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Control of Cucumber Downy Mildew by Some Plant Growth Promoting Rhizobacteria under Greenhouse Conditions

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ABSTRACT

Efficacy of six plant growth promoting rhizobacterial strains of *Pseudomonas* sp. and *Bacillus* spp. against cucumber downy mildew disease caused by *Pseudoperonospora cubensis* was assessed during two successive seasons, 2015 and 2016. Foliar application of the tested bacterial agents to manage the foliar disease incidence was carried out under greenhouse conditions in comparison to chemical fungicide Amistar. Results showed a significant effect of all treatments on disease severity as well as growth parameters (plant height, fresh and dry weight and crop yield) of cucumber plants relative to control. Application with either *B. subtilis* or the two strains of *P. fluorescens* showed significant reduction in disease incidence comparing with the other applied bacterial strains. Amistar showed the best performance followed by both *P. fluorescens* I and II with slight difference with respect to disease severity, area under disease progress curve (AUDPC), treatments efficacy (%) and plant growth during the two successive seasons. The bacterial application induced the defense-related enzymes involved in peroxidase and polyphenoloxidase in addition to direct antagonism and well bacterial colonization of leaves, which collectively contribute for enhancing resistance against invasion of *P. cubensis* in cucumber. The tested bacterial strains proved their antagonistic ability against cucumber downy mildew under greenhouse conditions, particularly both *P. fluorescens* strains and *B. subtilis*, which showed the highest performance against the pathogen. Environmentally friendly technologies, such as the use of bio-agents to control downy mildew disease of cucumber, represent promising alternative for the sustainability of agricultural ecosystems.

Key words: Biocontrol, downy mildew, cucumber, amistar, PGPR, *Bacillus* and *Pseudomonas*.

Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important cucurbit crops in Egypt and all over the world. Cucumber plants are subjected to many destructive fungal, bacterial and viral diseases. Downy mildew caused by *Pseudoperonospora cubensis* is one of the most serious foliar diseases that attack cucurbits on protected cultivation worldwide. It causes a major production problem in Egypt and in many areas of the world. The reduction of fruit quality and crop yield being the most striking aspects of disease loss (Reuveni and Raviv, 1997).

Plant growth promoting rhizobacteria are a group of bacteria that can actively increase plant growth and provide plants with protection against plant pathogens (Kloepper and Schroth, 1978). Most researchers have been concentrating on the use of Gram-negative bacteria belonging to *Pseudomonas* spp. and Gram-positive belonging to *Bacillus* spp. (Costa *et al.*, 2001; Slininger *et al.*, 2000). These microorganisms may have different mechanisms to provide protection or prevention against plant pathogens. They may improve the plant growth or combat the plant pathogen through niche exclusion, antibiosis, competition for nutrients, production of lytic enzymes and induced systemic resistance (Haas and Keel, 2003).

The most amazing features include their ability to produce numerous antimicrobials that are involved in disease suppression (Stein, 2005). *Bacillus* spp. are able to produce powerful plant growth promoting traits such as indole acetic acid (IAA) and gibberellic acid (GA3) besides, biocontrol attributes like production of HCN, hydrolytic enzymes and antibiotics (Senthilkumar *et al*., 2009).

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Recently, *Pseudomonas fluorescens* was shown to be as a biocontrol agent against seed, soil and foliar diseases on plant pathogens (Bharathi *et al*., 2004; Ramamoorthy *et al*., 2002). Florescent *Pseudomonas* are able to suppress diseases by producing protease, glucanase, HCN and defense enzymes, in addition to enhancement the induced systemic resistance (Ko *et al*., 2009). The present work aimed to study the impact of six different bacterial strains belonging to *Pseudomonas* spp. and *Bacillus* spp. to control cucumber downy mildew caused by *P. cubensis*, and their ability to produce plant growth promoters in order to enhance cucumber plant growth and increase crop yield under greenhouse conditions.

Materials and Methods

Tested bacterial strains:

Bacillus subtilis, B. pumilus, B. polymyxa, B, megaterium, *Pseudomonas fluorescens* I and *P. fluorescens* II used in this study were kindly obtained from the Department of Agric. Microbiology, (SWERI), ARC, Giza, Egypt. *Bacillus* strains were maintained on nutrient medium (Difco, 1985) for 48h at 28±2ºC and maintained refrigerated at 4ºC until use. Whereas, *P. fluorescens* I and *P. fluorescens* II were cultured and maintained on King's medium (King *et al.,* 1954) at 28±2ºC for 5 days.

Preparation of bacterial suspension.

Bacterial suspension was prepared by cultivating *Bacillus* strains in nutrient broth medium, while *Pseudomonas* strains in King's broth medium using shaking flask submerged culture, where 500 ml conical flasks containing 200 ml of the respective media were inoculated by one ml bacterial inoculums of 24 h old culture and incubated in rotary shaking incubator (120 rpm) at 28±2ºC for 72 h. The bacterial suspension density was adjusted to 10^8cfu/ml (Sun *et al*., 2013). The obtained cell suspensions were used directly on the cucumber plants as foliar spray.

Characterization of bacterial strains features.

