Effect of Three Species of Rhizobacteria (PGPR) in Stimulating Systemic Resistance on Tomato Plants against Cucumber Mosaic Virus (CMV)

Ramez Al Shami[1] Dr. Imad Ismail[2] Dr. Yaser Hammad[3]
[1]*Postgraduate Student, Plant Protection Dept., Faculty of Agriculture, Tishreen University, Lattakia, Syria. .*
[2] *Professor, Department of Plant Protection, Faculty of Agriculture, Tishreen University, Lattakia, SYRIA.*
[3] *Associate Professor, Department of soil and water sciences, Faculty of Agriculture, Tishreen University,*

Abstract

This experiment aimed to study the effect of three species of plant growth promoting rhizobacteria (Frateuria aurantia, Bacillus megaterium and Azotobacter chroococcum) inoculated to seeds and shoots of tomato plants on Cucumber mosaic virusdisease severity, salicylic acid and peroxidase activity content and their ability to suppress the effect of CMV in a plastic tunnel in Tartus-Syria. The results showed that, the treatment with single bacteria Frateuria aurantia produced significant reduction in disease severity and higher infree salicylic acid and peroxidase activity contents compared with Bacillus megaterium or Azotobacter chroococcumin CMV-infected or healthy controls. Mixed treatments with three bacterial species gave the highest reduction in disease severity and increasedof free salicylic acid and peroxide activity contained in both CMV-infected and healthy tomato plants. Such increase in free salicylic acid and peroxidase activity contents suggested the potential ability of rhizobacteria to stimulate mechanisms of systemic resistance and reduces the effect of CMV infection on tomato plants.

Keywords: *PGPR, CMV, Tomato, Disease Severity, Salicylic Acid, Peroxidase Enzyme.*

I INTRODUCTION

Tomato Lycopersicon esculentum Mill. takesa main placein vegetable crops because of its food and manufacturing value in Syria, The number of greenhouses planted with tomato were about 68 000 in 2014 [1]. More than 30 viruses from 16 different families infect tomato plants [2], Cucumber mosaic virus (CMV, Cucumovirus, Bromoviridae) infect more than 1000 plant species and one of them tomato plants [3].

Tomato Plants in all growth stages may show symptoms of CMV and cause a “shoestring” effect on young leaves with subsequent narrow, tendril-like leaflets. Plants with severe shoestring symptoms are stunted with little or no marketable fruit ([4], [5], [6], [7]). Cucumber mosaic virus which considered to be the most dangerous virus on tomato plantswas recordedin Central and coastal zone in Syria infecting tomato plants [8] and in southern reign [9].

Plant Growth Promoting Rhizobacteria (PGPR) is abeneficial microorganisms that inhabit the rhizosphere and promote qualitative and quantitativeplant growth, and facilitate absorption of plant material from soil ([10], [11], [12], [13]).

Many PGPRs protect plants by at least one of the following mechanisms: suppression of plant disease by induction of systemic resistance or antibiotic production (Bioprotectants), improved nutrition acquisition (Biofertilizers) and production of phytohormones (Bio-stimulants) ([14], [12]).

Because of the importance of tomatofruits in Syriaand the existence of CMV in tomato fields ([8], [9]), and the importance of PGPRs bacteria in systemic resistance against viruses, so this present research aimed to study the effect of PGPRs bacteria in suppression impact of CMV on tomato plant by measuring disease severity, determine freesalicylic acid andperoxidase enzyme activity within tomato plant tissues and their roles in stimulating systemic resistance.

II MATERIALS AND METHODS

A. Plant Material and Research Place

Tomato hybrid SweatyF1 unlimited growth wasused in this research (85% germination rate, 99% purity, origin of China,and treated with thiram). This research had beendoing between February and May in the Syrian coast onTartus in a plastic tunnel.

B. CM Visolate and Bacterial Species

Local CMV isolatefrom laboratory of viral diseases- Faculty of agriculture- Tishreen University was taken, and prepared according to [15].

