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Evaluation of Native Bacterial Isolates for Control of Cucumber Powdery Mildew under Greenhouse Conditions

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Abstract: Cucumber plants are often attacked by various pathogens, which can considerably decrease production and cause significant losses. One of the most prevalent fungal diseases is powdery mildew, caused by an obligate pathogen, *Podosphaera xanthii*. It is a serious disease that causes significant damage to the whole plant, i.e., leaves, fruits, and stems, under both greenhouse and field conditions. The main objective of this result is to assess the effectiveness of *Bacillus* spp. against cucumber powdery mildew under in vitro and in vivo conditions. Treatment with *B. licheniformis* and *B. aerius* culture filtrates reduced the conidial germination of the pathogen by 60 and 85%, respectively. Under greenhouse conditions, spraying cucumber plants with both microorganisms was effective at reducing powdery mildew disease severity. High reductions of disease severity were achieved by treatment of *B. licheniformis* as a cell suspension and *B. aerius strain* as culture filtrate, 45.3 and 77.3%, respectively, two days before inoculation. Additionally, treatment with these bacterial strains resulted in a significant increase in the fresh and dry weights of the cucumber plants. The highest increase of fresh and dry weight was found with *B. licheniformis* CS and *B. aerius strain* CF treatment at two days before or after infection. After treatment with the bioagents, the content of total phenols, polyphenol oxidase, and peroxidase was enhanced in treatment plants. The use of *B. licheniformis* and *B. aerius* as foliar sprays significantly induced resistance to *P. xanthii* in cucumber plants and stimulated many biochemical functions. Therefore, we propose *B. licheniformis* and *B. aerius* as an effective alternative to harmful chemicals.

Keywords: biological control; powdery mildew; cucumber; polyphenol oxidase; phenols contents

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1. Introduction

Cucumber (*Cucumis sativum* L.) is one of the most important vegetable crops grown under protected cultivation worldwide, including Saudi Arabia [1]. Saudi Arabia has a production rate of 1.27 tons/ha and an area under cultivation of 11,764 hectares, yielding 149,074 tons [1]. Cucumbers can be cultivated year-round in regulated environments, so there is much interest in greenhouse production of the vegetable. In addition, loans and subsidies are offered for the construction of greenhouses and polyhouses. Vegetables are being grown more frequently in greenhouses as the demand for organic produce rises. Under a safe environment, a grower can make a respectable profit. As compared to vegetables cultivated in the usual manner, plants grown in polyhouses are more frequently organic and offer health benefits [2].

Cucumber plants are frequently attacked by pathogens that can decrease production and cause significant losses [3]. One of the most common fungal diseases is powdery mildew, which is caused by *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff. It is a serious disease that causes significant damage to the whole plant, including the leaves,

fruits, and stems, under greenhouse and field conditions [4]. Powdery and downy mildews on cucumber can cause losses reaching 30–80% of yield [5].

Control of such diseases depend on the use of fungicidal treatment, but repeated use of fungicides in the control of plant disease has some problems, such as the development of tolerance to the fungicides used [6]. Additionally, the use of fungicides to treat plant diseases can pollute the environment and increase the level of hazardous compounds in the human food supply [7]. For this reason, researchers are searching for alternative methods to control such diseases, e.g., cultivation of resistant cultivars and biological control by various materials [8].

Increasing concerns for public health have encouraged researchers to find environmentally safe strategies to control plant diseases. Successful biological control methods for mildews caused by fungal and bacterial antagonists have been used under greenhouse conditions, such as the use of *Trichoderma* spp. and certain bacteria species. *Pseudomonas fluorescens* and *Bacillus subtilis* reduced powdery mildew of cucumber [9–11]. The efficiency of biological control depends on the potential of beneficial antagonistic microorganisms. However, further efforts are required to understand the mechanisms underlying the biological control of powdery mildew.