The ability of bacterial strains to produce hydrogen cyanide, glucanase enzyme, plant growth hormones, i.e. indole acetic acid and gibberellins were studied.

Hydrogen cyanide (HCN) production (qualitative assay).

Hydrogen cyanide was determined by modified method of Bakker and Schippers (1987). Bacterial strains were streaked on King's and nutrient agar media supplemented with or without 4.4 g glycien L^{-1} with simultaneous addition of filter paper soaked in 0.5% picric acid dissolved in 1% Na_2CO_3 in the upper lids of plates along with un-inoculcated control. The plates were sealed with Para film and incubated at 28±1ºC for 48 h. A change of color of the filter paper to brown was recorded as follows: light brown as week (+), brown as moderate (++) and dark brown as high (+++).

Production and determination of β- Glucanase.

β- Glucanase was produced and determined according to the method described by Dewi e*t al*. (2016).

Determination of indole acetic acid and gibberellic acid production.

Indole acetic acid was determined according to the method of Gordon and Weber (1951). Gibberellic acid was determined by colorimetric method of Holbrook *et al.* (1961).

Control of downy mildew on cucumber under greenhouse conditions.

The study was carried out under greenhouse conditions during the two successive seasons of 2015 and 2016 at Kaha Res. St., Qalyobia governorate, Egypt. Thirty days old cucumber seedlings c.v. Tifa obtained from Agrotech for modern agriculture Co. were transplanted at spacing of 50 cm on

two sides of the ridge. The treatments were arranged in randomized complete block design with four replicates; each replicate was 2 m length and 0.5 m width with 10 plants. Plants were fertilized with the recommended doses of mineral fertilizer. All bacterial strains were applied as foliar spray using bacterial suspension containing $1x10^8$ cfu/ml (Hamza *et al*., 2015). The antagonistic bacterial suspensions were sprayed with the sticking agent (1% Arabian gum), in the cucumber phylloplane, three times with 10 days intervals starting from one week of transplanting, at the rate of 5 L fed^{-1}. Disease severity of each season was assessed five times after the first spray with 7 days interval.

Tested fungicides

Amistar 25% SC (Azoxystrobin: methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxyJphenyl}-3- methoxyacrylate) was used as chemical control at the rate of 0.25 ml L^{-1} for comparison with the biocontrol agents.

Disease assessment

Plants were carefully examined to estimate the severity of downy mildew infection based on score chart of 0 to 5 scale (0: no infection, 1: 1 - 10, 2: 10.1 - 15, 3: 15.1 - 25, 4: 25.1 - 50 and 5: more than 50 percent of leaf area being covered with mildew growth as described by Singh *et al*. (1994).
The following equation was applied: $P = \Sigma (n \times y)/ SN \times 100$
Where: P = disease severity (%), n = number of infected leaves in each category, y = numerical values of each category, S = the highest rating value and N= total number of the infected leaves.
On the other hand, efficacy of each treatment was calculated as follows:
Efficacy (%) = (Control-Treatment / Control) x100 .

Area under disease progress curve (AUDPC) was estimated to compare different treatments on severity of downy mildew according to Pandey *et al*. (1989) using the following equation:
AUDPC = D [1/2 (Y1 +YK) +Y2 + Y3 +….Y (K-1)]
Where: D = days between readings, Y1 = first disease record and YK = last disease record.

Efficacy of bacterial strains related to fungicide

The efficacy of each treatment related to fungicide was calculated as follows:
% Efficacy = (Efficacy of treatment/ Efficacy of fungicide) x100.

Determination of peroxidase and polyphenol oxidase activity.

Treated cucumber plants were sampled and tested for the activity of peroxidase and polyphenoloxidase at plant age of 60 days. Plant tissues were cut into small portions and rapidly ground with 0.1 M sodium phosphate buffer at pH 7.1 (2 ml buffer g fresh $tissue^{-1}$) in a mortar. These triturated tissues were strained through four layers of cheese–cloth and the filtrates were centrifuged at 3000 rpm for 20 min at 6°C (Maxwell and Bateman, 1967). The supernatant fluids were used for enzyme assay.
- Peroxidase activity was determined according to Allam and Hollis (1972).
- Polyphenoloxidase activity was determined according to Maxwell and Bateman (1967**).**

Phylloplane bacterial population

Total bacterial counts were assessed to evaluate the survival of *Bacillus* and *Pseudomonas* strains on sprayed cucumber leaves by taking leaf samples at 0, 10, 20 and 30 days after 3rd foliar applications. Leaf samples (1 g) were transferred to a test tube containing 100 ml of sterile water. After thorough shaking, the bacterial population in the suspension was estimated by dilution plating, using nutrient agar medium at 28°C for 48 h.

Soil microbiological variables

Samples of cucumber rhizosphere soil were collected directly after the 3rd foliar applications to determine the total bacterial counts, which estimated on nutrient agar using the spread plate method (Difco, 1985) and total microbial activity was measured using dehydrogenase activity (µg TPF g rhizosphere^{-1}day^{-1}) as described by Skujins (1976).