Bacteria Azotobacter chroococcum was used: local air nitrogen fixation bacteria which isolated from soil planted with tomato plant [16], and was grown on a specialized medium Ashby's Mannitol Agar, [17], on Petri dishes and incubated at 28°C for three days. Bacteria Bacillus megaterium: phosphate solubilizingwas isolated from the commercial production (BIOPHOS /GET-PHOS) [16 and was grown on a specialized medium Pikoviskaya's Agar [17], on Petri dishes, and then

Incubated at 33°Cfor three days. And bacteria *Frateuria aurantia*: isolated from the commercial production (BIO-NPK/ BHARPUR) [16], and was grown on a specialized medium Glocuse- Yeast extract–$CaCO_3$ [18], and then the dishes incubated at 28°C for three days.

The bacterial inoculation was prepared with a liquid Tryptic Soy Broth medium (TSB) in a special bottle for growing bacteria BIOGEN/2 L/, then placed on shaking mortar on 100 rpm and incubated at 28°c for 48 hours, and used Bürkerslice for counting bacteria to estimate the density of the bacteria and regulated in the suspensoraccording to the required concentration 10^9 cells/ml.

C. Virus and Bacteria Inoculation

PGPRs bacteria were added before planting seeds by soaking treatments 4 hours and planted in Agricultural Vine and then watered shoots after transfer to Central agriculture 15 ml per plant of bacterial suspension 10^9 cells/ml.

The plants were inoculated with cucumber mosaic virus on stagethe fourth and fifth leaves in a week after transfer to plasticpots (one week after bacterial inoculation) including the treatment controlwith CMV infection, and taken control treatment without CMV inoculation.

D. Research Design and Statistical Analysis:

Soil of medium textures was used in this research, and added compost by 1/4volume, then covered with a clear plastic slide thickness 200 Micron with solar sterilization, After that,The agricultural mixture was filled to Plastic pots (30 x 40 cm) capacity 28 liters.

Treatment were 16 with 4 replicates and 3 plants per replicate. A total of plants was 192. The results were analyzed statistically by using One-way ANOVA test, Genstat-12 (no blocking), and compared the significant differences between means of treatments using LSD test (5%).

E. Readings:

1) Disease Severity (DS):

Disease assessments were done throughout the experiment; however, specific disease severity ratingswere taken at 14 and 28 dpi. Disease severity was measured using the following rating scale: 0 = no symptoms, 2 = mild mosaic symptoms on leaves, 4 = sever mosaic symptoms on leaves, 6 = mosaic and deformation of leaves, 8 = sever mosaic and severe deformation of leaves, and 10 severe mosaic and deformation of leaves with stunted growth.

Disease severity(DS) = Σ (disease grade × number of plants in each grade)/ Total number of plants × highest disease grade)× 100 [19].

2) Determination of Free Salicylic Acid:

Freesalicylic acid concentrations were measured in the tissues of the plant [20] after two weeks of artificial infection with CMV, we weighed 1 g of fresh leaves, then placed within mortar and added 1 ml of hydrochloric acid 6 regular and 10 ml of chloroform, after that,crushed and filtered the sample very well then added 5 ml of each sample Iron chloride FeCl3 solution (preparation of iron chlorine solution by adding 0.5 g powdered chlorine iron to 100 ml distilled water and stired well to fully melt) as a result of the interaction between salicylic acid and iron Fe3 + triple complex anion violet color varies depending on the concentration of salicylic acid in Plant sample tested, salicylic acid concentration (ppm) and absorbency of the resulting solution was read using a Spectrophotometer (JASCO-Japan) at 540 nm wavelength, after draw a line By using four concentrations of standard salicylic acid, 25 and 50, 75 and 100 ppm and 0 distilled water only.

3) Determination Peroxidase Enzyme Activity:

Peroxidase enzyme activitywas determined [21] by taking 1 g of fresh leavesfor each treatment after 14 and 28 days of artificial inoculation with CMV, Then was added 3 ml phosphate buffer PH = 7, 0.1M at 4°c, after that,placed witha mortar and crushed,put the extract into a tube 1.5 ml andclarified by centrifugation for 10 minutes at 15000 rpm speed and thesupernatant was used for the assay. peroxidase enzyme activity was measured after adding 1.5 ml Pyrogallol(0.05 M in 0.1M phosphate),0.5 ml of 1% H_2O_2,and 0.5 ml of enzyme extract.The mixture was incubation at (28°c). And Measured at wave lengths 420 nm. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer [22], the enzyme activity was calculated according to the equation:

Peroxidase activity = (B ×Simple Dilution Factor)/ (Reaction time×V)

B: the amount of low H_2O_2 between the first and ultimate timewith

Simple Dilution Factor: 20 in this essay.