Application of *Bacillus* spp. as a biotic inducer has been used to control plant diseases [12]. These bacteria have a positive effect on plant diseases directly or indirectly; directly by production of auxins or promoting plant growth along with indirect growth effects by reduction or killing of the pathogen via production of antibiotics or toxins and/or secondary metabolite production [13]. The use of *Bacillus* spp. can induce systemic resistance in plants after treatment. It can enhance the activation of peroxidase (PO) and polyphenol oxidase (PPO), which are involved in plant-induced systemic resistance (ISR) [14]. Additionally, it can increase the total phenol content (TPC) of tannins and flavonoids [15]. Biological control of powdery mildew diseases has been achieved by various bioagents under greenhouse and field conditions [16]. In the present study, we attempted to use culture filtrate and cell suspension to reduce disease symptoms before or after inoculation. The aim of this study was to assess the effectiveness of *Bacillus* spp. against cucumber powdery mildew under both in vitro and in vivo conditions, as well as the effect of those treatments on the induction of PO, PPO, and phenolic compounds.

2. Materials and Methods

2.1. Cucumber Powdery Mildew Pathogen Identification

Samples of powdery mildew of cucumber leaves were collected from Assiut Governorate, Egypt. The causal pathogen was identified using morphological properties of the pathogen according to Braun and Takamatsu [17], including the positioning of mycelia on leaf tissue, presence of dimorphic conidia, branching of the conidiophore, and size and form of the conidia.

2.2. Plant Materials

Cucumber seeds (cv. Ushuaia F1 hybrid, DP162-Holland) were planted in a 25 cm diameter pot with sand and clay soil that had been disinfected with 5% formalin. The pots were then watered, and two plants were left to grow up for 2 weeks before being thinned to two plants per pot.

2.3. Fungal Inoculum and Inoculation

Conidia of *Podosphaera xanthii* used in the experiment were obtained from plants that were naturally infected with powdery mildew. To obtain a concentration of 5×10^5 conidia/mL, conidia were gently brushed into 100 mL of distilled water that also contained two drops of Tween-20. The conidia were then counted using a hemocytometer. A conidial suspension was applied by hand sprayer to the upper surfaces of all the leaves for inoculation [18]. They were then sealed in plastic bags for 24 h to keep the humidity at 80%, high enough for disease

development. Control treatments were grown under the same conditions and sprayed with water.

2.4. Isolation of Native Bacterial Bioagents

Naturally existing native bioagents were isolated from the rhizosphere of healthy cucumber fields. Briefly, soil samples were collected from the rhizosphere of healthy cucumber fields at Hada-Al-Sham. The collected soil samples were packed into sterile polythene bags that were stored at 4 °C in a refrigerator. Next, several dilutions (10^{-4}, 10^{-5}, 10^{-6}, 10^{-8} and 10^{-9}) were prepared from each sample [19] by dissolving 2 g of soil into 5 mL of sterilized double distilled water and mixed by vortex. Nutrient agar (NA) (1.5 g of yeast extract, 5 g of peptone, 1.5 g of beef extract, 5 g of NaCl, 15 g of agar, 1000 mL of distilled water and final pH 7.4 ± 0.2) plates were used to isolate the bacterial colonies. The dilutions were inoculated onto plates and then incubated at 27 °C for 48 h. Germinated colonies were purified by the single colony transfer method [19], and the plates were again incubated at 27 °C for 3 days. Purified colonies were preserved in sterilized glass slants containing NA. These were stored at 4 °C for further use.

2.5. Screening of Certain Bioagents against Conidiospore Germination

Ten isolates were tested against conidiospore germination as follows. A glass rod was used to gently shake young sporulating lesions to obtain viable *P. xanthii* conidial spores [20]. The newly collected spores were placed on glass slides [21]. Slides were covered by thin layers of 2% water agar and amended with the cell suspension of the tested bioagents. The control slides were the same, except the slides, covered by agar-free cell suspension, were then laid over glass rods in sterilized Petri dishes containing fully water-moistened filter papers, and incubated at 25 °C for 24 h under light conditions [22]. Spores were examined at 40× magnification to determine germination; spores producing a germ tube as long as the width were considered germinated. The spore germination percentage was calculated for 100 spores [23]. Three replicates were examined for each treatment.