Growth measurements

To determine whether the selected antagonistic microorganisms under test have any positive or negative effects on cucumber plants during two successive seasons (2015 and 2016), some of botanical and yield measurements were determined in the experimental plants as follows:-
-*Plant height (cm):* From the base of stem up to the apex for each plant.
-*Plant fresh and dry weight* (g): Cleaned uprooted plants free from lateral root-hairs were weighted freshly and then after drying at 70°C until weight to be stable.
-*Average weight of fruits/plant (kg):* Fruits, at marketable size, were harvested. Harvesting was repeated every three days and extended for 90 days, and the accumulated yield was expressed as weight of fruits / plant.

Statistical analysis

Data were analyzed statistically by the analysis of variance test (ANOVA) using Wasp1 and the different means were compared by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Control of downy mildew on cucumber under greenhouse conditions

Results presented in tables (1 & 2) illustrated the impact of six tested rhizobacterial strains on biocontrol of cucumber downy mildew disease under greenhouse conditions. The check treatment recorded the highest final disease severity (68.2 and 62.3 % in 2015 and 2016, respectively). The fungicide (Amistar) achieved the least final disease severity (12.41 and 8.21% in 2015 and 2016, respectively). The six tested bacterial strains play a major role in reducing final disease severity but in fewer rates than the fungicide. *Pseudomonas fluorescens* I exhibited the second best performance as it reduce disease severity to 17.98 and 13.09% during the two successive seasons, respectively. Generally, in 2015, *B. subtilis*, *P. fluorescens* I and *P. fluorescens* II caused the highest reduction in disease severity. Whereas, in 2016, *P. fluorescens* I was shown to be highly potential compared with the rest agents in this respect.

All treatments reduced the area under disease progressive curve (AUDPC) compared to the check during the two successive seasons. *Pseudomonas fluorescens* I exhibited the best performance after fungicide treatment (221.55; 353.85 and 222.18; 415.35 in 2015 and 2016, respectively).

On the other hand, *P. fluorescens* I exhibits the highest bio-control efficacy percentage related to the fungicide during the two successive seasons (90.02 and 90.98 %) followed by *P. fluoresens* II (89.47 and 83.98) and *B. subtilis* (89.94 and 78.58), respectively. Whereas *B. Pumilus* exhibits the least efficacy during the two growing seasons (72.67 and 71.67%), respectively. These results could be explained by Cawoy *et al.* (2011) who reported that the ability of *Bacillus* species members such as *B. subtilis*, *B. megaterium* and *B. pumilus* to produce very efficient antibiotic molecules. Many microorganisms produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, and glucan. Secretion of these enzymes by different microbes may result in suppression of plant pathogen activities directly (Palumbo *et al.* 2005. In conformity with Maksimov *et al*. (2014) spraying of the bio-agent on the leaves and stems of cucumber markedly reduced the downy mildew disease severity.

Table 1: Effect of tested bacterial strains on disease severity, AUDPC and biocontrol efficacy of cucumber downy mildew disease under greenhouse conditions during season of 2015.

Treatments	Final disease severity %	AUDPC	Efficacy I %	Efficacy II %
B. subtilis	18.02^{e}	500.50^{d}	73.58	89.94
B. pumilus	27.66^{b}	732.90^{b}	59.44	72.67
B. polymyxa	25.32^{c}	729.40^{b}	62.87	76.86
B. megatrium	20.68^{d}	614.95^{c}	69.68	85.18
P. fluorescens I	17.98^{e}	353.85^{e}	73.64	90.02
P. fluorescens II	18.28^{e}	485.80^{d}	73.19	89.47
Amistar	12.41^{f}	221.55^{f}	81.8	-
Control (un-treated)	68.20 ^{a}	1458.45^{a}	-	-

- AUDPC: Area under disease progressive curve
- Values in the same column with the same letter aren't significant at P ≤ 0.05
- Efficacy I: related to control; Efficacy II: related to fungicide

Table 2: Effect of tested bacterial strains on disease severity, AUDPC and biocontrol efficacy of cucumber downy mildew disease under greenhouse conditions during season of 2016.

Treatments	Final disease severity (%)	AUDPC	Efficacy I (%)	Efficacy II (%)
B. subtilis	19.80^{d}	567.35^{e}	68.22	78.58
B. pumilus	23.54^{c}	594.44^{d}	62.22	71.67
B. polymyxa	31.12^{b}	656.95^{c}	50.05	57.65
B. megatrium	24.92^{c}	674.80^{b}	60.00	69.12
P. fluorescens I	13.09^{f}	415.35^{g}	78.99	90.98
P. fluorescens II	16.87^{e}	477.05^{f}	72.92	83.98
Amistar	8.21^{g}	222.18^{h}	86.82	-
Control (un-treated)	62.30^{a}	1429.4^{a}	-	-

- AUDPC: Area under disease progressive curve
- Values in the same column with the same letter aren't significant at P ≤ 0.05
- Efficacy I: related to control; Efficacy II: related to fungicide

Mode of action of the tested bacterial strains:

Hydrogen cyanide production:

Results presented in table (3) exhibited that both *Pseudomonas fluorescens* I and II produced moderate amount of Hydrogen cyanide HCN (++) and *Bacillus subtilis* show low production (+), where the other three *Bacillus* strains (*B. pumilus*, *B. polymyxa* and *B. megaterium*) didn't produce HCN. Production of the volatile metabolites (HCN) plays a major role in control of plant pathogens (Ko *et al*., 2009). Also exposing plants to the volatile metabolites of the bio-agents causes' significant increase in peroxidase activity, which plays an important biological role in the plant defense response against biotic or abiotic stresses (Senthilkumar *et al*., 2009).The production of HCN by some strains

of *Bacillus* and *Pseudomonas* species confirmed the use of these species as strong inhibitors against pathogenic fungi. Similar results have been reported by Datta *et.al.* (2011). Likewise, Ko *et al*. (2009) reported that some fluorescent pseudomonas produced protease, siderophore, and HCN acted as antimicrobial agents against *Pythium* sp. and *Phytophthora nicotianae*. Mayak *et al*. (2004) proved that *Pseudomonas* spp. received great attention as bio-agents because of their potentially in producing broad range of antifungal metabolites such as pyoluteorin, pyrrolinitrin and hydrogen cyanide cyanogens.

β- glucanase production:

Table (3) illustrated that, both *P. fluorescens* strains I and II exhibit the best performance as produces for the defense enzyme glucanase (0.241 and 0.236 U), respectively, followed by *Bacillus subtilis* (0.231 U). While, *B. polymyxa* and *B. megatrium* strains produced moderate amount of glucanase (0.112 and 0.100 U). On the other hand, *Bacillus pumilus* secreted slight amount (0.003 U). Glucanase enzyme which probably degrade the glucans of fungal cell wall (glucan and glucosidic bonds). Our results coincide with Manjula and Podile (2005) who reported that *Bacillus subtilis* AF1 displays some fungitoxicity through the secretion of *N*-acetyl glucosaminidase and glucanase. The action of lytic enzymes by some bacterial strains was quite effective due to concentrated action of both chitinase and β-1,3glucanase (Gupta *et al*., 2006 and Chaiharn *et al.*, 2008).

Table 3: Secondary metabolites produced by the tested of bacterial strains.

Bacterial strains	Antimicrobial features	Growth promoting features		
	Hydrogen cyanide	β-glucanase (U)	IAA (μg ml^{-1})	GA (μg ml^{-1})
B. subtilis	+	0.231	5.70	2.03
B. pumilus	-	0.003	4.10	1.30
B. polymyxa	-	0.112	22.41	3.19
B. megatrium	-	0.100	14.13	2.50
P. fluorescens I	++	0.241	10.32	1.30
P. fluorescens II	++	0.236	12.20	1.80

- U: Enzyme amount that releases 1 mg of glucose in 1 min. I ml^{-1} of culture filtrate under assay conditions.
- IAA: Indole acetic acid; GA: Gibberellic acid

Indole acetic acid (IAA) production:

Data also in Table (1) show that, all the tested bacterial strains produced IAA in variable values. *Bacillus polymyxa* exhibited the best performance (22.41 μg ml^{-1}) followed by *B. megatrium*, *P. fluorescens* II and *P. fluorescens* I (14.13, 12.20 and 10.32 μg ml^{-1}, respectively), meanwhile, *B. pumilus* and *B. subtilis* showed the lowest performance (4.10 and 5.70 μg ml^{-1}, respectively). IAA production by *Bacillus* spp. in significant amounts has been reported by Singh *et al.* (2008) and Mehta *et al.* (2010).

Gibberellic acid (GA) production:

All *Bacillus* strains were positive for gibberellic acid (GA) production. The amount of GA produced varied from 1.30 to 3.19 μg ml^{-1}, *B. polymyxa* achieved the highest performance 3.19 μg ml^{-1}, followed by *B. megaterium* and *B. subtilis* (2.50 and 2.03 μg ml^{-1}, respectively), while *B. pumilus* showed the least one (1.30 μg ml^{-1}). On the other hand, both *P. fluorescens* strains didn't produce GA. IAA and GA are plant growth hormones, which have stimulatory effects on growth of vegetable crops

in particular and all plants in general. Moreover, hormones, including the auxin indole acetic acid (IAA) are essential regulators of a multitude of biological functions, including plant responses to biotic and abiotic stressors (Petti *et al.*, 2012).

Concerning to induction of defense enzymes foliar application of all tested bacterial strains induced cucumber plants to synthesize high level of defense-related enzymes, compared to un-treated control. Increased in peroxidase (PO) activity was observed in plants treated with bacterial strains and the fungicide. The highest level of PO activity was achieved according to application of fungicide as well as *P. fluorescens* I&II in growing season of 2015 (1.51, 1.49 and 1.45 Umg^{-1}), respectively, meanwhile the maximum PO activity recorded in season of 2016 was accomplished to application of *P. fluorescens* I&II (1.91 and 1.99 Umg^{-1}), respectively. On the other hand, the least values of PO activity were recorded with plants treated with pathogen only (control). Similar trend was observed in polyphenoloxidase (PPO) activity. Induction of PPO activity was more with fungicide and *P. fluorescens* I, followed by *P. fluorescens* II, during the two growing seasons. It appears that among the tested bacterial strains, the highest induction of both PO and PPO activities were recorded with both *P. fluorescens* strains. Our results are in agreement with Nandeeshkumar *et al.* (2008) they found that plant growth-promoting rhizobacteria can elicit plant defenses throw synthesis of defense enzymes like catalase, chitinase, phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase.