V: sample volume.

Reaction time: The end time (3 minutes)-primeval time (0.5 minutes).

III RESULTS AND DISCUSSION:

A. Disease Severity (DS) for CMV onTomato Plants Inoculated with PGPRs Bacteria:

Table (1) showed that tomato plants inoculated with PGPRs (seeds+shoots) and infected with CMV exceeded significantly in decrease disease severity in all treatments compared with infected control, The differences by taking readings was so increasingly influence progress in time, The largest reduction of disease severitywas for treatments F+ CMVs+ sh, BF+ CMVs+ sh and ABF+ CMVs+ shat after 14 days of viral infection 13.33%, 10%, 5% and after 28 days of viral infection 20%, 13.33%, 8.33%, respectively, compared with 48.33% control and 61.66%.

Through previous results was found the difference in reducing the disease severity of the virus and stimulatingthe resistance of CMV according to treatments and time. It turns out that three bacterial species have the ability to reduce the disease severity of virus, Bacteria *Frateuria aurantia* was the best in reducing disease severity compared with other species.

Table 1: Effect of PGPRs Bacteria Ondisease Severity of CMV on Tomato Plants

Treatments	Disease Severity	
	After 14 Days Inoculation	After 28 Days Inoculation
A+CMV s+sh	23.33^{ef}	30^{c}
B+CMV s+sh	20^{g}	26.66^{d}
F+CMV s+sh	13.33^{j}	20^{g}
AB+CMV s+sh	16.66gi	23.33^{e}
AF+CMV s+sh	15^{ij}	20^{g}
BF+CMV s+sh	10^{k}	13.33^{j}
ABF+CMV s+sh	5^{l}	8.33^{k}
Control –CMV	48^{b}	61.66^{a}
LSD 5%	3.12	

Azotobacter chroococcum (A), *Bacillus megaterium* (B), *Fraturia aurantia* (F), CMV: *Cucumber mosaic virus.* s+sh: Inculation seeds+ shoots. Values followed by the same letters in the same column are not significantly different at P=0.05

Our current study agree withprevious studies ([23], [19]) were found different strains of PGPRs were declared its ability to stimulate resistance against Cucumber mosaic virus, and was less in treating plants with bacteria compared with control-CMV. Re. [19] also was indicated in 2003 that strains of PGPRs bacteria were protected the tomato plants from virus Tomato mottle virus (ToMoV) by reducing the disease severity under greenhouse conditions.

The correlation between more than one bacterial strain increases the plant resistance to various diseases and under different environmental conditions [24]. Effect of mixture bacteria strains withcucumber seed treatmentswere reduced the infection with CMV more than each strain alone [25].

The disease severity of CMV virus on cucumber plants which treated with 8 species PGPRs was reduced and stimulated growth plants [26] in another study were found the bacteriaBacillus subtilus IN937b decreased developing CMV and stimulated resistance of tomato plants against CMV ([27], [28], [29]).

Sweating kombosha(beneficial bacteria and yeast) was reduced the disease severity of cucumber mosaic virus and increased the reduction of infection [30].

In another similar study 5 isolates Streptomyces spp. were stimulated Systemic acquired resistance (SAR) against CMV by reducing disease severity [31]. Bacteria Bacillus mycoides (BmJ) was reduced the rate of potato virus Y (PVY) infection on potato plants.

B. Effect of PGPRs Bacteria on Free Salicylic Acid on Tomato Plants:

In table (2) results showed the increasing on free salicylic acid content in leaves of tomato plants in all treatment inoculated with PGPRs (seeds+shoots) (none and infected) with CMV compared with the control healthy or infected. The biggest significantly increase was on immixed treatments ABF+ CMVs+ sh and ABF that free salicylic acid content in leaves was 199.3 and 136.9 u/g fresh weight, respectively, compared with the control healthy or infected 33.38 and 81.45 µg/g fresh weight, respectively, Our results indicated treatments which contained Fraturia aurantia alone or mixed were significant on treatments in other species Azotobacter chroococcum and Bacillus megaterium.

