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Efficiency of Using Some Biological Organisms as Biological Catalysts to Reduce the Incidence of Cantaloupe Downy Mildew Disease Under Greenhouse Conditions

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Abstract: Infection of cantaloupe plant with Downy mildew caused by the fungus *Pseudoperonospora cubensis* leads to significant losses in the amount of the crop, in addition to, reducing its marketing value in many countries around the world, therefore, the efficacy of each of the organisms (*Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis*, *Bacillus megaterium* and *Pseudomonas fluorescens*), and the fungicide Ridomil gold plus 71.5 WP for the control of cantaloupe downy mildew under laboratory and greenhouse conditions was studied, initially, under laboratory conditions, the results obtained showed that, when using bio filters, in addition to, the recommended concentration of the fungicide to inhibit the germination of *P. cubensis* sporangia, a significant reduction in the germination rate of the pathogenic fungus sporangia was observed, also, under greenhouse conditions, treatment of cantaloupe with the tested organisms and with the fungicide significantly reduced the severity of downy mildew, compared to the control treatment, where, the best of these treatments in reducing the severity of the disease was the use of the fungicide, while, the organisms were arranged according to their efficiency in reducing the severity of the disease in the following descending order: *B. subtilis*, *Ba. megaterium*, *P. fluorescens*, *T. viride* and *T. harzianum*, In addition, cantaloupe plants showed a significant increase in plant growth parameters, for example, (number of leaves, leaf area and total chlorophyll content), also, an increase in yield components, For example, (number of fruits/plant and fruit weight /plant), the above treatments also increased the activity of defense-related enzymes, for example, peroxidase and polyphenol oxidase enzymes, as well as, total phenol content compared to the control treatment.

Keywords: *Pseudoperonospora cubensis*, Biological Organisms, Fungicide, Enzyme Activity

1. Introduction

Cantaloupe (*Cucumis melo* L. var. reticulate Ser.), is one of the most common vegetable crops grown under greenhouses in Egypt. Cantaloupe plants are infected with many fungal and viral diseases, in addition to, infection with nematodes [30, 5, 7, 14, 9].”Downy mildew is one of the most important diseases of cantaloupe, caused by the fungus *Pseudoperonospora cubensis*. It affects the vegetative total of plants in most countries of the world, which leads to a reduction in the quantity and marketing quality of the crop [3, 1]. The use of fungicides, has been one of the most effective methods of combating plant diseases, but the uncontrolled use of these substances in many agricultural areas around the world, has raised serious concerns about health issues, and environmental pollution in particular, after it was published [11, 47]. Furthermore, many fungi can develop strains that are resistant to most of the fungicides used, [12, 34]. Under the above conditions, it becomes necessary to develop plant-derived plant-based pesticides or microbial pesticides, whether biological, environmentally friendly or biodegradable, in order to, control plant pathogens. Bio stimulants have potential in agriculture for plant disease control [8, 17, 38]. Currently, biological control using antimicrobials, resistance inducers, and growth stimulators, provides an excellent practical and economical alternative in controlling plant pathogens [13, 19]. Saturated microbes, have been documented for many organisms living in the plant cover and/or roots and/or inducing systemic resistance mechanisms within the plant [10, 29, 15]. Recently, induction of plant resistance by application of various microorganisms has emerged as a novel strategy in plant disease management [33]. Bio stimulators, are known to have activities that trigger a variety of defensive reactions in host plants, in response to microbial infection, including defense-related enzymes and the accumulation of phenolic compounds, as well as, specific flavonoids [36, 21, 20, 4, 35, 32, 24]. The activity of the defensive enzyme, β-1, 3-glucanase, is known to induce systemic resistance of many plants infected with fungal pathogens [36, 21]. Also, this enzyme acts synergistically in the partial degradation of fungal cell walls. Furthermore, a parallel increase in the activities of these enzymes is important for optimal function in plant defense [36]. In addition, peroxidase enzymes (PO), phenylalanine ammonia (PAL), and polyphenol oxidase (PPO), have been reported as inducers of systemic induced resistance (ISR) in plant disease control [45, 18, 15]. These enzymes act as catalysts of the phenylpropanoid pathway, leading to the biosynthesis of a variety of plant metabolites, such as, phenolic compounds, flavonoids, tannins and lignin [39]. These products can provide defense in plants against pathogen attack [23]. Several studies, indicated an increased accumulation of phenols as a result of increased activities of these oxidizing enzymes that can provide protection against plant diseases [40, 4, 24, 18, 15].

