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Research Article

Application of some plant growth-promoting rhizobacteria to enhance plant growth and protection against *Cucumber mosaic virus* in cucumber

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Abstract: The current study evaluated *Pseudomonas fluorescens* VUPf5 and three isolates of *Bacillus subtilis* (GB32, GB12, and VRU1) for induction of resistance against *Cucumber mosaic virus* (CMV) in cucumber *Cucumis sativus* L. (cultivar Sultan) plants. Seed treatment with plant growth-promoting rhizobacteria (PGPR) strains significantly reduced the number of symptomatic plants when CMV was mechanically inoculated. Serological analysis using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) also showed a significant reduction in the CMV accumulation in plants treated with PGPR strains. In every treatment, growth indices, leaf chlorophyll content, leaf carotenoid content, leaf, and root Iron, Zinc, Copper, and Manganese concentration of virus-infected plants were significantly increased. The highest reduction in CMV concentration was observed in plants treated with VRU1. The maximum chlorophyll concentration, leaf Iron, copper, and manganese were observed in plants treated with GB32.

Nevertheless, the highest carotenoid content was measured in the VUPf5 treatment. In the case of growth indices, the best results were obtained by VUPf5 compared to untreated control. In addition, the production of lipase, siderophore, protease, cellulase, HCN, auxin, and phosphate carbonate was determined under *in vitro* conditions. All four strains were positive for siderophore and auxin production. These results suggest that *P. fluorescens* and *B. subtilis* should be further evaluated for their potential to contribute to CMV management under *in vivo* and *in situ* conditions.

Keywords: *Bacillus subtilis*, *Cucumber mosaic virus*, DAS-ELISA, *Pseudomonas fluorescens*

Introduction

Virus-induced diseases are a global problem for cucurbit production and cause serious economic losses. Indeed, more than 35 different viruses have been isolated from cucurbits (Provvidenti, 1996). These viruses cause complex and dynamically changing problems (Nameth *et al*., 1986). Mosaic and yellowing are the most prevalent symptoms in cucumber greenhouses worldwide. Among plant viruses, cucumber mosaic virus (CMV) is considered one of the most economically damaging viruses in the field-grown vegetables (Sevik and Deligoz, 2017). This

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virus infects more than 1200 plant species worldwide, and it is efficiently transmitted by more than 80 aphid species (Duber *et al*., 2010; Palukaitis and Garcia-Arenal, 2003). 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 strategies. Besides, the growing cost of pesticides and the customer demand for pesticide-free food has led to a search for substitutes for these products. There are also some diseases, mainly viral and viroid diseases, for which there are no effective chemical solutions (Shehata and El-Borollosy, 2008). Therefore, many screening studies have been conducted on antiviral agents from different sources (Kubo *et al*., 1990). Plant growth-promoting bacteria (PGPB) are associated with many plant species and are commonly present in many environments. The most widely studied group of PGPB are (PGPR) colonizing the root surfaces (Saharan and Nehra, 2011), i.e., Azotobacter, Azospirillum, Rhizobium, Bacillus, Pseudomonas, and Serratia (Compant *et al*., 2005). Systemic resistance to the virus infection can be induced in plants treated with certain bacteria or bacterial products and chemicals (Bakker *et al*., 2003; Shoman *et al*., 2003). Many studies indicated that some plant growth-promoting rhizobacteria act as inducers of systemic resistance in plants (Jamali *et al*., 2004; Zeynadini-Riseh *et al*., 2018). Ryu *et al*. (2004) reported the plant growth-promoting rhizobacteria-mediated protection against CMV in *Arabidopsis thaliana*.

