

Effect of Some Bioagents on *Cladosporium cucumerinum* Causing Cladosporium Leaf Spot: A New Challenge in Watermelon Production in Egypt.

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Seven isolates of bioagents, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Anabena oryzae*, *Nostoc muscorum*, *Chaetomium globosum* and *Gliocladium catenulatum* were used to control cladosporium leaf spot. *Nostoc muscorum* mixed with *Pseudomonas fluorescens* successfully reduced the growth and spore germination of *Cladosporium cucumerinum*. The potentially of the bioagents significantly reduced the cladosporium leaf spot disease incidence and inhibiting its progress when used each alone or as mixtures under field conditions. The bioagents generally improved the watermelon plants and significantly increased the plant height, number of branches, fruit numbers and fruits weight due to treatment with the tested bioagents either each alone or in mixture compared to the untreated control.

Keywords: bio-agents, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Cladosporium cucumerinum*, leaf spot, watermelon

Watermelon (*Citrullus lanatus*) is considered as fruited crop belonging to the family Cucurbitaceae (Edwards *et al.*, 2003), and is considered one of the most important vegetable crops in Egypt. It is an annual creeping, commercial crop grown throughout the world as it is sugary, fleshy edible fruit. It is eaten especially during hot seasons (Bharath *et al.*, 2005). Watermelon contains high levels of lycopene that is very effective in protect cells from damage and lower the risk of heart disease. Watermelon extracts help to reduce hypertension and lower blood pressure in obese adults. Watermelon fruit is also a good source of potassium. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure. Consequently, it prevents against stroke and coronary heart diseases (Le *et al.*, 2005). Lycopene is an inhibitor for various inflammatory processes and also works as an antioxidant to neutralize free radicals. It also contains a good amount of vitamin-B6 (pyridoxine), vitamin-C and manganese. Consumption of food rich in vitamin-C helps the body to develop resistance against infectious agents

and scavenge harmful oxygen-free radicals. Manganese is used by the body as a co-factor for the antioxidant enzyme, superoxide dismutase.

Watermelon is an excellent source of Vitamin A, which is a powerful natural antioxidant. It is one of the essential vitamins for vision and immunity (Edwards *et al.*, 2003).

Cladosporium leaf spot was recorded in Egypt as the first report in 2018 on watermelon plants. *Cladosporium cucumerinum* is the causal pathogen of this disease lesions were observed on leaves and petioles brown to dark brown in color with or without a chlorotic halo. Shape of lesions was circular to oval and on leaves they were generally 1 to 14 mm in diameter. The conidia measured 2 to 8 × 1 to 3 µm (average 4.94×1.94 µm) (Farang, 2018).

Chemicals have played an important role in manage the diseases management in various vegetable crops over the years. Due to the excessive use of fungicides, the cost of crop production has also increased. Fungicides are toxic in nature and are equally harmful to human beings if applied injudiciously (Abhilash and Singh 2009). However, the continuous use of chemical fungicides led to the development of resistance in pests and residue in food chains and effect on human health and environment have been realized. Biological control may offer a good substitute to fungicides. Fungal and bacterial antagonists are one of the chief biological agents that have been studied for the control of foliar diseases (Hussein *et al.*, 2007; Zegeye *et al.*, 2011 and Abo-Elyousr *et al.*, 2017). Bio-control agents such as *C. globosum*, *G. catenulatum*, *T. harzianum*, *B. subtilis*, and *P. fluorescens* have the ability to control cladosporium leaf spots (Huang 1978; Xiao and Li. 2013; Sandoval, *et al.*, 2009 and Wang *et al.*, 2018) and enhance the plant growth (Compant *et al.*, 2005).

Various strains of cyanobacteria such as *Nostoc muscorum* and *Anabaena oryzae* are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal and antiviral activity. These biologically active compounds include antibiotics and toxins (De Caire *et al.*, 1990; Abo-Shady *et al.*, 2007 and Abedin & Taha, 2008.). This study was promised to evaluate the potential of *C. globosum*, *G. catenulatum*, *T. harzianum*, *P. fluorescens*, *B. subtilis*, and various strains of cyanobacteria such as *Nostoc muscorum* and *Anabena oryzae* when they were used each alone or in mixture to control leaf spot on watermelon and improvement its productivity using two application methods: seed soaking and/or foliar spray under field conditions.