The current study aims to evaluate the effectiveness of some organisms in inducing resistance to cantaloupe plants against downy mildew by stimulating the systemic defense activity under the conditions of greenhouses. Estimation of the activity of defense-related enzymes, phenolic compounds, growth parameters of cantaloupe plants and yield.

2. Materials and Methods

2.1. The Source of Biological Organisms

Pure isolates of the tested fungal biota, such as, *Trichoderma harzianum* and *Trichoderma viride*, and tested bacterial biota, such as, *Bacillus subtilis*, *Bacillus megaterium* and *Pseudomonas fluorescens,* were obtained from, Integrated Disease Management Department (IDMD), Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

2.2. Preparing the Inocula for the Tested Organisms

T. harzianum and *T. viride* isolates, were grown singly on PDA medium for 10 days. Spores of each fungus were prepared separately at a concentration of 10^7 spore /ml−1 with sterile water, using a hemocytometer slide. The tested bacteria isolates, of *B. subtilis*, *Ba. megaterium* and *P. fluorescens*, were grown in bottles containing 250ml liquid nutrient medium, for 3-4 days. The concentration, of each bacterial suspension at 10^9 cells /ml −1, was calculated using a slide hemocytometer.

In vitro tests:

Efficiency of culture filters and fungicide Ridomil gold plus 71.5% WP for tested organisms on the sporangial germination of Pseudoperonospora cubensis:

Leaves samples naturally infected with Downy mildew disease caused by the fungus *P. cubensis*, were taken from cantaloupe plants of Gallia cultivar, which, were cultivated under the conditions of greenhouses in Nub aria area, Beheira governorate. Infected leaves were incubated at 20 ± 1°C under humid conditions, for ten days to encourage sporangia formation. Sporangia were collected from the lower surface of cantaloupe leaves, with a sterilized brush. Then, the pathogen sporangia were placed in bio filters and the recommended concentration of the fungicide, Ridomil gold plus 71.5% WP, (250 g / 100 L water), at a ratio of 1:1. One mL, of the treated sporangial suspension, was placed on two sterile slides, mounted on two glass rods in a sterile Petri dish, containing a cotton swab moistened, with sterile distilled water, to provide high relative humidity. A comparative treatment was carried out using sterile distilled water, as an alternative to the previous treatments. All treatments were incubated under dark conditions at 20 ± 1°C, for 48 hours. One drop of lacto-phenol cotton blue stain was added, at the time of slide examination to fix and killing germinated sporangia. The Percentage of sporangial germination, was counted as empty sporangium (cleared sporangium, which zoospores released out the sporangium) in a total of 100 sporangium. The germinated sporangia were counted and mean of percentages of germination was calculated and recorded for each treatment [25]. Three replicates were examined for each treatment.