Table (2): Effect of PGPRs Bacteria in Content of Salicylic Acid on Tomatoleaves (u/g fresh weight)

Inoculation / Treatments	Bacterial Inoculation (seeds+shoots)	Bacterial Inoculation (seeds+shoots)+CMV
A	86.4[e]	97.1[i]
B	69.4[b]	83.4[d]
F	88.2[f]	155.1[n]
AB	87.7[f]	92.5[g]
AF	95.7[h]	123.1[l]
BF	112.5[j]	114.2[k]
ABF	136.9[m]	199.3[o]
Control-CMV	81.5[c]	
Control Healthy	33.4[a]	
LSD 5%	1.28	

***Azotobacter chroococcum* (A), *Bacillus megaterium* (B), *Fraturia aurantia* (F), CMV: *Cucumber mosaic virus.* s+sh: Inculation seeds+ shoots. Values followed by the same letters in the same column are not significantly different at P=0.05**

Our results agree with other studies, Salicylic acid was stimulated systemic resistance of plants against cucumber mosaic virus by inhibition systemic transition movement of the virus within the plant as affected by salicylhydrocamicacid [32]. Also Murphy said that increase the Salicylic acid in plants was linked to increase systemic resistance of plants against viral pathogens ([33], [34]). In another study salicylic acid stimulated Formation of anti pathogen-PR1, PR2 in tomato plants [35].

Re. [36], [37], and [38] indicated to Salicylicacid stimulating SAR within many plants against plant viruses by activating Pathogen Related Proteins (PRs). Re. [39] observed that some species of bacteria PGPRs stimulated systemic resistance by production Salicylic acid on the surface of plant roots.

Our results are conforming with anther similar studies conducted by re. [26] proved some of PGPRs bacteria increased on free Salicylic acid level within cucumber plantsand stimulated SAR and disease severity against CMV virus.Re. [30] found kombosha leaky that reducing in disease severity of cucumber mosaic virus accompanied with an increase in the amount of salicylic acid.

C. Effect of PGPRs Bacteria in Peroxidase Enzyme Activity in Tomato Plants:

Table (3) showed that peroxidase enzyme activity was increased on tomato leaves within progress in time and increased its activity in all treatments compared withcontrol (healthy and infection) after 14 and 28 Days after infection, and the most of the treatments were significant compared with (healthy and infection) control.

The largest of enzyme activity was in mixed treatments ABF+ CMVs+ sh and ABF 0.103 and 0.083 n.mol after 14 days and 0.233 0.273 n. mol after 28 days of infection, respectively, compared with (healthy and infection) control 0.019 and 0.044 n.mol after 14 days of infection and 0.035 and 0.067 n.mol after 28 days of infection, respectively. In our study were found significant insingular treatment with *Fraturia aurantia* compared with *Bacillus megaterium* and *Azotobacter chroococcum*. And by the results were found that PGPRs bacteria increased in activity of peroxidase enzyme with and without infection CMV in all treatmentsin comparing with (healthy and infection) control.

Table (3): Effect Of PGPR Bacteria in Enzyme Peroxidase Activity in Tomato Leaves (Nanomol)

Treatments	Activity of Peroxidase Enzyme in Tomato Leaves	
	After 14 Days Inoculation	After 28 Days Inoculation
A	0.063[e]	0.106[f]
B	0.054[cd]	0.083[d]
F	0.098[k]	0.105[g]
AB	0.056[d]	0.143[h]
AF	0.092[i]	0.161[i]
BF	0.083[h]	0.123[g]

ABF	**0.105^{m}**	**0.273^{m}**
A+CMVs+sh	**0.096^{j}**	**0.107^{f}**
B+CMV s+sh	**0.052^{c}**	**0.078^{c}**
F+CMV s+sh	**0.085^{h}**	**0.202^{k}**
AB+CMV s+sh	**0.074^{g}**	**0.104^{e}**
AF+CMV s+sh	**0.098^{k}**	**0.186^{j}**
BF+CMV s+sh	**0.065^{f}**	**0.144^{h}**
ABF+CMV s+sh	**0.103^{l}**	**0.233^{l}**
Control Healthy	**0.019^{a}**	**0.035^{a}**
Control –CMV	**0.044^{cd}**	**0.067^{c}**
LSD 5%	**0.0023**	**0.0024**

***Azotobacter chroococcum* (A), *Bacillus megaterium*(B), *Fraturia aurantia* (F), CMV: *Cucumber mosaic virus.* s+sh: Inculation seeds+ shoots. Values followed by the same letters in the same column are not significantly different at P=0.05**

Re. [40] was pointed out that salicylic acid stimulatedthe activity of peroxidase enzyme and prevented the accumulation and replication of *Potato virus Y.* As well as, re. [41] proved a positive correlation between the increased level of salicylic acid and peroxidase enzyme activity and increased kitinase enzyme in plants.