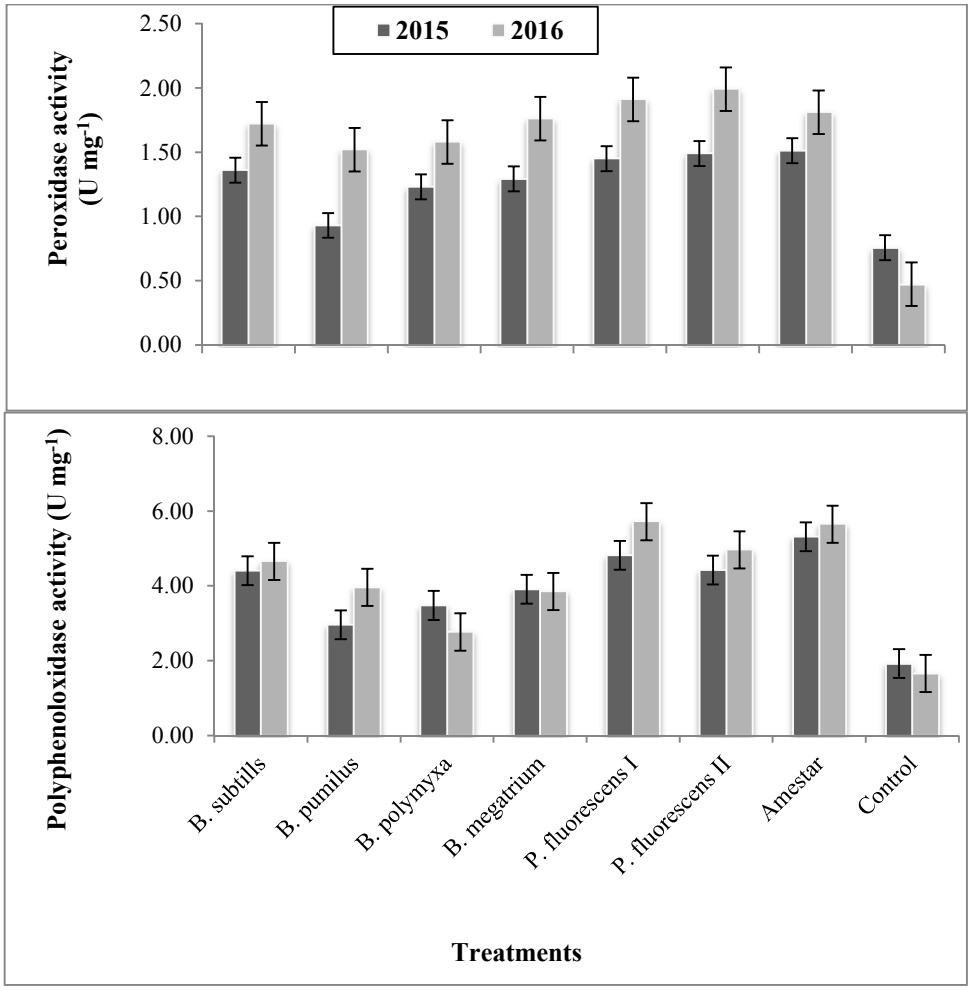

Fig. 1: Peroxidase and polyphenoloxidase activities in cucumber plants treated with the tested bacterial strains in two successive seasons (2015 and 2016).