In vivo tests:

Greenhouse experiments

A greenhouse experiment was conducted in Nub aria area, Beheira governorate, season 2020, to study the efficiency of the tested organisms and the fungicide Ridomil gold plus 71.5% WP on reducing the severity of cantaloupe downy mildew. A plastic greenhouse (40 m long x 9 m wide) was selected and the experiment was designed with a completely random block design. I used cantaloupe c. v Gallia seeds. The seeds were planted inside the eyes of rectangular foam trays (5 × 5 × 7 cm). The eyes of the trays were filled with a mixture of vermiculite and peat moss in a ratio of 1: 1 w/w. The seeds sown inside the eyes of the trays were covered with the same mixture mentioned above. The trays were well irrigated, covered with reinforced plastic and left until the seeds germinate. After germination, the trays were spread out on iron supports, irrigated and fertilized to obtain 21-day-old cantaloupe seedlings. The seedlings were planted inside the plastic greenhouse on terraces (0.7 m wide, 2 m long), in the form of crow's feet, between each seedling at a distance of 50 cm. Cantaloupe seedlings were distributed on 3 terraces. Seven treatments were performed including the comparison treatment, each treatment containing 3 replicates. Each repetition contains 12 seedlings. The plants were irrigated and fertilized according to the guidelines of the Ministry of Agriculture and Land Reclamation. Plants were sprayed separately two days before infection with pre-prepared isolates of fungal and bacterial organisms, as well as, the fungicide Ridomil gold plus 71.5% WP, {copper oxychloride + metalaxyl-M (mefenoxam)}, at a rate of 250 g / 100 liters. Water. Cantaloupe plants were infected with a suspension of pathogenic fungus sporangia, at a rate of 1x103 sporangia/ml [1]. Post-infection cotyledons were covered with plastic sheets, for 24 h. to provide high humidity. The comparative treatment was sprayed with water only.

2.3. Estimate the Severity of the Injury

The severity of the disease was estimated after 15 days of infection. Cantaloupe plants were checked periodically for symptoms, and disease severity was estimated according to the scale (0-4) described by [43]. Ten randomly selected plants/replicators were examined. The severity of downy mildew disease was estimated using the following equation:

$$P = \sum n \text{ x } v) \text{ x } 100$$

Where;
n = number of leaves within infection grade.
v = numerical value of each grade.
N = total number of leaves.

Then efficacy of biological control agents and fungicide was calculated using Abbott's formula [2], as follows:

$$\text{Efficacy (\%)} = (X - Y) / X * 100$$

Where, X = disease severity in untreated control. Y = disease severity in each treatment.

Some growth measurements, such as, the number of leaves, leaf area in centimeters, and components of the cantaloupe plant yield, such as, the number and weight of fruits for each plant, were also estimated.

2.4. Determination of Chlorophyll Content

The chlorophyll content, was estimated in the fifth leaf of treated and untreated cantaloupe plants (comparison treatment), using vegetation measurements, the SPAD-501 Portable Leaf Chlorophyll meter (Minolta Corp [44].

2.5. Estimation of Growth Rate and Yield Measurements

Some growth parameters and yield components of treated and untreated cantaloupe plants (comparison treatment), were estimated after 50 days of sowing, where the average number of leaves for each plant, was calculated, in addition to, the leaf area in square centimeters for each plant, was calculated using the CI-202 CID Portable Laser Leaf Area Meter (Bio-Science, Inc 1554 NE 3rd Avenue, Camas, WA, 98607, USA). Some components of the yield were estimated, such as, calculating the average number and weight of fruits per plant, after 100 days of sowing, so that, the size of the fruits reached the size The marketing campaign, where the harvest was done every three days. The amount of the crop, was expressed by the number and weight of fruits for each plant.

2.6. Estimation of Biochemical Changes

2.6.1. Determination of Oxidative Enzymes

Treated and untreated cantaloupe cv Gallia plants, which were artificially infected by *P. cubensis*, were collected to estimate the activity of enzymes related to activation of the systemic defense system, such as, peroxidase and polyphenol oxidase enzymes. The enzyme extract was obtained from the collected plants by the method described by [26]. One gram of plant tissue from each treatment, was placed in a crucible, Then 1 ml of 0.1 ml sodium phosphate solution was added to it at pH 7. It was ground well. Then, the extracts were filtered through four layers of cheesecloth, and then centrifuged under refrigeration for 20 minutes at 3000 rpm, and the clear supernatant was obtained and kept as a crude enzyme source to estimate the activity of the enzymes under study.