Increasing on plant enzymes such asperoxidase enzyme maybe was Accompanying directly with the ability to protect tissues Systematically with cells lining when plants attacked by plant diseases ([42], [43]). Kmbosha leaky (beneficial bacteria and yeast) in tomato plants infected with cucumber mosaic virus were working to increase the activity of peroxidase enzymes and in the amount of salicylic acid with low disease severity of the virus, theyincreased, with lignin in cell walls which affected transport and movement of systemic virus within the plant and increased resistance to the virus [44].

In another study were found the effect of three species of PGPRs bacteriaof CMV reduced symptoms as caused an increase in the concentration of enzyme b-1, 3-glucanase and peroxidase enzyme this refers to the role of peroxidase enzyme activation mechanisms of resistance within the cucumber plants [45].

As re. [46] pointed out mechanisms of systemic resistance (ISR and SAR) within plant byexisting pathogen and PGPRs bacteriawere biological inter relationship between others to formalize the genetic expression of resistance and provide a new strategy in resistance to pathogens plants.

CONCLUSIONS

Previous results showed that, thethree bacterial species reduced from disease severity of CMV on tomato plants and was the biggest influence for treatments F +CMV, FB + CMV and ABF + CMV. Accompanies increased both the content of salicylic acid and peroxidase enzyme activity with a low disease severity, which refers toActivation systemic resistance mechanisms in the tomato plants against CMV. Bacteria Frateuria aurantia showed the largest increase in the content of free salicylic acid and peroxidase enzyme activity in tomato leaves, followed by species Bacillus megaterium and Azotobacter chroococcum. Thus the possibility of using bacterial species studied together on a cucumber mosaic virus resistance, and studies on its effectiveness against other pathogens.

REFERENCES

[1] Group agriculture statics (2014). The Directorate of statistics and planning, Ministry of agriculture and agrarian reform, Syrian Arab Republic. In Arabic.

[2] G. P. Martelli, & A. Quacquarelli, (1983). "The present status of Tomato and pepper viruses". Acta Horticulturae. (ISHS). 127: 39-64.

[3] P. Soleimani, G. Mosahebi, M. K. Habibi, (2011). Identification of some viruses causing mosaic on lettuce and characterization of Lettuce mosaic virus from Tehran Province in Iran. Afr. J. Agric. Res. 6 (13), 3029-3035.

[4] A. K. Brunt, M. Carbtree, A. Dallwitz, Gibbs and L. Watson. (1996). Viruses of plants: descriptions and lists from the VIDE database. CAB. International. Printed and bound in the UK at the University press, Cambridge. 1484pp.

[5] P. Sutic, D. D. For, R. E. Tosic, (1999). Hand book of plant virus diseases. CRC prees. 553pp.

[6] G. N. Agrios, (2005). Plant pathology, 5thed. Elsevier,. 922p.

[7] R. Cerkauskas, (2004). Cucumber mosaic virus (CMV). Published by AVRDC- The World Vegetable Center, P.O. Box 42. Shanhua, Taiwan 741, ROC.

[8] H. Khalil, (2007). Investigation of viral diseases on tomato plants in Central and coastal area. Journal of Albaath University. Syria, volume 29 number (2), p231-246. In Arabic.

[9] H. Kawas, (2006). Studies on tomato viral diseases in southern Syria, and screening cultivars for resistance to infection with viruses. Ninth Arab Congress of Plant Protection, Damascus, Syria. 19-23 November, 2006, Damascus. Syria.

[10] T. M. Abdel Ghany, M. M. Alawlaqi, M. A. Al Abboud, (2013). Role of Biofertilizers In Agriculture: A Brief Review. Review Article. Mycopath 11, 2. p 95-101.