Zehnder *et al*. (2000) reported that *Bacillus amyloliquefaciens*, *B. pumilus*, and *B. subtilis* reduced the CMV infection and promoted tomato growth under greenhouse conditions. Additionally, tomato plants treated with a *Bacillus* sp. showed increased resistance against CMV (Wang *et al*., 2009). In another research, selected PGPR strains shown to induce resistance in previous experiments were evaluated as a paired combination for inducing resistance in tomato plants against CMV. Results showed that plants treated with bio-preparation had greater height, fresh weight, and flower and fruit numbers than plants in the control treatment. Moreover, CMV disease severity ratings and the CMV accumulation were significantly lower for bio-prepared plants (Murphy *et al*., 2003). Induction of CMV resistance in cucumber and tomato plants was observed by two PGPR strains, which had previously induced resistance against bacterial and fungal pathogens in cucumber under *in vivo* conditions (Raupach et al., 1996). El-Borollosy and Oraby (2012) found that Azotobacter followed by Pseudomonas irrigation crude culture treatment remarkably decreased the symptom of the virus in cucumber plants.

The objective of this study was to evaluate the ability of some PGPR strains to control CMV infection and enhance the growth of cucumber plants under pot conditions.

Materials and Methods

PGPR isolates

*Pseudomonas fluorescens*VUPf5 and *Bacillus subtilis* VRU1 were obtained from the culture collection unit at the Vali-e-Asr University of Rafsanjan. *B. subtilis* GB12 and GB32 were obtained from the Plant Protection Research Department, Agricultural Research Center of Kerman, Iran. For further studies, bacterial isolates were kept at -80 ℃ in 15% glycerol and Luria Broth (LB).

Evaluation of biocontrol ability of bacterial isolates under *in vitro* conditions

Siderophore production

The isolates were checked for the production of siderophores on blue agar containing chromeazurol S (CAS) medium and hexadecyltrimethylammonium bromide (HDTMA) as indicators (Schwyn and Neilands, 1987). The blue agar CAS medium was prepared by adding 850 ml of autoclaved MM9 salt medium [added with 32.24 g piperazine-N, N0 - bis 2- ethane sulfonic acid (PIPES) at pH 6], 100 ml of blue dye, 30 ml of filter-sterilized 10%

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Casamino acid solution and 10 ml of 20% glucose solution. The blue agar medium was aseptically poured onto sterile plates and allowed to solidify. All the bacterial isolates were inoculated into the CAS medium and incubated at 28 °C for 24 h. The development of a golden yellowish-orange halo around the growth was considered positive for siderophore production (Jasim *et al*., 2014).

Lipase production

The medium containing peptone 10 g, calcium chloride 0.1 g, sodium chloride 5 g, agar 15 g, distilled water 1 L, 10 ml sterile tween 20 was used to determine the lipase enzyme activity (Omidvari, 2015). The bacteria were streaked on this medium and incubated at 27 ˚C for 48 h. Depositions around the bacterial colonies indicated the activity of the lipase enzyme.

Cellulase production

To evaluate the production capacity of cellulase enzyme, a medium containing 0.5% CMC, 0.1% $NaNO_3$,0.1% K_2HPO_4,0.1% KCl,0.05% $MgSO_4$, 0.05% yeast extract, 1.5% agar in one liter of distilled water with pH = 7 was used. After 24 h of culture, the desired bacteria were cultured on a plate containing the above culture medium. The plates were incubated at 28 °C for five days, then flooded with 0.1% Congo red solution for 15 min, and then washed three times with 0.1 M NaCl solution. The clear zone diameter around the colony on CMC agar was measured (Kasana *et al*., 2008).

Hydrogen cyanide

Production of HCN was assessed on King's B medium containing 4.4 g/L of glycine. The indicator was Whatman paper soaked in 0.5% (v/v) picric acid and 2% (w/v) sodium carbonate and placed inside plates incubated at 27 °C for 48 h–72 h. Any positive response caused the indicator paper to turn from yellow to cream, light brown, dark brown, and brick brown scaled 1-4 (Alstrom and Burns 1989).

Protease

Production of extracellular protease was tested by spotted bacterial strains on skim milk agar (SMA) plates (Maurhofer *et al*. 1995). The experiments were performed in triplicate. Semi quantification evaluation of protease production measured the clear halo zone around the bacterial colonies.