2.6.2. Estimation of Peroxidase Enzyme Activity

The peroxidase activity was determined using a spectrophotometer, according to the method described by [6], by measuring the oxidation of pyrogallol to pyrogalene in the presence of H_2O_2 at 425 nm. The cuvette sample included the following mixture:

0.5 ml of 0.1 M sodium phosphate solution at pH 7, 0.3 ml of the enzyme extract that was previously boiled to inactivate the enzyme, then 0.3 ml of 0.05 M pyrogallol, and 0.1 ml 1% H_2O_2, were added, then, the mixture was completed by Sterile distilled water up to 3 ml. Peroxidase activity was expressed as the change in absorbance/min. The readings were recorded every 15 seconds for 2.5 minutes.

2.6.3. Estimation of Polyphenol Oxidase Enzyme Activity

The activity of the enzyme polyphenol oxidase was estimated using a spectrophotometer, according to the method described by [26]. The reaction mixture contained the following components:

0.5 ml of crude enzyme, which was previously boiled to inactivate the enzyme, then, 0.5 ml of sodium phosphate solution at pH 7 and 0.5 ml of catechol were added, the volume was supplemented with sterile distilled water to 3 ml. The activity of the polyphenol oxidase enzyme was expressed by the change in absorbance/min at the optical density of 495 nm. The readings were recorded every 30 seconds for 5 minutes.

2.6.4. Estimation of Total Phenolic Compounds

The 10 ml of 80% methanol alcohol was added to one gram of treated and untreated cantaloupe extract, the mixture was incubated for 15 min at 70°C. The total phenolic components were determined by the method described by [48]. The total content of fresh weight mg GAE/g phenolic compounds was expressed.

2.7. Statistical Analytics

Statistical analyzes of the obtained results were conducted using the MSTAT program [28], where the results were compared in all experiments using L.S.D. Test ($P \leq 0.05$).

3. Results

In vitro tests:

Efficiency of culture filters and fungicide Ridomil gold plus 71.5% WP for tested organisms on the sporangial germination of Pseudoperonospora cubensis:

The efficiency of the tested bio filters, *i.e. T. harzianum*, *T. viride*, *B. subtilis*, *Ba. megaterium* and *P. fluorescens* plus the fungicide (Ridomil gold plus 71.5% WP), on the percentage of germination of *P. cubensis* sporangia under laboratory conditions.

The results obtained in Table 1, showed that all the bio filters, as well as, the fungicide inhibited the germination of *P. cubensis* sporangia, compared to the control treatment. While, the fungicide gave the highest inhibition of Sporangia germination, giving an efficiency of (95.56%). On the other hand, the isolates of the biological organisms were arranged according to their efficiency to the following descending order *B. subtilis*, *Ba. megaterium*, *P. Fluorescens*, *T. viride* and *T. harzianum*, which gave an efficiency of (89.64%, 86.90%, 84.36%, 80.76% and 74.83%), respectively.

Table 1. *Effect of bio filters and fungicide Ridomil gold plus 71.5% WP on the germination of Pseudoperonospora cubensis sporangia, under incubation conditions at 20 ± 1°C.*

Treatments	Spore or culture filtrate Concentration	Germination%	Efficiency (%)
Trichoderma harzianum	10^7 spore/ ml	15.24	74.83
Trichoderma viride	10^7 spore/ ml	11.63	80.76
Bacillus subtilis	10^9 CFU	6.26	89.64
Bacillus megaterium	10^9 CFU	7.87	86.90
Pseudomonas fluorescens	10^9 CFU	9.65	84.36
Ridomil gold plus 71.5% WP	250 g/100 L	2.68	95.56
Control		60.45	00.00
L. S. D at 0.05%		3.19	

Efficiency of the tested biological organisms and fungicide Ridomil gold plus 71.5% WP in controlling downy mildew disease on cantaloupe plants under greenhouse conditions:

The results presented in Table 2, showed that, all tested organisms, in addition to, the fungicide Ridomil gold plus 71.5% WP, caused a significant decrease in the severity of downy mildew infection, compared to the control treatment. The organisms were arranged according to their efficiency in reducing the severity of the disease, in descending order:

B. subtilis, *Ba. megaterium*, *P. Fluorescens*, *T. viride* and *T. harzianum*, which gave an efficiency in reducing the severity of infection (62.12%, 58.42%, 51.35%, 42.53% and 39.22%), respectively, compared to the control treatment, which gave an efficiency of 00.00%. On the other hand, the fungicide gave the highest efficiency in reducing the severity of the disease, as it scored (63.89%).

Table 2. *Efficiency of the biological organisms and the fungicide Ridomil gold plus 71.5% WP on the severity of downy mildew infestation of cantaloupe plants 15 days after infection under greenhouse conditions.*

Treatments	Disease severity%)	Efficiency (%)
Trichoderma harzianum	37.47	*39.22*
Trichoderma viride	35.43	*42.53*
Bacillus subtilis	23.35	62.12
Bacillus megaterium	25.63	*58.42*
Pseudomonas fluorescens	29.99	51.35
Ridomil gold plus 71.5% WP	22.26	63.89
Control	61.65	00.00
L. S. D at 0.05%	0.05	

Efficiency of the tested biological organisms and fungicide Ridomil gold plus 71.5% WP on chlorophyll content and cantaloupe plant growth measurements:

The obtained results, which are shown in Table 3, showed a significant increase in both chlorophyll content and growth measures, such as, (leaf area and number of leaves), in cantaloupe plants treated with the tested organisms and the fungicide Ridomil gold plus 71.5% WP, compared to the control treatment. The highest chlorophyll content, was recorded in the fungicide-treated cantaloupe plants (43.88), while, *B. subtilis* recorded the second highest chlorophyll content (42.89). Whereas, *Ba. megaterium*, *P. Fluorescens*, *T. viride* and *T. harzianum* (40.39, 37.84, 34.39 and 29.74), respectively, compared to the control treatment recorded (24.29). Also, an increase in the number of leaves and leaf area of treated cantaloupe plants was observed. The fungicide recorded the highest increase in number of leaves/plant (36.00). While the tested organisms were arranged according to their efficiency in increasing the number of leaves/plant in descending order *B. subtilis*, *Ba. megaterium*, *P. Fluorescens*, *T. viride* and *T. harzianum* were recorded (35.30, 33.90, 31.60, 26.40, and 21.80, respectively). Compared to the control treatment, which was recorded (20.20). On the other hand, an increase in the leaf area of treated cantaloupe plants was observed in the same table. Both the fungicide and the bacteria *B. subtilis* recorded the maximum increase in leaf area (360.30 and 351.20 cm^2), respectively, followed by the treatment of plants with the organisms *Ba. megaterium*, *P. Fluorescens*, *T. viride* and *T. harzianum* recorded (346.40, 335.60, 323.60 and 268.00 cm^2, respectively) compared to the control treatment, which recorded (207.00 cm^2).

***Table 3.** Efficiency of the bio-organisms and the fungicide Ridomil gold plus 71.5% WP on chlorophyll content and growth measurements of Cantaloupe Downy mildew plants.*

Treatments	Chlorophyll content	Number of leaves/ plant	Leaf area ($cm^{2)}$
Trichoderma harzianum	29.74	21.80	268.00
Trichoderma viride	34.39	26.40	323.60
Bacillus subtilis	42.89	35.30	351.20
Bacillus megaterium	40.39	33.90	346.40
Pseudomonas fluorescens	37.84	31.60	335.60
Ridomil gold plus 71.5% WP	43.88	36.00	360.30
Control	24.29	20.20	207.00
L. S. D at 0.05%		3.44	0.91