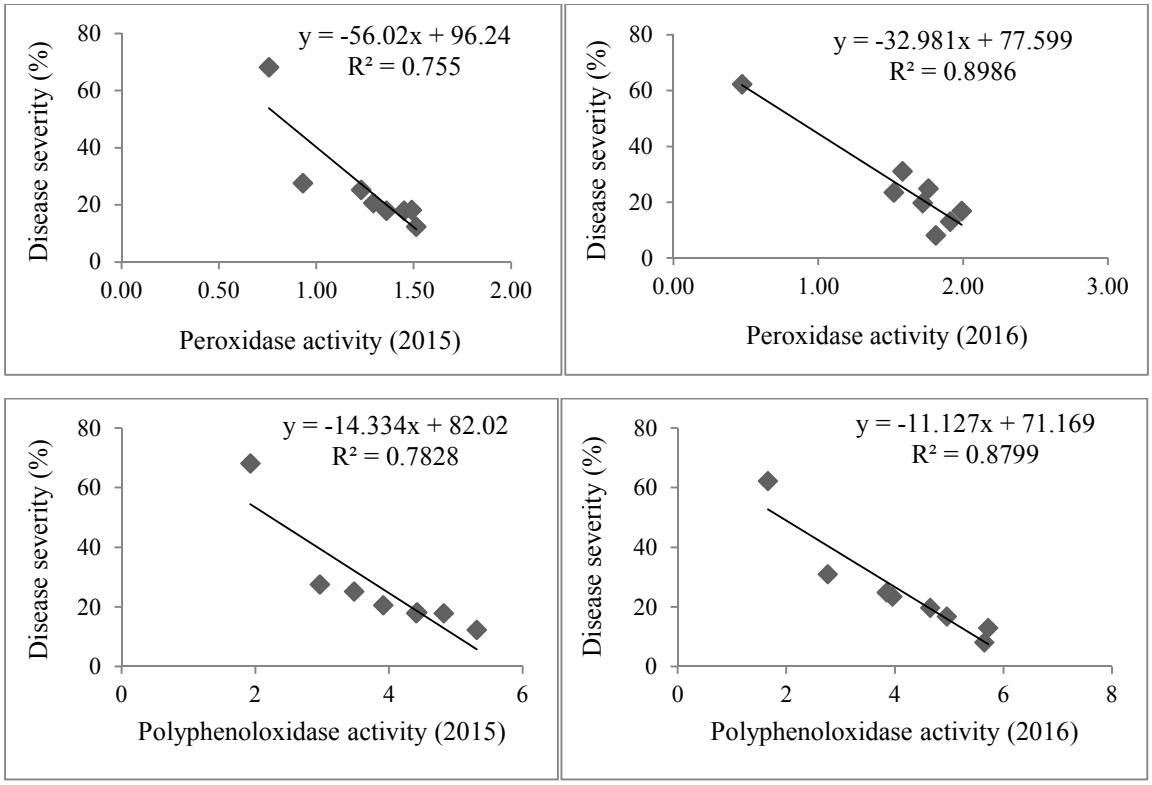

Fig. 2: Relationship between disease severity (%) and peroxidase and polyphenoloxidase activities during 2015 and 2016 growing seasons.

Relationship between disease severity and activities of peroxidase and polyphenoloxidase were analyzed by correlation and regression coefficient during 2015 and 2016 growing seasons. There were a strong correlation between disease severity and activities of peroxidase and polyphenoloxidase during 2015 and 2016. In season 2015, the relation was 0.755 and 0.782 between them, respectively. In season 2016, the relation was 0.898 and 0.879, respectively. Accordingly, the relation in the second season was stronger than the first season. As a result, it could be predicted about the level of disease severity from activities of enzymes. Our results coincide with those obtained with Nandeeshkumar *et al.* (2008).

An important and necessary attribute to consider for a given bacterial control activity is its ability to efficiently colonize plant surfaces by producing microcolonies (Romero *et al*., 2004). The colonization characteristics of total bacterial population in response to *Bacillus* and *Pseudomonas* strains on cucumber phylloplane under greenhouse conditions (Table 4) revealed that the ability of tested bacterial antagonist to survive on cucumber leaves but with different levels. Two of the tested *Bacillus* spp., *B. subtilis* and *B. pumilus*, were well survived and established on cucumber leaf surface at all sampling time, resulted in higher total bacterial count than those recorded with *B. polymyxa* or *B. megatrium*, during the two growing seasons. On the other hand, the two strains of *P. fluorescens* were more efficient colonization and long-term maintenance on cucumber phylloplane, resulted in the highest total bacterial counts all over the treatments, particularly *P. fluorescens* II which surpassed the effect of *P. fluorescens* I, during the two growing seasons. The indigenous bacterial microbiota, represented by analysis of untreated-infected leaves (control), was affected due to the presence of pathogen, which helps in increasing the permeability of some nutrients. The bacterial survival results indicated that the tested bacterial strains applied upon the leaves were able to remain in this habitat, probably as stable cellular aggregates. It also appeared that the *Pseudomonas* strains multiplied on cucumber leaves, which recorded maximum level of 1.9-fold on 20th day, which surpassed the multiplication rate of *B. pumilus* (1.4-fold on 20th day), in season of 2015. Therefore, these results suggesting that this number of bacteria is suitable for effective control of downy mildew disease. These findings, in close agreement with those reported by (Karthikeyan *et al*., 2007) who stated that

Pseudomonas and *T. viride* well colonized and multiplied on rose leaves and suppressed the pathogen population. Efficiency of bacteria to colonize and form microcolonies provides protection to it and exclusion of other microorganisms from the occupied niche or the increased production of antimicrobials (Stein, 2005). The good colonization capabilities of diverse bacteria seem to be related to the ability to form stable biofilms upon the leaf surface, protecting the cells against the harshness of environmental flux as found by Demoz & Korsten (2006). The ability of these bacterial strains to efficiently colonize leaf surfaces and revealed the occurrence of antagonistic interactions between control agents and *Podospheara fusca* structures (Romero *et al*., 2007).

Table 4: Total bacterial count on cucumber phylloplane in response to foliar application of *Bacillus* and *Pseudomonas* strains, expressed as log cfu g fresh leaf^{-1}.