Auxin

Strains were grown in 100 ml L-broth medium in 250 ml Erlenmeyer flasks supplemented with 10 ml filter-sterilized solution of L-tryptophan to a final concentration 1 mg/ml). The flasks were inoculated with 100 μL of bacterial cell suspension adjusted to an optical density of 10^7 CFU ml^{-1} measured at 600 nm by spectrophotometer (S-300D; R & M Marketing, Hounslow, UK). All flasks were incubated at 37 ºC for 72 h at 120 rpm) in triplicate. After incubation, cells were removed from the culture medium by centrifugation at 2300 g for 15 min (Sigma 2-5; Sigma Laborzentrifugen, Osterode, Germany). A 1-ml aliquot of the supernatant was mixed vigorously with 4 ml of Salkowski's reagent (150 ml of concentrated H_2SO_4, 250 ml of distilled H_2O, 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$), and allowed to stand at room temperature for 20 min before the absorbance at 535 nm was measured. The concentration of IAA was determined by comparison with a standard curve (Ali and Hasnain, 2007).

Phosphate solubilization

Phosphate solubilization was studied using Pikovskaya Agar plate assay, an in vitro assay for phosphate solubilization following Paul and Sinha (2015). Phosphate solubilization index and phosphate solubilization efficiency were calculated from inhibition zones using the following formulas:

Index: PSI ¼ (Colony diameter + Halo zone diameter)/colony diameter

Efficiency: PSE (%) ¼ ((Halo zone diameter - Colony diameter)/Colony diameter) × 100

CMV inoculation

Cucumber mosaic virus (CMV) was obtained from the culture collection unit at the Vali-e-Asr University of Rafsanjan. The virus was maintained on cucumber plants under greenhouse conditions and was used in all greenhouse experiments.

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***In vivo* assay**

Seed bacterization

Bacteria were applied to surface-disinfested cucumber seeds (*Cucumis sativus* L. cultivar Sultan) according to Ownley *et al*. (1992). Briefly, bacteria were cultured on nutrient agar and incubated at 27 °C for 48 h. The bacterial lawns were suspended in 5 ml of sterile deionized water. Then 2 ml of the suspension was inoculated onto each of two King medium B agar plates and incubated at 27 °C for 24 h. Bacteria from both King medium B plates were suspended in 20 ml of medium-viscosity 0.5% (wt./vol) methylcellulose (MC) (Sigma, St. Louis, Mo.) in a shaking incubator with 120 rpm at 39 °C for 12 h. Seeds were coated with bacteria by mixing 2.5 ml of bacterial suspension with 5 g of seed. The coated seeds were dried for 1.5 to 2 h under a stream of sterile air at room temperature. This process consistently yielded densities from 10^7 to 10^8 CFU/seed, verified by dilution plating before planting. The control was treated with 0.2 M phosphate buffer.

Greenhouse assay

In each experiment, 4 PGPR strains were tested along with the control (CMV inoculation, no PGPR) and the healthy control (no CMV inoculation, no PGPR). Three replications per treatment were arranged in a randomized block design consisting of 10 cucumber plants. Seeds were planted in plastic pots containing sterilized soil. Experiments were conducted under greenhouse conditions at 32/25 °C day/night temperatures. Four weeks after planting, the first two leaves (cotyledonary leaves) of each cucumber plant were lightly dusted with carborundum and then mechanically inoculated with CMV inoculum. Inoculum consisted of CMV-infected cucumber leaf tissue ground in 50mM KPO_4, pH 7.5, containing 10 mM sodium sulfite at a ratio of 1g tissue to 5 ml buffer. The healthy control plants were inoculated with sterile buffer instead of CMV.

Disease assessment

Disease incidence was recorded as the CMV concentration in different treatments. The CMV accumulation in the leaves of untreated and treated test plants was measured using the indirect Enzyme-linked Immunosorbent Assay (ELISA) method described by Clark and Adams (1977). Each plant was sampled after one month by collecting three young non-inoculated leaves. To normalize the ELISA samples, 0.5 g of plant tissue was mixed with 10 × (w/v) coating buffer (15mM sodium carbonate, 35mM sodium bicarbonate, pH 9.6, containing 2% polyvinyl pyrrolidone: CB-PVP), then homogenized using a mortar and pestle for sap extraction. Extracted crude sap was filtered through cheesecloth and centrifuged at 6000 rpm for 2 min. The clarified extract was pipetted into wells of polystyrene plates. The antigen solution was stored overnight at 4℃ in or at 37℃ for three hours. After three washes with phosphate-buffered saline (PBS) containing 0.5% Tween-20 (PBS-T) for 3 min, the plates were blocked by incubation in 1% bovine serum albumin (BSA) in PBS, for 30-60 min. The blocking solution was replaced by appropriately diluting a specific monoclonal antibody against CMV.