[11] J. S. Singh, (2013). Plant Growth Promoting Rhizobacteria Potential Microbes for Sustainable Agriculture. (Central) University, Raibarely Road, Lucknow 226025 Uttar Pradesh, India. 2013.pp7.

[12] B. S. Saharan, and V. Nehra, 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. Life Sciences and Medicine Research, Volume: LSMR-21.

[13] B. Bouizgarne, (2013). Bacteria for Plant Growth Promotion and Disease Management. Springer 454p 40 illus. hardcover.

[14] Z. A. Siddiqui, and M. S.Akhtar, (2007). Biocontrol of a chickpea root-rot disease complex with phosphate

solubilizing microorganisms. Journal of Plant Pathology. 89:31-42.
[15] C. J. Jeffries, (1998). Potato. FAO/IPGRI technical guidelines for the safe movement of germplasm. 19, 62-63.
[16] Y. Hammad, and R. Al-Shami, (2017). Identification some species of Plant Growth Promoting Rhizobacteria from some Biofertilizer and Soils. Journal of Albaath University. Syria. Vol 39. P 25. In Arabic.
[17] Technical Data. (2011). Pikovskayas Agar M520. HiMedia Laboratories. P2. AshbysMannitol Agar M706. HiMedia Laboratories. P3.
[18] P. Lisdiyanti, Y. Yamada, T. Uchimura, K. Komagata, (2003). Identification of Frateuriaaurantia Strains Isolated from Indonesian Sources. Microbiol.Cult. Coll. Vol. 19, No. 2. Dec. 2003. p. 81 -90.
[19] J. F. Murphy, M. S. Reddy, Ch.-M. Ryu, J. W. Kloepper and R. LI, (2003). Rhizobacteria- Mediated Growth Promotion of Tomato Leads to Protection Against Cucumber mosaic virus. Phytopathology. 93. p1301-1307.
[20] J. Maria Gil and V J ctor Mart J nez-Merino. (2007). Determination of The Free Salicylic Acid Concentration in Aspirin Byforming Fe+3 Complexes. P8.
[21] R. Hammerschmidt, E. M. Nuckles, and J. Kuc, (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrichum lagenarium. Physiol. Plant Pathol., 20: 73-82.
[22] B. Behera, S. Ghanty, F. Ahmad, S. Santra, S. Banerjee. (2012). UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. Analytical &Bioanalytical Techniques. J Anal Bioanal Techniques. Volume 3 . Issue 6.
[23] G. Raupach, S. Liu, L. John, F. Murphy, S. Tuzun, and J. W. Klopper, (1996). Induced Systemic Resistance in Cucumber and Tomato Against Ccumber Mosaic Cucumo virus Using Plant Growth Promoting Rhizobacteria (PGPR). Plant Dis. 80:891-894.
[24] J. W. Kloepper, C-M. Ryu, S. A. Zhang, (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94, 1259-1266
[25] K. Jetiyanon, and J. W. Kloepper, (2002). Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biological Control 24. 285-291.
[26] KH. A. El-Douggoug, A. A. Megahed, B. A. Othman, S. M. Lashin, M. A. Ibrahim and I. H. Attitalla, (2013). Induction of Salicylic Acid in Cucumber Plants Against Cucumber Mosaic Cucumovirus Using Biotic Inducers. American Journal of Biochemistry and Molecular Biology 3(2): 258-255.
[27] G. W. Zehnder, Y. Changbin, J. F. Murphy, E. R. Sikora, and J. W. Klopper, (2000). Induction of Resistance in Tomato Against Cucumber mosaic virus by Plant Growth- Promoting Rhizobacteria. Printed in the Netherlands Biocontrol 45.. p127-137.
[28] G. W. Zehnder, J. F. Murphy, E. J. Sikora, and J. W. Kloepper, (2001). Application of rhizobacteria for induced resistance. European Journal .of Plant Pathology 107: P 39-50.
[29] S. Sivasakthi; G. Usharani & P. Saranraj, (2015). Biocontrol potentiality of plant growth promoting bacteria (PGPR) Pseudomnas fluorescens and Bacillus subtils: A review. African Journal of Agriculture research. 9(16) .8 pp. 1265-1277.