2.6. Identification of Unknown Bioagents Isolates Using Polymerase Chain Reaction Nucleotide Sequence (PCR-Seq)

The unknown bioagent isolates (KAUBL1 and PST13) showing the highest in vitro antibiosis against *P. xanthii* was sent to Solgent Company, Daejeon South Korea for rRNA gene sequencing. Sequences were further analyzed using BLASTn from the National Center for Biotechnology Information (NCBI) website. Multitudinous alignments were performed for closely related sequences of Bacillus similarity calculations using CLUSTAL X [24].

2.7. Efficacy of Tested Bioagent Culture Filtrates or Cell Suspension on Spore Germination of Podosphaera xanthii

The same methods were used to test the two best isolates using cell suspension and culture filtrate of both isolates (KAUBL1 and PST13). Three replicates were examined for each treatment.

2.8. Antagonistic Test against P. xanthii In Vivo

Cucumber seedlings were transplanted to pots four weeks after germination (25 cm diameter) and filled with garden soil (two seedlings per pot). Twenty-five days after planting, the leaves were inoculated as described previously. *Bacillus licheniformis* and *B. aerius* were tested under greenhouse conditions. Eight replicates were used for each treatment in a randomized block pattern. Spraying with *B. licheniformis* and *B. aerius* was performed 2 days before and after inoculation with the pathogen [25]. The control treatment was sprayed with water.

Disease severity was estimated 15 days after inoculation. The evolution was classified into five categories based on Morishita et al. [26] according to the following scale:

0 = no visual infection, 1 = 1–5% infection, 2 = 6–25% infection, 3 = 26–50% infection and 4 = more than 50% of leaf area covered by fungal colonies. Final disease assessment was conducted at 15 days after inoculation.

Disease severity (%) = (Σ (n $\times$ v)/5 N) $\times$ 100, where n = number of infected leaves in each category, v = numerical values of each category and N = the total number of infected leaves.

The fresh and dry weight along with the number of leaves of plant were recorded at the end of the experiment.

2.9. Biochemical Assays

Leaf samples from various treatments under greenhouse conditions were taken 10 days after spraying and used for biochemical analysis. For enzyme extraction, leaf samples from each treatment were powdered separately in 5 mL of 0.1 M sodium phosphate buffer (pH 7.0) [27]. The pulverized materials were centrifuged for 15 min at 10,000 rpm, and the supernatant was then employed as an enzyme source. PPO, PO, TPC, and their activity were assessed in tissues from cucumber leaves treated with bioagents as well as the untreated control treatment [28].

2.10. Sample Collection

2.10.1. Determination of Peroxidase

Using absorbance at 430 nm/g fresh weight/15 min, the activity of PO was measured using the spectrophotometric method of Allam and Hollis [29]. Enzyme unit/mg protein/min was the unit used to express PO activity.

2.10.2. Determination of Polyphenol Oxidase

At an absorbance of 405 nm, the activity of PPO was assessed using the [30] spectrophotometric method. The unit of measure for PPO activity was enzyme unit/mg protein/min.

2.10.3. Determination of Total Phenol Content

Each cucumber leaf sample was extracted with 10 mL of 80% methanol at 70 °C for 15 min. TPC was determined using the Folin-Ciocalteu reagent colorimetric analysis method as reported by Zieslin and Ben-Zaken [31]. TPC was measured as micrograms of GAE per gram of fresh weight.

2.11. Statistical Analysis

All experiment was conducted twice. The computer program MSTATC was used to perform variance analyses. According to Gomez & Gomez [32], least significant difference (LSD) was calculated at $p < 0.05$.

3. Results

3.1. Screening of Bioagents against Conidiospore Germination

Nineteen biocontrol agents were evaluated for their antagonistic activity against conidiospores of *P. xanthii*. It is apparent from the data in Table 1 that KAUBL1 and PST13 inhibited the germination of the *P. xanthii* conidiospores. The biocontrol agent KAUBL1 caused a reduction in germination of 90%, followed by isolate PST13 at 85%. The lowest reduction was achieved by isolates PST1, PSR7 and KAUBL3. The other tested bacterial bioagents were not effective. Based on these results, isolates KAUBL1 and PST13 were selected for use in the following experiments.