Evaluation of plant growth characteristics

One month after inoculation with different treatments, the plants were harvested following the procedure described by Radwan *et al*. (2007). Plants were carefully dislodged from the soil. After washing the roots, the leaf numbers, heights, and fresh weights were measured. After that, the plants were kept in paper bags and dried in an oven for 2-3 days for dry weight determination.

Chlorophyll and carotenoid assays

At the end of the experiment, to evaluate chlorophyll a, b, total chlorophyll, and total carotenoid, 0.25 g of fresh and old cucumber leaves were chopped and ground in a laboratory mortar with 10 ml of acetone (80%). The resulting mixture was then poured into a 10 ml Falcon tube and centrifuged at 3500 rpm for 10 minutes. The absorption of supernatant was measured with a spectrophotometer (PG, T80UV/VIS spectrometer) at 480, 510, 625, 645, and 663 nm. The chlorophyll a, chlorophyll b, total chlorophyll, and total carotenoids were determined according to the formula of Arnon (1949):

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Chl a (mg. g^{-1}fw) = [[(12.7 × OD 663) – (2.69 × OD 645)] × V] ÷ [1000 × V]
Chl b (mg. g^{-1}fw) = [[(22.9 × OD 645) – (4.68 × OD 663)] × V] ÷ [1000 × V]
Total chl (mg. g^{-1}fw) = [[(8.02 × OD 663) + (20.2 × OD 646)] × V] ÷ [1000 ×V]
Total cha (mg. g^{-1}fw) = [[7.6 × OD 480 – 1.49 × OD 510] × V] ÷ [1000 × V]

Plant analysis
The percentage of total Iron, Zinc, copper, and manganese in cucumber shoots was determined according to Chapman *et al*. (1983). First, the shoots of the cucumber plant in different treatments were dried in an oven at 70 ° C for 72 h; then, 0.5 g of dried tissue was ground and poured into a laboratory porcelain plate. The samples were dried in an oven at 250 °C for half an hour and then at 550 °C for 3 h to be reduced to ashes. After cooling, the samples were transferred from the oven, and 5 ml of 2 N hydrochloric acid was added to each porcelain plate. The samples were then poured through a filter paper into a 50 mm volumetric balloon, and each balloon was double-distilled with water. The concentrations of Iron, Zinc, Copper, and Manganese in the extract were measured using an atomic absorption spectrometer (GBC Avanta, Australia).

Statistical analysis
An analysis of variance (ANOVA) and Duncan's multiple tests (at $P \leq 0.05$) were performed to analyze statistical differences and discriminate among means.

Results

Biochemical characterization
Siderophore
All bacterial strains produced siderophore on CAS blue agar and were able to change the color of the CAS medium from blue to orange. Among them, VUPf5 showed a high level of siderophore production.

Protease
Extracellular protease can contribute to the ability of bacteria to suppress diseases. Strain JB32 protease test was negative. The diameter of the halo zone around strain VUPf5 was the largest, followed by JB12 and VRU1with a clear zone of 2.2 mm, 2 mm, and 1.7 mm, respectively.

Cellulase
Strains VRU1, JB32, and JB12 produced cellulase with 3.2 mm, 3 mm, and 3mm zone diameters. VUPf5 was cellulase-negative.

HCN
VUPf5 and VRU1 were able to produce higher quantities of HCN. JB32 and JB12 had little or no HCN production.

Auxin
The auxin production was determined on TSB medium containing L-Tryptophan. All bacterial strains were able to produce auxin; however, the highest amount of production was by VUPF5 with 3.28 µg/ml, and the lowest amount was by JB32 with 26.9 µg/ml.