Treatments	Season 2015				Season 2016			
	Days after applications							
	0	10	20	30	0	10	20	30
B. subtilis	5.65^{d}	5.72^{d}	5.70^{d}	5.62^{c}	5.59^{d}	5.60^{d}	5.65^{c}	5.48^{d}
B. pumilus	5.74^{c}	5.86^{c}	5.90^{c}	5.85^{b}	5.81^{c}	5.85^{c}	5.86^{b}	5.84^{c}
B. polymyxa	5.20^{e}	5.12^{e}	5.06^{e}	5.00^{d}	5.32^{e}	5.15^{e}	5.00^{e}	4.95^{e}
B. megatrium	5.05^{f}	5.00^{f}	4.90^{g}	4.98^{d}	5.16^{f}	5.10^{e}	5.11^{d}	4.96^{e}
P. fluorescens I	5.80^{b}	6.04^{b}	6.08^{b}	6.00^{a}	5.95^{b}	6.08^{b}	6.23^{a}	6.18^{b}
P. fluorescens II	5.98^{a}	6.15^{a}	6.23^{a}	6.12^{a}	6.16^{a}	6.20^{a}	6.30^{a}	6.27^{a}
Amistar	3.81^{h}	3.78^{h}	3.74^{h}	3.70^{f}	3.89^{h}	3.90^{g}	3.92^{f}	3.91^{f}
Control (un-treated)	4.90^{g}	4.95^{g}	4.98^{f}	4.80^{e}	4.95^{g}	4.98^{f}	5.00^{e}	4.90^{e}

**Values in the same column with the same letter aren't significant at P ≤ 0.05*

Concerning to total bacterial populations in rhizosphere of cucumber plants in response to foliar spray of tested bacterial strains (Table 5). The rhizosphere soil had great variation in total bacterial counts, where data recorded significantly augment in plants treated with either *Bacillus* or *Pseudomonas* strains, compared with those obtained with check treatments, and the superiority was owing to application of both *Pseudomonas fluorescens* strains, followed by *B. subtilis* during the two growing seasons. Foliar application of *P. fluorescens* I resulted in pronounced increases in rhizosphere bacterial population, wherein led to increase by 3.6 and 3.9-fold over the control, in season of 2015 and 2016, respectively. Moreover, the bacterial populations in all tested treatments were higher in 2nd season than 1st season. Similarly, enhancement was observed in the microbial activity (expressed as dehydrogenase activity) in all plants treated with the tested bio- agents compared with control, and the promotion was higher in the 2nd season.

Both *P. fluorescens* strains (I&II) and *B. subtilis* exhibited the superiority in rhizosphere microbial activity among all biological treatments, while chemical fungicide treatment was the least among all treatments, due to its mortal action on soil microflora. This indirectly confirms the involvement of bacterial synthesizing IAA and GA in enhancing the plant growth and health of plants along with root volume, which subsequently reflected in significantly augment in microbial status in rhizosphere area. Our results close agreement with those reported by Ismail *et al*. (2014) who stated that the foliar application of onion plants with microorganisms resulted in increasing in microbial activity in rhizosphere area. The promotive effect of the induced plant growth promoting bacteria (PGPB) that improves the plant growth directly through the production of plant hormones, i.e., indole acetic acid production, Gibberellic acid as founded by Bottini *et al*. (2004).

Table 5: Effect of tested bacterial strains on microbial status in rhizosphere of cucumber plants under greenhouse conditions during two growing seasons.

Treatments	Total bacterial count ($x10^8$ CFU g dry rhizoshere^{-1})		Dehydrogenase activity (µg TPF g dry soil^{-1} day^{-1})	
	Season 2015	Season 2016	Season 2015	Season 2016
B. subtilis	7.3^{c}	9.4^{c}	39.51^{b}	55.31^{ab}
B. pumilus	4.9^{e}	5.0^{e}	28.09^{e}	36.97^{d}
B. polymyxa	6.3^{d}	6.5^{d}	34.34^{c}	40.31^{d}
B. megatrium	5.0^{e}	4.6^{ef}	30.66^{d}	47.00^{c}
P. fluorescens I	11.5^{a}	14.0^{a}	40.10^{b}	51.04^{bc}
P. fluorescens II	9.7^{b}	12.1^{b}	46.51^{a}	57.26^{a}
Amistar	2.4^{f}	3.4^{g}	17.03^{g}	19.32^{f}
Control (un-treated)	3.2^{f}	3.6^{fg}	22.12^{f}	24.00^{e}

**Values in the same column with the same letter aren't significant at P ≤ 0.05*

Regarding to growth measurements, Tables 6 & 7 showed that the fungicide (Amistar) treatment recorded the best growth and yield parameters, which slightly surpassed some bio-agents during the 2nd season. Data showed that, the highest plant height recorded during season 2015 was attained in plants treated with the fungicide (228 cm), followed by *P. fluorescens* I (227 cm), while the least plant height was attained due to application of both *B. polymyxa* and *B. pumilus* (208.00 and 209.00 cm), respectively. Similarly, plants treated with the fungicide and *P. fluorescens* I were recorded the highest plant height in season 2016 (228 and 224 cm), respectively.

Table 6: Effect of tested bacterial strains on some growth and yield parameters of cucumber during season of 2015.