Efficiency of bio-organisms and the fungicide Ridomil gold plus 71.5% WP on the production and components of the cantaloupe crop:

Recorded results in Table 4, showed a significant increase in the components of the crop of cantaloupe plants, treated with the tested biological organisms and the fungicide Ridomil gold plus 71.5% WP, compared to the control treatment. The best of these treatments was the treatment of cantaloupe plants with the fungicide, which recorded an increase in the number of cantaloupe fruits/plant (4.00 fruits/plant), with an average increase of 200.75%, compared to the control treatment, which recorded (1.33 fruits/plant). On the other hand, the tested organisms were arranged according to their efficiency in increasing the number of cantaloupe fruits in the following descending order: *B. subtilis*, *Ba. megaterium*, *P. fluorescens*, *T. viride* and *T. harzianum*, which recorded (3.66, 3.33, 3.00, 2.33 and 2.00 fruits/plant), respectively, with an average increase of 175.18, 150.37, 125.56, 75.18 and 50.37%, respectively. Also, observed in the same table, a significant increase in yield kg fruit/plant, compared to the comparison treatment. Where, the best of these treatments in increasing the yield of cantaloupe fruits was the treatment of the plants with the fungicide, which recorded (3.60 kg / plant) with an average increase of 207.69%. Also, the tested biological organisms were arranged according to their efficiency in increasing the yield of cantaloupe fruits as mentioned previously, as they were recorded (3.29, 2.99, 2.70, 2.17 and 1.80 kg/plant), respectively, with an average increase of 181.19, 155.55, 130.76, 85.47 and 53.84%, respectively.

***Table 4.** Efficiency of the bio-organisms and the fungicide Ridomil gold plus 71.5% WP on the components and yield of Cantaloupe Downy mildew.*

Treatments	Number of Fruits /plant	Average increase over the control%	Fruits yield (kg/ plant)	Average increase over the control%
Trichoderma harzianum	2.00	50.37	1.80	53.84
Trichoderma viride	2.33	75.18	2.17	85.47
Bacillus subtilis	3.66	175.18	3.29	181.19
Bacillus megaterium	3.33	150.37	2.99	155.55
Pseudomonas fluorescens	3.00	125.56	2.70	130.76
Ridomil gold plus 71.5% WP	4.00	200.75	3.60	207.69
Control	1.33	-	1.17	-
L. S. D at 0.05%	0.13		0.25	

Efficiency of the bio-organisms and the fungicide Ridomil gold plus 71.5% WP on biochemical changes in cantaloupe Downy mildew:

Spraying cantaloupe plants infected with downy mildew with *T. harzianum, T. viride*, *B. subtilis*, *Ba. megaterium* and *P. fluorescens*, which were used as bio stimulants, as well as, fungicide Ridomil gold plus 71.5% WP, resulted in an increase in the activity of defense related enzymes, such as, peroxidase and polyphenol oxidase, as well as, an increase in the total phenol content, compared with control. The results presented in Table 5, showed an increase in the activity of the peroxidase enzyme, when the cantaloupe was treated with *B. subtilis*, which was recorded (226.5), followed by the treatment of the cantaloupe with the organisms *Ba. megaterium*, *P. fluorescens*, *T. viride* and *T. harzianum* were recorded (185.9, 178.2, 167.4 and 153.2), respectively. While, the lowest activity of the peroxidase enzyme was obtained by the fungicide Ridomil gold plus 71.5% WP, which scored (147.8). At the same time, results were obtained consistent with the enzyme polyphenol oxidase when applying the same treatments mentioned above. Where, *B. subtilis* recorded the highest level of polyphenol oxidase activity (125.9), followed by treatment of cantaloupe with *Ba. megaterium*, *P. fluorescens* and fungicide were recorded (117.6, 110.8 and 94.5), respectively. On the contrary, the lowest polyphenol oxidase activity, was obtained when cantaloupes were treated with *T. viride* and *T. harzianum*, (82.3 and 81.6), respectively. Also, it was noticed in the same table an increase in the content of total phenols in all the tested treatments, especially when the cantaloupe was treated with *Ba. megaterium* which scored (66.4), compared to the control treatment which scored (22.9), followed by *B.*

subtilis which recorded (56.7), *P. fluorescens* (50.8), *T. viride* (47.8), and *T. harzianum* (45.6), while, the fungicide showed less efficiency on the total phenols content, as it scored (43.4).