Phosphate solubilization
The results of phosphate dissolution of bacterial strains after five days with the appearance of a colorless halo around the colony showed that this test was positive in VUPF5 and VRU1. The results showed that VUPF5 strain with a halo diameter of 2.11 cm had the highest activity and VRU1 strain with a halo diameter of 1.4 cm had the lowest ability to solubilize phosphate. Strains JB32 and JB12 could not dissolve phosphate.

Greenhouse experiments
The CMV accumulation in systemically infected cucumber leaves was measured by ELISA (Fig. 1). The results provided evidence of PGPR-mediated induced resistance against CMV in cucumber. Compared to the challenge control, CMV accumulation decreased significantly in plants treated with VUPf5, VRU1, GB12, and GB32. The most pronounced effect was obtained by applying VRU1 followed by VUPf5 and GB32. The highest percentage of CMV infection was recorded in challenge control plants. However, the use of bacterial strains alone induced resistance in cucumber plants against CMV.

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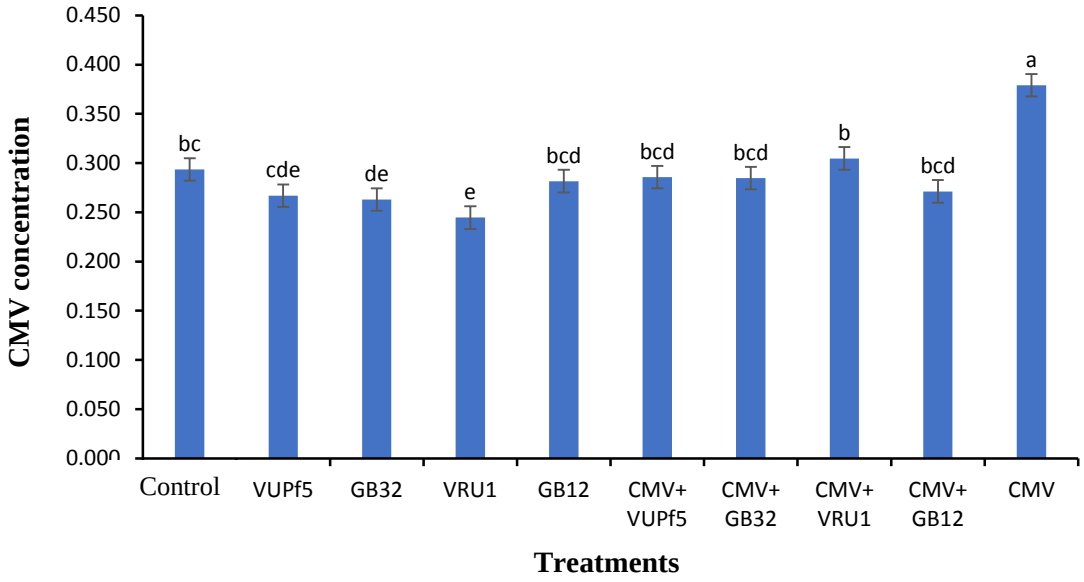

Figure 1 Mean comparison of the Cucumber mosaic virus concentration based on the light density in different treatments using a spectrophotometer at 450 nm. Columns with the same letters are not significantly different from each other (Duncan's multiple range test, $P \leq 0.05$). Value was the average of three replicates.
Abbreviations: *Pseudomonas fluorescens* VUPf5 and three isolates of *Bacillus subtilis* (GB32, GB12, and VRU1)

Plant growth

Tables 1 and 2 show that CMV-infected cucumber plants had significant decreases in shoot length, root length, fresh and dry weight of shoots, fresh and dry weight of roots, and leaf numbers compared with control plants. On the other hand, plants treated with VUPf5, VRU1, GB12, and GB32 significantly increased all the above-mentioned morphological characteristics compared to the challenge control. Specifically, VUPf5 resulted in the highest shoot lengths (73.08 mm), root length (21.99 mm), leaf numbers (9.25), dry weight of shoots (3.11 g), and fresh weight of roots (2.42 g). In the case of the dry weight of roots and fresh weight of shoots, GB12 and VRU1 with 0.185 and 25.74g, respectively, gave the best results.