Table 1. In vitro inhibition of conidiospore of *P. xanthii* by bacterial bioagents isolates.

Bacterial Isolate	Reduction of Conidiospore (%)
PTS1	15 d
PTS2	30 c
PTS3	0 e
PTS4	35 c
PTS5	0 e
PPR6	0 e
PPR7	10 e
PPR8	0 e
PPR9	0 e
PPR10	0 e
PTS11	0 e
PTS12	0 e
PST13	85 b
KAUBL1	90 a
KAUBL2	0 e
KAUBL3	20 d
KAUBL4	0 e
KAUBL5	0 e
KAUBL6	0 e
Control	-

Values in the column followed by the same letter within a column are not significantly different as determined by the LSD test $p = 0.05$.

3.2. Identification of Unknown Bacterial Antagonist

Pure cultures of two unknown bioagent isolates were identified by molecular characterization using 16S rRNA sequencing. Based on BLAST searches on the NCBI data libraries (16S ribosomal RNA sequences and Nucleotide Collection Database) for similarities of the 16S rRNA sequences, the isolate KAUBL1 was found to be most similar to *B. licheniformis* (GenBank accession No. KT758463.1). The 16S rRNA sequences of isolate KAUBL1 were lodged in the GenBank sequence database under accession number MW165779.1. Furthermore, the bacterial isolate PST13 was found to be most similar to *B. aerius* strain 24K (NCBI accession number NR_118439.1) and was deposited in the GenBank database under accession number MT409890.1 (Table 2).

Table 2. Molecular characterization of unknown isolates by 16S rRNA analysis.

Bioagent Isolate	Maximum Score	Total Score	Query Cover	E Value	Percent Identity	Most Similar Organism	GenBank Accession No.
KAUBL1	1732	1732	100%	0.0	100%	*Bacillus licheniformis*	KT758463.1
PST13	1135	1143	99%	0.0	99%	*Bacillus aerius*	NR_118439.1

3.3. Effect of Bacillus licheniformis and B. aerius as Culture Filtrate or Cell Suspension on Conidiospore Germination

The data in Figure 1 show the effects of cell suspension and culture filtrate of *Bacillus licheniformis* and *B. aerius*. The *B. licheniformis* cell suspension was more effective at reducing spore germination of *P. xanthii* compared to the other treatments, followed by the cell suspension of *B. aerius* and culture filtrate of *B. licheniformis*. The lowest effect was exhibited by the culture filtrate of *B. aerius* as compared to the untreated control.

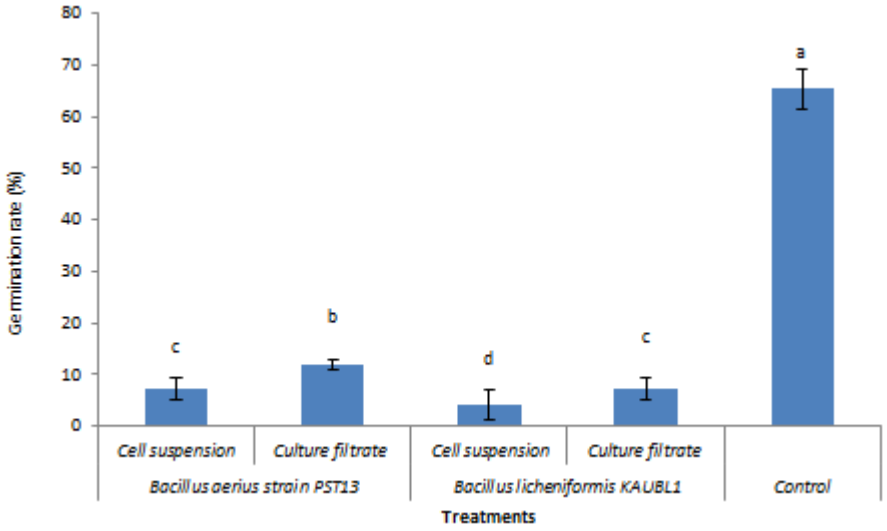

Figure 1. Effect of *Bacillus licheniformis* and *B. aerius* on conidial germination 24 h after treatment. Values in the column followed by different letters indicate significant differences among treatments according to Duncan's multiple range test ($p \leq 0.05$).