Materials and Methods

Seven bio-agents isolates representing in *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Anabena oryzae* and *Nostoc muscorum* were kindly obtained from the Department of Microbiology, Soil, Water & Environment Res. Inst., ARC, Giza, Egypt in addition to *Chaetomium globosum* and *Gliocladium*

catenulatum, isolated during this study from watermelon leaves and identified at Assiut University Mycological Center based on their morphological characteristics were cultured and maintained on potato dextrose agar (PDA). All these bioagents were used each alone or in mixtures to evaluate their potential on *Cladosporium cucumerinum* spore germination, the antagonistic effect on *C. cucumerinum* growth *in vitro* as well as to control cladosporium leaf spot on watermelon and to improve its productivity using two application methods: seeds soaking and/or foliar spray under field conditions.

Bacterial suspension of *B. subtilis* was grown in nutrient broth medium for multiplication, while *P. fluorescens* was multiplied on King B (KB) liquid medium (King *et al.*, 1954) using shaking flask submerged culture, where 500 ml conical flasks, each containing 200 ml of the respective media was inoculated by one ml bacterial inoculum of 24 h old culture and incubated in rotary shaking incubator (120 rpm) at $25 \pm 1^\circ\text{C}$ for 48 h. *Chaetomium globosum* and *Gliocladium catenulatum* were grown on potato dextrose broth medium. *T. harzianum* was grown in *Trichoderma*-selective medium broth (TSM) (Elad *et al.*, 1981), amended with streptomycin and $50 \mu\text{g ml}^{-1}$ rose Bengal in conical flask, incubated at 25°C for 15 days

The effect of tested cyanobacteria on linear growth of C. cucumerinum:

The effect of *N. muscorum* and *A. oryzae* against the growth of *Cladosporium cucumerinum* was determined using a linear growth method. Dry *N. muscorum* and *A. oryzae* (0.03 g) were added to melted, autoclaved PDA medium. About 15 ml of the medium were poured into each Petri dish and allowed to solidify. Inocula (5mm) taken from a 7-day-old culture of *C. cucumerinum* were placed in the center of the prepared Petri dishes. For the other tested bioagents (fungi and bacteria), a 5mm disk was taken from the growth of each antagonistic fungus and/or bacterium and was placed onto the PDA, 10mm from the edge of the Petri dish and 5mm disc from *C. cucumerinum* was placed on the opposite side and then incubated for seven days at $25 \pm 2^\circ\text{C}$. PDA medium without the cyanobacterial filtrates, fungal and bacterial tested served as control (Singh & Tripathi, 1999). Both experimental and control dishes were assigned to a completely randomized design, with five replicates per treatment. The antagonistic effect measurements were carried out when the pathogenic fungal growth filled all Petri dishes in control. The effect of the cyanobacterial filtrates and bioagents growth in terms of percentage inhibition of mycelial growth was calculated using the formula:

$$\text{Reduction in linear growth} = [(C - T)/C] \times 100$$

Where: C = growth of *C. cucumerinum* on control, T = growth of *C. cucumerinum* with cyanobacterial extracts and bioagents.

Effect of the tested bioagents on spore germination of C. cucumerinum:

Drops of the tested bioagents suspension were placed separately or in mixture on glass slides and spore suspension drops of *C. cucumerinum* were placed exactly on these respective drops. These slides were kept in Petri plates lined with moist blotting paper and then placed in moist chamber. Spores immersed in distilled water as well as fungicide suspension (Ridomil gold MZ 68 WG) served as control. Three replicates were made for each treatment. The slides were incubated at $25\pm 2^{\circ}\text{C}$ for 24 h and the percent of germination was calculated under a light microscope.