Chlorophyll and carotenoids content

Chlorophyll a and b content in leaves of cucumber plants showed a significant decrease after being challenged with CMV compared to non-inoculated control plants; however, the carotenoid content in both CMV-inoculated and non-inoculated controls was the same. Chla, Chlb, and carotenoid content increased significantly after treatment with VUPf5, VRU1, GB32, and GB12. The most pronounced increase in Chla content was detected in plants treated with GB32 (74.26%). GB12+CMV treatment had the highest effect in increasing Chlb content. Cucumber plants treated with VUPf5 showed the highest carotenoid content compared to other treatments. Infection with the virus caused a notable reduction in Chla, Chlb, and carotenoid content compared to non-inoculated control plants (Table 3 and 4).

Plant analysis

The quantities of Fe, Zn, Mn, and Cu, in the infected and healthy control cucumber plants are presented in Tables 3 and 4. The obtained data showed that Fe, Zn, Mn, and Cu in leaves and roots markedly decreased with the CMV infection. In contrast, these micronutrients slightly increased when cucumber plants were treated with bacterial isolates. No significant changes were observed in the Zn content of roots.

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Table 1 Mean squares of cucumber parameters when seeds were treated with plant growth-promoting rhizobacterial strains.

Source of variation	Df	Shoot length	Root length	Leaf numbers	Shoot fresh weight	shoot dry weight	Root fresh weight	Root dry weight
Treatments	9	503.33	29.94	9.04	63.47	0.640	0.370	0.00090
Error	30	3.37	1.60	0.35	0.47	0.037	0.007	0.00006
CV%		11.00	11.01	15.42	5.98	22.350	20.390	11.74

CV: Coefficient of variation.

Table 2 Evaluation of cucumber growth parameters when seeds were treated with plant growth-promoting rhizobacterial strains under *in vivo* conditions.

Treatments	Shoot length (cm)	Root length (cm)	Leaf numbers	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	46.53e	15.21d	6.79b	17.23d	2.39b	1.66e	0.152e
VUPf5	73..08a	21.99a	9.25a	25.3a	3.11a	2.42a	0.180ab
GB32	68b	19.70b	8.71a	23.89b	3.02a	2.18b	0.175abc
VRU1	69b	19.93b	8.67a	25.74a	2.82a	2.20b	0.177abc
GB12	66.70b	19.61b	9.13a	24b	2.98a	2.35a	0.185a
CMV + VUPf5	64.89c	18.04bc	7.25b	18.95c	2.25b	1.99c	0.159de
CMV + GB32	64.64c	18.66bc	7.25b	19c	2.39b	1.97cd	0.167bcd
CMV + VRU1	54d	16.93cd	6.58b	18.46c	2.80a	1.92cd	0.158de
CMV + GB12	62.33c	17.49c	7b	19.3c	2.5b	1.85d	0.164cd
CMV	37.71f	12.2e	4.33c	13.57e	1.88c	1.41f	0.132f

Means with the same letters in each column are not significantly different (Duncan's multiple range test, $P \leq 0.05$).

Table 3 Mean squares of chlorophyll a, chlorophyll b, carotenoid, and some microelements in cucumber plants treated with plant growth-promoting rhizobacterial strains.

Source of variation	df	Chla	Chlb	Carotenoid	Fe		Zn		Cu		Mn	
					Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Treatments	9	4.92	0.509	0.26	0.077	0.203	0.0024	0.0016	0.0012	0.0033	0.022	0.0309
Error	30	0.034	0.00034	0.0042	0.0014	0.0017	0.0022	0.00005	0.00001	0.000009	0.0008	0.0006
CV%		25.98	23.94	22.25	25.78	26.05	21.66	24.69	11.61	15.5	18.59	5.45

CV: Coefficient of variation.

Table 4 The chlorophyll, carotenoid, Fe, Zn, Cu, and Mn content in cucumber plants when seeds were treated with plant growth-promoting rhizobacterial strains.