3.4. Enzymatic Activity of Effective Bacterial Bioagents

A screening for lytic exoenzymes in the bacterial bioagents was performed, and the results are presented in Table 3. The enzyme activity of *B. licheniformis* was found to be highest for amylase, pectinase, and protease, while *B. aerius* was found to be highest for cellulase and showed the lowest activity for all tested enzymes.

Table 3. Extracellular enzymatic activities of *Bacillus licheniformis* and *B. aerius*.

Bacterial Bioagents	Zone of Hydrolysis (mm)			
	Amylase	Cellulase	Pectinase	Protease
B. licheniformis KAUBL1	15 a	63 b	37 a	16 a
Bacillus aerius PST13	10 b	75 a	20 b	9 b

Values in the column followed by different letters indicate significant differences among treatments according to LSD test at 0.05.

3.5. Effect of Bioagents on Disease Severity of Powdery Mildew

Cucumber plants treated with both isolates as cell suspensions or culture filtrate two days before or after inoculation caused a reduction in disease severity compared to the infected control. The greatest reductions were found with treatment of *B. licheniformis* KAUBL1 as a cell suspension and *B. aerius* as culture filtrate. The other treatments also reduced the disease severity before or after infection with the pathogen (Table 4).

Table 4. Effect of *Bacillus licheniformis* and *B. aerius* on disease severity under artificial infection in pots.

Treatments	Method of Application	2 Days before Infection		2 Days after Infection	
		Disease Severity (%)	Reduction (%)	Disease Severity (%)	Reduction (%)
B. licheniformis	Cell suspension (CS)	30.1 c	45.3	39.4 c	39.7
	Culture filtrate (CF)	35.5 b	35.6	40.2 b	38.4
B. aerius	Cell suspension (CS)	12.5 b	77.3	19.7 b	69.8
	Culture filtrate (CF)	29.1 c	47.2	39.2 c	39.9
Control		55.1 a	-	65.3 a	

Values in the column followed by different letters indicate significant differences among treatments according to LSD test at 0.05.

3.6. Effect of Bioagents on Vegetative Growth

The data presented in Table 5 show that the fresh weight (FW) and dry weight (DW) of the cucumber plants were significantly increased by both treatments as CS or CF compared to the control group (Table 5). The height, FW and DW as well as number of leaves per plants were increased significantly in all treatment groups compared to the infected control (Table 5). The highest increase was found with *B. licheniformis* CS and *B. aerius* CF treatment at two days before or after infection, followed by the other treatments.

Table 5. Effect of *Bacillus licheniformis* and *B. aerius* treatments on fresh and dry weight of cucumber plants.

Treatments	Method of Application	2 Days before Infection			2 Days after Infection		
		Plant Fresh Weight FW (g/plant)	Plant Dray Weight DW(g/plant)	No. Leaves Plant^{-1}	Plant Fresh Weight FW (g/plant)	Plant Dray Weight DW (g/plant)	No. Leaves Plant^{-1}
B. licheniformis	Cell suspension (CS)	287.9 a	27.7 a	25 a	255.3 a	29.3 a	23 a
	Culture filtrate (CF)	198.5 b	12.3 e	14 d	185.5 b	10.3 e	12 e
B. aerius	Cell suspension (CS)	165.3 b	21.1 c	18 c	165.9 b	20.1 c	18 c
	Culture filtrate (CF)	250.3 a	25.1 b	21 b	256.1 a	29.2 b	23 a
Infected Control		150.2 bc	19.2 d	12 e	155.3 bc	19.2 d	15 d

Values assigned to similar letters are not significantly different among treatments according to LSD test at 0.05.