Treatments	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Yield (fruit/plant) (Kg)
B. subtilis	223.67^{c}	286.7^{a}	54.67^{c}	4.6^{bc}
B. pumilus	209.00^{e}	282.3^{b}	46.00^{e}	2.8^{d}
B. polymyxa	208.00^{e}	260.7^{d}	47.00^{e}	3.2^{c}
B. megatrium	215.30^{d}	214.7^{e}	40.33^{f}	3.1^{c}
P. fluorescens I	227.00^{b}	282.3^{b}	56.67^{b}	4.3^{b}
P. fluorescens II	214.60^{d}	272.7^{c}	51.3^{d}	5.9^{a}
Amistar	228.00^{a}	283.7^{b}	61.00^{a}	5.5^{a}
Control (un-treated)	178.00^{f}	214.7^{e}	41.67^{f}	1.4^{e}

-Values in the same column with the same letter aren't significant at P ≤ 0.05

In concern to fresh weight (g) during season 2015, *B. subtilis* treatment attained the highest value (286.7 g), which surpassed all other treatments including the fungicide treatment, whereas the fungicide treatment gave 283.7 g. The highest values of cucumber plant dry weight, recorded in season of 2015, were due to application of the fungicide and *P. fluorescens* I (61.00 and 56.67g), respectively. Meanwhile, at season 2016, the fungicide and *B. subtilis* recorded the maximum plant dry weight (63.56 and 54.35 g), respectively.

Regarding to fruit yield, *P. fluorescens* II exhibited the highest yield (5.9 kg fruit/plant), followed by fungicide treatment (5.5kg fruit/plant) in season 2015. Meanwhile, the application of fungicide, *P. fluorescens* I and *P. fluorescens* II resulted in the highest fruit yield in season of 2016 (5.2, 4.7 and 4.4 kg fruit/plant), as there was no significant difference between their values.

Table 7: Effect of tested bacterial strains on some growth and yield parameters of cucumber during season of 2016.

Treatments	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Yield (fruit/plant) (Kg)
B. subtilis	209.00^{g}	276.5^{c}	54.35^{b}	4.2^{b}
B. pumilus	226.67^{b}	220.1^{f}	44.56^{f}	3.6^{b}
B. polymyxa	219.33^{d}	261.3^{e}	52.21^{cd}	3.4^{b}
B. megatrium	217.70^{e}	280.6^{b}	48.72^{e}	3.1^{c}
P. fluorescens I	224.00^{bc}	273.9^{d}	52.97^{c}	4.7^{a}
P. fluorescens II	212.70^{f}	280.4^{b}	50.16^{d}	4.4^{a}
Amistar	228.00^{a}	286.4^{a}	63.56^{a}	5.2^{a}
Control (un-treated)	165.00^{h}	210.8^{g}	40.87^{h}	1.5^{d}

-Values in the same column with the same letter aren't significant at $P \leq 0.05$

During season of 2015, the bio-agents treatments recorded slightly less growth and yield parameters than fungicide, particularly *P. fluorescens* I, consequently, the tested bio-agents are considered the ones to be used to control the downy mildew disease as they are safe and have no toxicity effect. Both bio-fertilization and phytostimulation are important phenomena. The microorganisms that possess a combination of these growth promoting activities and biocontrol potential offers the advantage to supply the crop in one application with a bio pesticide and a bio fertilizer ((Kumar *et al*., 2012).

Use of *Bacillus* species mainly, *Bacillus subtilis, B. megaterium, B. pumilus* and *B. polymyxa* as biocontrol agents is attributed to this genus encompasses a large genetic biodiversity. Bacilli are present in an extreme large palette of environments, this bacterium could be used as one of the major sources of bio pesticides become it retains several valuable traits. Bacilli such as *B. subtilis* are well studied organisms which are facilitates their rational use (Kumar *et al*., 2012). *Bacillus subtilis* which is recognized non-pathogen this is of course essential regarding its application as bio-pesticide, moreover *bacilli* have the capacity to produce spores (Piggot and Hilbert, 2004), which are extremely resistant dominancy forms capable to withstand high temperatures, unfavorable pH, lack of nutrients and water. The bacterial suspensions of this genus can be converted into easy handle powder formulations without the impressive bacterial mortality observed with non-sporulation bacteria (Lolloo, *et al*., 2010). The group of bacilli can efficiently colonize leaf surfaces and root systems and their surrounding soil layer.

Plant protection from infection through competition for ecological niche/substrate production of inhibitory allele chemicals and induction of systemic resistance in host plants (Lolloo *et al*., 2010). *Pseudomonas fluorescens* is considered as a strong bio-control agents through induction of some metabolites auxins, gibberellins, siderphores, antibiotics, hydrogen cyanide and volatile compounds (Alizadeh and Parsaeimehr, 2011).

In conclusion, results confirmed the usefulness of the tested bacterial strains, particularly *Pseudomonas fluorescens* I and II strains as well as *Bacillus subtilis* to control downy mildew of cucumber. Their non-chemical properties suggest potentials for broad application under greenhouses conditions.

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