Treatments	Chla (mg)	Chlb (mg)	Carotenoid (mg)	Fe (µg)		Zn (µg)		Cu (µg)		Mn (µg)	
				Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Control	3.32e	1.24fd	2.17f	3.23bc	3.89d	0.32ef	0.23cd	0.063bc	0.034d	0.75cd	0.72d
VUPF5	5.51b	1.56c	4.96a	4.03a	5.46a	0.45bc	0.32b	0.080b	0.065bc	0.91bc	1.36a
GB32	5.89a	1.56c	4.43b	4.10a	4.92b	0.38cde	0.30b	0.286a	0.034d	1.44a	1.20b
VRU1	4.68c	1.54c	3.78c	3.97a	4.37c	0.39cde	0.37a	0.081b	0.078b	0.80c	1.07bc
GB12	5.24b	1.54c	4.61ab	3.29bc	5.28a	0.51ab	0.39a	0.082b	0.109a	0.91bc	1.45a
CMV + VUPf5	3.37e	1.22de	3.34d	3.26bc	4.78b	0.44bcd	0.23cd	0.062bc	0.049cd	0.83c	0.93c
CMV + GB32	3.54e	1.65b	3.12d	3.45b	3.40e	0.57a	0.24c	0.068bc	0.120a	1.02b	0.94c
CMV + VRU1	3.28e	1.82a	2.93e	3.19c	3.13f	0.35def	0.18e	0.054cd	0.030d	0.60d	1.05c
CMV + GB12	4.21d	1.20e	3.40d	3.2c	4.32c	0.28f	0.19de	0.040d	0.034d	0.43e	0.38e
CMV	2.64f	0.55f	1.78g	2.21d	2.81g	0.27f	0.16e	0.041d	0.006e	0.41e	0.47e

Means with the same letters in each column are not significantly different (Duncan's multiple range test, $P \leq 0.05$).

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Discussion

Considering the disadvantages of chemical pesticides, there are increasing efforts aiming to utilize PGPRs against many phytopathogens to improve crop production (Saberi Riseh *et al*., 2018; Moradi pour *et al*., 2019; Saberi Riseh and Moradi pour, 2021; Fathi *et al*., 2021; Saberi Riseh *et al*., 2021). Most of the bacterial isolates used in this study increased growth of cucumber inoculated with CMV. Although, VUPf5 isolate was the best in improving plant growth. *Pseudomonas* sp. produces a wide variety of antibiotics, growth-promoting hormones, HCN and can solubilize phosphorous (Rodriguez and Fraga, 1999). Better inhibition of CMV and growth promotion was achieved by isolates that produced more siderophore, protease, cellulase, and HCN. There are a lot of strains with the ability to induce resistance in plants by siderophore production (Höfte and Bakker, 2007). Some authors found that the production of pyoverdines contributes to the biocontrol capacity of the fluorescent Pseudomonads (Becker and Cook, 1988; Loper and Buyer, 1991). Siderophore-mediated competition for iron is one of the mechanisms of the bacterial antagonism against soil-borne pathogens (Loper and Buyer, 1991). It has been reported that the lack of exoprotease reduces the biocontrol activity of CHA0 against Meloidogyne incognita (Siddiqui *et al*., 2005). Pseudomonas spp. has been reported to inhibit potato root development by HCN production (Bakker *et al*., 1989). HCN produced by rhizosphere Pseudomonads had a detrimental effect on plant establishment in several crops (Schippers *et al*., 1987) and suppressed root pathogens (Défago *et al*., 1990). Murphy *et al*. (2003) evaluated the effect of *Bacillus subtilis* GB03, *B. amyloliquefaciens* IN937a, *B. pumilus* SE34, *B. pumilus* T4, *B. subtilis* IN937b, and *B. pumilus* INR7 for the ability to induce growth promotion of tomato plants and resistance to infection by CMV. Their results indicated that tomato plants treated with PGPR had significantly greater height, fresh weight, and flower and fruit numbers than CMV-inoculated control treatments. Moreover, treating tomato plants with biopreparations resulted in significant protection against infection by CMV. Similarly, 1 out of 4 plants irrigated with *Pseudomonas* culture showed virus but with a lower density compared with control plants (El-Borollosy and Oraby, 2012). Results of physiological characteristics of cucumber plant′s were much better in treated plants compared with controls. Statistical analysis of plant growth data showed significant differences between the four treatments. Our findings were in harmony with those of El-Borollosy and Oraby (2012). Khalimi and Suprapta (2011) studied the efficacy of *Pseudomonas aeruginosa* in inducing plant growth on soybean against *Soybean stunt Cucumovirus* under in vivo conditions. They found that these bacteria increased plant growth parameters compared with untreated plants. In cucumber plants, infection with the virus caused a notable reduction in Chla, Chlb, and carotenoid content compared to non-inoculated control plants (Table 3 and 4). In similar studies, the CMV infection reduced photosynthetic pigments and plant growth in cucumbers (Farahat *et al*., 2018; Sofy *et al*., 2020). Also, Vitti *et al.* (2016) found that photosynthetic pigments and plant growth were reduced in CMV- infected tomato plants. The reduction in chlorophyll content in virus-infected plants can be caused by the stimulation of specific cellular enzymes such as chlorophyllase (Rahoutei *et al*., 2000) or by the effect of the virus on pigment synthesis (Balachandran *et al*., 1997). The cucumber plants treated with VUPf5, VRU1, GB32, and GB12 significantly enhanced chlorophyll content, contributing to the improvement and sustainable plant production and increasing plant tolerance through better uptake of essential nutrients by PGPRs (Adesemoye and Egamberdieva, 2013).