3.7. Effect of Some Bioagents on Biochemical Changes in Cucumber Plants

The data presented in Table 6 show that all treatments had greater PO and PPO activity and TPC compared to the infected control. In addition, cucumber plants treated with *B. licheniformis* as CS exhibited the greatest increase in PO and PPO activity and TPC, followed by those treated with *B. aerius* as CF two days before or after infection with the pathogen. In general, enzyme activity and TPC were higher two days before infection, followed by the content two days after inoculation.

Table 6. Effect of *Bacillus licheniformis* and *B. aerius* treatments on biochemical changes of powdery mildew cucumber plants after 10 days from treatment.

Treatments	Method of Application	Peroxidase (Enzyme Unit/mg Protein/min)		Polyphenol Oxidase (Enzyme Unit/mg Protein/min)		Total Phenols (µg GAE/g Fresh Weight)	
		2 Days before Inoculation	2 Days after Inoculation	2 Days before Inoculation	2 Days after Inoculation	2 Days before Inoculation	2 Days after Inoculation
B. licheniformis	(CS)	180.3 a	121.2 a	125.3 a	113.8 a	51.6 a	45.3 a
	(CF)	98.3 d	81.2 d	101.5 d	80.7 c	35.6 b	35.2 c
B. aerius	(CS)	134.2 c	100.2 c	119.3 b	102.1.3 b	44.6 b	40.3 b
	(CF)	122.1 b	108.1 b	111.2 c	101.8 b	43.2 b	39.6 b
Infected Control		51.7 e	41.2 e	85.2 e	45.8 d	30.7 c	29.3 d

Values assigned to similar letters are not significantly different among treatments according to LSD test at 0.05.

4. Discussion

In the present study, the effects of 19 isolates from the cucumber rhizosphere were tested in vivo against *P. xanthii* spore germination. Only seven isolates were able to reduce spore germination to various degrees. Of the seven isolates, two showed great reduction of the spore germination (KAUBL1 and PST13) and were selected for further experiments. The antifungal effects of bacterial isolates can be due to the production of direct inhibitory substances such as hydrogen cyanide, hydrolytic enzymes (amylase, cellulase pectinase, and protease), and siderophores or antibiotics [13]. Present results showed that both biagents *(B. licheniformis* and *B. aerius*) produced amylase, cellulase pectinase and protease. These findings are consistent with those of Elsisi [14], who demonstrated the ability of the bioagents to prevent the germination of powdery mildew conidial spores maybe due to hydrolytic enzymes. According to research by Sarhan et al. [33], the culture filtrate of

the examined bioagents, including *B. subtilis*, *P. polymyxa*, and *S. marcescens*, significantly reduced the germination of *P. xanthii* conidia in vitro.

The in vitro and in vivo investigations revealed that both of the bioagents examined significantly reduced the severity of the disease. Highly reductions of disease severity were achieved by treatment of *B. licheniformis* as a cell suspension and *B. aerius* strain as culture filtrate 45.3 and 77.3%, respectively, two days before inoculation. Additionally, they increased the fresh and dry weight and number of leaves per plant as compared to the control. It is well known that bioagents are effective methods of treating a number of fungal diseases, such as cucumber powdery mildew [33]. In general, antibiosis, competition, mycoparasitism, and induced resistance are among the mechanisms associated with biological control of phytopathogenic fungi [34].

Romero et al. [35] mentioned that the *Bacillus* species can used to control powdery mildew on melon (*Podosphaera fusca*), they suggested that the bacteria antagonistic can reduced percentage the infection through inhibition of spore germination by production of antifungal compounds.