Field experiments:

Two application methods: seeds soaking and foliar spray/or just foliar spray only under field experimental conditions were carried out at the farm of Agricultural Research of Sids Station, Agric. Res. Center, Beni Sweif governorate, during early summer growing seasons (from middle of February to April 2018 to 2019) to evaluate the potential of *Bacillus subtilis*, *Pseudomonas fluorescens* (3×10^8 cfu), *Chaetomium globosum*, *Gliocladium catenulatum*, *Trichoderma harzianum* (3×10^6 conidia per ml) and cyanobacteria (*Nostoc muscorum* and *Anabena oryzae*, 50 L/Fed.), each alone or in mixture to control cladosporium leaf spot on watermelon and increasing its productivity.

Field design and soil analysis:

The soil of the experimental field was clay in texture (16.5 % sand, 30.1 % silt, 53.4 % clay), pH of 8.1, EC 1.2 dSm^{-1} ; 1.3 % organic matter and 26.2, 10.1 and 176 ppm available N, P and K, respectively. The experiment was set in a randomized complete blocks design with two factors, three replications for each treatment. The first factor was assigned to the bioagents tested and the second one to application methods. The field plots were 6×3.5 m.

Field Preparation:

In each season, the soil was mechanically plowed and planked twice prepared into ridges (2 m width). During the preparation for cultivation, calcium superphosphate (15.5 % P_2O_5) as a source of phosphorus was added in the rate of 200 kg/fed. The watermelon seeds (Giza 1 cultivar) were soaked in the bioagents suspension for 24h and the treated and untreated seeds were sown at 75 cm spacing between plants on February 15th in the two experimental seasons. Weeds were removed by manual operations as needed and plants were irrigated regularly as necessary, throughout the growing season to maintain constant growth. Nitrogen was applied in the form of ammonium nitrate (33 % N), at the rate of 400 kg/fed. (recommended rate). Potassium sulfate (48 % K_2O) as a source of potassium was added at the rate of 75 kg/fed. The plants were sprayed every 10 days, always performed early in the morning, with the tested bioagents, 1% Tween 20 as well as the fungicide Ridomil gold MZ 68 WG at the rate of 50 gm/100L water before the appearance of first symptoms until run off and distilled water (control). Monitoring

and scouting the plants weekly for the appearance of cladosporium leaf spot and disease incidence and severity were estimated as follow:

Disease assessment:

Percentage of disease incidence was recorded as the number of diseased plants relative to the number of growing plants for each treatment and then the average of disease incidence was calculated. Disease severity (DS) was recorded using the following 0 to 5 scale: 0, no incidence; 1, lesions limited to lower 1/5 of leaf area; 2, lesions present on lower 1/3 of leaf area; 3, lesions present on more than 1/3 of leaf area; 4, lesions present on more than 2/3 of leaf area; and 5, severe infections on all leaves determined using the following formulas Shi *et al.* (2012):

$$DS (\%) = \frac{\sum \text{disease scale} \times \text{number of leaves}}{\text{The most serious disease scale} \times \text{total number of leaves}} \times 100$$

Evaluation of watermelon yield and its components:

Plant length (m), branches numbers/plant, fruits number/plant and fruits weight/plant (kg) were determined as averages from the replicates.

Evaluation total phenols and growth hormones:

Total phenols was estimated using Folin ciocalteau reagent method described by Lafka *et al.* (2007). Plant hormones (abscisic acid, gibberellic acid and indole acetic acid) were determined according to the method described by (Shindy and Smith, 1975).

Data were statistically analyzed for computing L.S.D. test at 5 % probability according to the procedure outlined by Snedecor and Cochran (1989).