In conclusion, the four PGPR strains studied here could induce resistance against CMV. However, VUPf5 and VRU1 had a better effect on decreasing CMV concentration and promoting plant growth parameters. Based on the obtained results, it may be recommended to use *P. fluorescens* and *B. subtilis* for plant

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growth promotion and as a systemic resistance inducer against viral infections.

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استفاده از تعدادی ریزوباکتری افزایش‌دهنده رشد گیاه به‌منظور افزایش رشد بوته خیار و محافظت در برابر ویروس موزاییک خیار

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چکیده: مطالعه حاضر به‌منظور ارزیابی *Pseudomonas fluorescens* VUPf5 و سه جدایه از *Bacillus subtilis* (GB12 GB32 و VRU1) برای القاء مقاومت در برابر ویروس موزاییک خیار در گیاهان خیار رقم سلطان انجام شد. مایه‌زنی بذر با سویه‌های PGPR هنگامی‌که CMV به‌صورت مکانیکی تلقیح شد، به‌طور قابل‌توجهی تعداد گیاهان دارای علایم را کاهش داد. تجزیه‌وتحلیل سرولوژی با استفاده از روش الیزا (DAS-ELISA) کاهش قابل‌توجهی در تجمع CMV در گیاهان تلقیح شده با سویه‌های PGPR نسبت به گیاهان تلقیح نشده نشان داد. در هر تیمار، شاخص‌های رشد، محتوای کلروفیل برگ، محتوای کاروتنوئید برگ و همچنین غلظت آهن، روی، مس و منگنز ریشه و برگ گیاهان آلوده به ویروس به‌طور معنی‌داری افزایش یافت. بیش‌ترین کاهش غلظت CMV در گیاهان تحت درمان با VRU1 مشاهده شد. با این‌حال، تفاوت معنی‌داری بین VRU1 و سایر تیمارهای باکتریایی وجود نداشت. حداکثر غلظت کلروفیل، آهن برگ، مس و منگنز در گیاهان تیمار شده با GB32 مشاهده شد. اما، بیش‌ترین مقدار روی و کاروتنوئید در تیمار VUPf5 اندازه‌گیری شد. در مورد شاخص‌های رشد، بهترین نتایج توسط VUPf5 در مقایسه با کنترل حاصل شد. علاوه براین، تولید لیپاز، سیدروفور، پروتئاز، سلولاز، HCN، اکسین و کربنات فسفات توسط باکتری‌های ذکر شده در شرایط آزمایشگاه مورد آزمایش قرار گرفت. هر ۴ سویه برای تولید سیدروفور و اکسین مثبت بودند. این نتایج نشان می‌دهد که باید پتانسیل *P. fluorescens* و *B. subtilis* برای کمک به مدیریت CMV در شرایط in vivo و in situ بیش‌تر مورد ارزیابی قرار گیرد.

واژگان کلیدی: الیزا، *Pseudomonas fluorescens*، CMV، *Bacillus subtilis*

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