The greenhouse results showed that cucumber plants treated with both isolates either as a cell suspension or culture filtrate showed a significant reduction in disease severity two days before or after inoculation with the pathogen, and treatment two days before was better than two days after inoculation. These results are in line with a previous study by Punja et al. [36], who observed that *Bacillus* spp. can be used to control cucumber powdery mildew disease. This reduction of the disease may be due to the bioagent's ability to reduce spore germination as well as to enhance plant growth [37,38]. Recently, several studies have investigated the effect of various bioagents, such as *Bacillus* spp. for controlling airborne pathogens, e.g., powdery mildew disease [39–41]. El-Sharkaway et al. [42] found that spraying cucumber plants with *Pseudomonas fluorescens* and *B. subtilis* significantly reduced the disease severity of cucumber powdery mildew. According to research by Punja et al. [36], the severity of the powdery mildew disease was reduced when *B. subtilis* was applied to greenhouse cucumber plants as a preventative or eradicative treatment.

Using PGPR to induce resistance in plants is an important method of suppressing plant diseases caused by fungi or bacteria [20,42,43]. Induced resistance in plants is directly linked to the accumulation of phenolic compounds and/or induction of defense-related enzymes PO, PPO, LOPX and PAL [15]. The results presented here indicated that cucumber plants treated with *B. licheniformis* as CS caused the highest increase in antioxidant enzyme activities and TPC two days before or after infection with the pathogen. The reduction in disease severity of cucumber powdery mildew is associated with increased phenolic compounds and antioxidant enzymes PO and PPO [42]. In addition, Elsisi [14] demonstrated that the reduction of powdery mildew of squash under greenhouse conditions is correlated with increased defense-related enzymes PPO and PO as well as TPC in squash plants.

The current findings demonstrated that both bioagents used as cell suspension or culture filtrate could induce plant systemic resistance by PO and PPO activities, thus aiding in the management of cucumber powdery mildew. The prevention and control of disease by bacterial bioagents may occur by a variety of mechanisms [44], including host resistance induction [45], ecological place and nutrient competition, or production of antibacterial substances and colonization ability [46–48].

The stimulation of phenolic compounds is directly interconnected with disease resistance as well as plant resistance against fungal plant pathogens [47]. TPC was increased in treated cucumber plants compared to the infected and healthy control plants. The accumulation of phenolic compounds at the infection site has been linked to the restriction of pathogen development because such compounds are toxic to pathogens [48]. A change in the pH of plant cell cytoplasm due to an increase in phenolic acid content may also increase resistance, thereby inhibiting pathogen development [43,49]. In the present study, treatment with bacterial bioagents resulted in an increased accumulation of phenolic compounds in response to infection by the pathogen.

It has been reported that after inoculation with biocontrol bacteria or other non-biological factors, the levels of oxidase activities, such as POD and PPO, are increased, thereby inducing resistance to pathogen invasion and expansion [50]. PO activity was significantly increased in infected plants treated with all bacteria. These results suggest that endophytic bacteria promote cucumber plant growth by increasing defense-related PO enzymes. Several investigators have reported that the enhancement of PO activity is associated with resistance of plants to fungal, bacterial and viral pathogens [51]. The highest PPO activity was obtained by treatment with both bioagents in the present study. These results are in agreement with those reported by Esmaeili [52]. The importance of PPO activity in plant disease resistance probably stems from its ability to oxidize phenolic compounds to quinines, which are often more toxic to microorganisms than the original phenols [51,53].

5. Conclusions

Cell suspension or culture filtrate of *B. licheniformis* and *B. aerius* induced potential antifungal activity against *P. xanthii* under in vitro and in vivo conditions. Both bioagents decreased the conidia germination of *P. xanthii*. Also, *B. licheniformis and B. aerius* reduced the disease severity of powdery mildew under greenhouse conditions and increased the fresh and dry weight compared to untreated plant. These effects were accompanied by high-value total phenol contents and along with increased activities of PO and PPO enzymes in treated cucumber plants. The results of this study both in vivo and in vitro suggest that the use of both isolates of *Bacillus* spp. (*B. licheniformis and B. aerius*) as either cell suspensions or culture filtrate could protect or reduce the disease severity of powdery mildew by changing biochemical compounds in cucumber plants.

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