيار من الاصابة بفيروس موزيك الخيار. أدت معاملة رش النباتات براشح المزرعة لعزلات الأستربتومايسيز إلى انخفاض ملحوظ فى مستوى الشدة المرضية للإصابة بالفيروس. على العكس كانت معاملة التربة أقل فاعلية فى تثبيط الفيروس. وقد أعطت معاملة رش النباتات براشح المزرعة لكلا من ستربتومايسيز جريسيوربينز, ستربتومايسيز كافيورينسيز قبل العدوى بالفيروس بـ 48 ساعه أعلى تأثير فى تثبيط النسبة المئوية للإصابة بالفيروس حيث كانت (90% , 85%) على الترتيب. من ناحية أخرى أدى نقع بذور الخيار لمدة ساعتين فى راشح المزرعة لعزلات الأستربتومايسيزإلى تثبيط النسبة المئوية للإصابة بالفيروس وقد أعطى الستربتومايسيز جريسوربينز أعلى نسبة مئوية للإصابة بالفيروس (65%). تم استخدام الأليزا لتأكيد تعريف الفيروس فى بداية وأثناء الدراسة.أدى إستخدام التفريد الكهربى للبروتين لأوراق نباتات الخيار المعاملة براشح مزرعة الأستربتومايسيز إلى ظهور عدد من البروتينات المستحدثة وغير موجودة فى النباتات غير المعاملة وقد أدت المعاملات برواشح المزارع أيضا قبل الحقن بالفيروس إلى زيادة ملحوظة فى كمية الفينولات الكلية ومستوى نشاط كلا من إنزيمى البيروكسيديز والبولى فينول أكسيديز وقد أعطى ستربتومايسيز جريسيوربينز أعلى القيم فى نباتات الخيار المعاملة وذلك عندما تم قياسها بعد 14 يوم من حقن الفيروس.