Table 5. *Efficiency of the biological organisms and the fungicide Ridomil gold plus 71.5% WP on the biochemical changes of Cantaloupe Downy mildew plants 15 days after inoculation.*

Treatments	**Peroxidase (enzyme unit / mg) Protein / min.**	**Polyphenol oxidase (enzyme unit /mg) Protein / min.**	**Total phenols (mg GAE / g Fresh weight)**
Trichoderma harzianum	153.2	81.6	45.6
Trichoderma viride	167.4	82.3	47.8
Bacillus subtilis	226.5	125.9	56.7
Bacillus megaterium	185.9	117.6	66.4
Pseudomonas fluorescens	178.2	110.8	50.8
Ridomil gold plus 71.5% WP	147.8	94.5	43.4
Control	79.6	50.3	22.9
Correlation coefficient	-0.83	-0.82	-0.94

4. Discussion

Modern methods of combating fungal plant diseases depend on the use of safe alternative tools instead of using traditional methods (pesticides), as the use of traditional methods leads to contamination of food and the environment, which negatively affects human and animal health. Therefore, it has been recommended by scientists to use anti-organisms, especially in the proposed protocols to combat downy mildew disease that affects plants of the cucurbit family, as these organisms are used at an early stage in the life of the plant before the fruits ripen, so that the plant can stimulate the systemic defense system before Harvest [1, 17, 25]. Downy mildew disease, caused by the fungus *Pseudoperonospora cubensis* is considered one of the most dangerous diseases that affect the cantaloupe plant, as it leads to the cessation of plant growth and thus a significant decrease in the yield of the crop [14].

The current study aims to use some organisms to combat cantaloupe downy mildew disease, by activating the plant's systemic defense system, under greenhouse conditions.

Initially, under laboratory conditions, the obtained results indicated that, all tested organisms filters, such as, *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis*, *Bacillus megaterium* and *Pseudomonas fluorescens*, as well as, the fungicide Ridomil gold plus 71.5 WP, induced a decrease in the germination of *P. cubensis* sporangia. The fungicide was the most effective of these treatments in reducing the germination of the pathogenic fungus spores, followed by treatment with filters of *B. subtilis* and *Ba. megaterium.* These results are in agreement with [17], where they showed that the tested organisms had a significant ability to inhibit the germination of *P. cubensis* sporangia. The effectiveness of bio filters in inhibiting the germination of *P. cubensis* sporangia was explained by [37, 33, 32, 41], who reported that these organisms produce substances such as, Antibiotics and/or other direct inhibitors, such as, hydrolytic enzymes, hydrogen cyanide, or ferric acid, inhibit the germination of *P. cubensis* sporangia.