[30] A. M. M. Mahdy, M. A. Hafez, Kh. A. EL-Dougdoug, R. N. Fawzy, and S. M. Shahwan, (2010). Effect Of Two Biotic Inducers on Salicylic Acid Induction in Tomato Infected with Cucumber Mosaic Cucumovirus. 3rd Inter. Conf. Virol., Cairo Univ. Center, Nov. 24-25. Egyptian J. Virol, SP. Issue, 355-372.
[31] Kh. A. El-Dougoug, M. F. Ghaly and M. A. Taha, (2012). Biological Control of *Cucumber Mosaic Virus* by Certain Local *Streptomyces* Isolates: Inhibitory Effects of Selected Five Egyptian Isolates. International Journal of Virology 8 (2): 151-164.
[32] M. Naylor, A. M. Murphy, J. O. Berry, J. P. Carr, (1998). Salicylic acid can induce resistance in plant virus movement. Mol Plant Microbe Interact 11:860-868.
[33] A. M. Murphy, A. Gilliland, C. J. York, B. Hyman, J. P. Carr, (2004). High-level expression of alternative oxidase protein sequences enhances the spread of viral vectors in resistant and susceptible plants. J Gen Virol 85:3777-3786.
[34] A. M. Murphy, S. Chivasa, D. P. Singh, J. P. Carr, (1999). Salicylic acid-induced rsistance to viruses and other pathogens: a parting of ways? Trends Plant Sci 4:155-160.
[35] D. Hondo, S. Hase, Y. Kanayama, N. Yoshikawa, S. Takenaka, H. Takahashi, (2007). The LeATL6 –associated ubiquitin/ proteasome system may contribute to fungal elicitor-activated defense response via the jasmonic acid-dependent signaling pathway in tomato. Mol Plant-Microbe Interact 20:72-81.
[36] L. C. Van Loon, (1997). Induced resistance in plants and the role of pathogenesis-related proteins. European Journal Plant Pathology, 103:753-765.
[37] L. C. Van Loon, C. M. J. Bakker, and P. A. H. M. Pieterse, (1998). Systemic resistance induced by rhizosphere bacteria. Annual Review of Phytopathology, 36: 453-483.
[38] L. C. Van Loon, and E. A. Van Strien, (1999). The families of pathogenesis related proteins and comparative analysis of PR-1 type proteins. Physiological and Molecular Plant Pathology, 55:85-97.
[39] D. K. Choudhary, A. Prakash, and B. N. Johri, (2007). Induced systemic resistance (ISR) in plants: mechanism of action. Indian Journal of Microbiology. 47 (4): 289-297.
[40] X. Nie, (2006). Salicylic acid suppresses Potato virus Y isolate N:O-induced symptoms in tobacco plants. Phytopathology 96:255-263.
[41] R. Hammerschmidt, J. P. M´etraux, and L.C. van Loon. (2001). Inducing resistance: a summary of papers presented at the First International Symposium on Induced Resistance to Plant Diseases, Corfu, May 2000. European Journal of Plant Pathology 107: 1-6,
[42] J. M. Chittoor, J.E. Leach and F.F. White, (1999). Induction of peroxidase during defense against pathogens. In: Pathogenesis: Related proteins in plants. S.K. Datta, S. Muthukrishnan (eds.). CRC Press, Boca Raton, FL. 291 pp.
[43] S. Ebrahim, K. Usha and B. Singh, (2011). Pathogenesis Related (PR) Proteins in Plant Defense Mechanism. Science against microbial pathogens: communicating current research and technological advances.pp12.
[44] E. Shahwan, S. M. El-Din. (2010). Inducing systemic resistance against some tomato virus diseases. Dissertation Submitted in Partial Fulfillment of the Requirements for The Degree Of DOCTOR Of PHILOSOPHY In PLANT PATHOLOGY (Viral Diseases). Pp: 256.
[45] A. M. El-Borollosy, And M. M. Oraby, (2012). Induced systemic resistance against Cucumber mosaic cucumovirus and promotion of cucumber growth by some plant growth-promoting rhizobacteria. Faculty of Agriculture, Ain Shams University, Annals of Agricultural Science 57(2). p 91-97.
[46] P. Lancioni, (2008). Studies on biotic and abiotic elicitors inducing defense responses in tomato. Ph.D. Thesis, Phytopathology Dept., Fac. Agric., University of Bologna, Italy 125pp.