Under greenhouse conditions, the obtained results indicated that, all tested organisms and the fungicide had a significant decrease in the severity of cantaloupe downy mildew, compared to the control treatment. The fungicide was the best of these treatments in reducing the severity of the disease. Then, the plants were treated with *B. subtilis* and *Ba. megaterium*. These results are similar to what was decided by [1], which indicated that, the organisms belonging to the genus *Bacillus* have a high ability to reduce the severity of cantaloupe downy mildew disease. The reaction of the organisms in reducing the severity of cantaloupe downy mildew was explained by [37, 31], where they indicated that, the reaction of the organisms might be due to the widespread use of the tested organisms, such as, PGPR, in the fight against plant diseases. It has also been explained, [16, 41], that antimicrobial organisms produce compounds, such as, puromycin, which has been classified as an antifungal antibiotic, and plays a role in reducing the severity of plant diseases. The efficacy of the tested biological organisms, in addition to, the fungicide, was studied on the chlorophyll content, in treated and untreated cantaloupe, as well as, leaves number and area of leaves. The obtained results indicated that, all treatments led to an increase in chlorophyll content, number and leaf area of cantaloupe, compared to the control treatment. The fungicide was the best of these treatments in increasing the content of chlorophyll, number and area of leaves, followed by, treating the plants with anti-organisms, *B. subtilis* and *Ba. megaterium*. These results are consistent with many researchers, who reported that, treatment of plants with biological organisms led to a decrease in the incidence of pathogens, and thus improved measurements of plant growth. It was indicated by [35, 27, 39, 22], when treating cucumber and squash plants with probiotics and *Bacillus spp.*, *Serratia spp.* and *Trichoderma spp.* To combat powdery mildew, these treatments improved the growth parameters of those plants, in addition, a significant increase in chlorophyll content, as a result of their efficiency in reducing the severity of the disease. Also, noted [46], showed that when tomato plants were treated with *Serratia proteamaculans*, this resulted in a significant increase in chlorophyll content in treated leaves, compared to untreated ones.

The activity of the tested organisms was studied, in addition to, the fungicide, on the components of the cantaloupe crop, such as, the number and weight of fruits for each plant. The obtained results indicated that, all treatments led to an increase in the number and weight of cantaloupe fruits, compared to the control treatment. The fungicide was the best of these treatments, in increasing the number and weight of fruits, while, the tested biological organisms were arranged according to their effectiveness in increasing the number and weight of fruits in the following descending order:

B. subtilis, *Ba. megaterium*, *P. fluorescens*, *T. viride* and *T. harzianum*. These results are consistent with those mentioned by [1], where they reported that, treating cantaloupe plants with the bio-organisms led to an increase in the number of fruits and the quantity of cantaloupe yield, as a result of its efficiency in reducing the severity of downy mildew disease. The reaction of biological organisms in combating pathogens has been explained by many researchers, who found that, these biological organisms have the ability to produce plant growth regulators, such as, (indoleacetic acid, gibberellin and cytokinin), which leads to improved plant growth and increased fruit productivity [35, 39].

The effectiveness of the tested biological organisms and the fungicide on the biochemical changes of cantaloupe in treated and untreated plants was studied. The results obtained indicated a significant increase in the activity of enzymes related to stimulating the systemic defense activity, as well as, an increase in the total phenol content, in the treated plants, compared to the untreated plants.

The use of organisms, such as, *Trichoderma spp.* and *Bacillus spp.*, as inducers of systemic defense activity in plants, is one of the modern methods used to combat plant pathogens. Plant resistance to pathogens is associated with the induction of defense-related enzymes, such as, peroxidase and polyphenol oxidase, as well as, the accumulation of phenolic compounds [42, 39]. Several researchers have indicated positive relationships between biota and increased activity of defense-related enzymes, phenolic compounds, and resistance to plant pathogens. [17, 27], that treatment of cucumber plants with microorganisms, has significantly reduced the severity of powdery and downy mildew diseases, as the organisms activate the production of defense-related enzymes, such as, peroxidase and polyphenol oxidase, as well as, increase the accumulation of phenolic compounds. Reported by [22, 18], show that, resistance to powdery mildew is negatively correlated with increased biochemical changes, such as, defense-related enzymes and increased accumulation of phenolic compounds, in probiotic-treated squash plants.

5. Conclusions

It can be concluded from this study that, the use of organisms, such as, *Bacillus spp., Pseudomonas fluorescens* and *Trichoderma spp.,* It leads to the protection of cantaloupe plants from infection with downy mildew, by stimulating the systemic resistance of plants, Then these isolates can be used in integrated disease control programs.

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