Results

The effect of tested bioagents on spore germination and linear growth of C. cucumerinum:

In the antagonism experiments, clear inhibition zones on the PDA plates were observed, and the mycelium of *C. cucumerinum* stopped growing confronted to *A. oryzae*, *B. subtilis*, *C. globosum*, *G. catenulatum*, *N. muscorum*, *P. fluorescens*, *T. harzianum* and their combinations after 7-10 days of incubation (Table, 1). Treatment of *N. muscorum* mixed with *P. fluorescens* recorded the highest reduction in linear growth (83.3%) and spore germination of *C. cucumerinum* (82.7%). followed by *N. muscorum* mixed with *T. harzianum* (76.7 and 80.8%), *N. muscorum* mixed with *B. subtilis* (75.6 and 81.4%) and *A. oryzae* mixed with *P. fluorescens* (73.3 and 80.5%) treatments with no significant differences among them except with treatment of *A. oryzae* + *P. fluorescens* in case of linear growth. In general, the fungicide Ridomil gold MZ 68 WG was more effective than the other treatments in reducing the linear growth (91.1%) and spore germination (88.6%) of *C. cucumerinum*. On the other hand, *C. globosum* recorded the lowest effect on the linear growth reduction and spore germination, being 38.9 and 69.7%.

Table (1): Effect of different bioagents on spore germination and linear growth of *C. cucumerinum* in vitro

Treatments	Germination %	Reduction* %	Linear growth	Reduction* %
<i>A. oryzae</i>	8.8	76.2	3.4	62.2
<i>B. subtilis</i>	9.3	74.9	4.5	50.0
<i>C. globosum</i>	11.2	69.7	5.5	38.9
<i>G. catenulatum</i>	10.5	71.6	4.8	46.7
<i>N. muscorum</i>	8.7	76.5	3.2	64.4
<i>P. fluorescens</i>	9.2	75.1	3.8	57.8
<i>T. harzianum</i>	9.7	73.8	5.0	44.4
<i>A. oryzae</i> + <i>B. subtilis</i>	8.3	77.6	2.9	67.8
<i>A. oryzae</i> + <i>P. fluorescens</i>	7.2	80.5	2.4	73.3
<i>A. oryzae</i> + <i>T. harzianum</i>	8.2	77.6	2.5	72.2
<i>N. muscorum</i> + <i>B. subtilis</i>	6.9	81.4	2.1	75.6
<i>N. muscorum</i> + <i>P. fluorescens</i>	6.4	82.7	1.5	83.3
<i>N. muscorum</i> + <i>T. harzianum</i>	7.1	80.8	2.2	76.7
Ridomil gold MZ 68 WG	4.2	88.6	0.8	91.1
Control	37.0	----	9.0	----
L.S.D. at 0.05	0.9	----	0.4	----

* Reduction % related to the control.

The effect of tested bioagents on disease incidence and severity:

Data in Tables (2 and 3) focus on the potentialities of the bioagents to reduce significantly the cladosporium leaf spot disease incidence and inhibit the progress of disease compared with the untreated control (Fig.1) when they were used individually or as mixtures under field conditions. Among all treatments, the fungicide Ridomil gold MZ 68 WG was the most efficient in this regard which recorded the lowest disease incidence, being 16.8, 13.4 and 7.9 %, respectively and significantly delayed the progress of disease to 9.8, 6.7 and 3.9%, respectively as a result of the three sprays. *Nostoc muscorum* mixed with *P. fluorescens* came in the second rank and recorded the best value on reducing disease incidence and severity followed by *N. muscorum* mixed with *B. subtilis* without significant differences between them. The corresponding mean values in disease incidence were 19.2, 15.4, 13.5 & 20.1, 16.9, 14.5 %, respectively and 12.1, 7.8, 4.8% & 12.4, 8.8, 5.2, respectively for disease severity. Moderate disease incidence and severity were observed in plots received *A. oryzae* mixed with *P. Fluorescens* and *N. muscorum* mixed with *T. harzianum* without significant differences between them followed by *A. oryzae* mixed with *B. subtilis* and *A. oryzae* mixed with *T. harzianum* treatments. Meanwhile, treatment of *C. globosum* was the least effective in this concern. Overall

the method of seed soaking and spraying the watermelon plants with the tested bioagents was the best method to reduce the incidence and severity of cladosporium leaf spot.

The interaction effect of the different bioagent treatments and their application methods was significant during the two successive growing seasons. The lowest values of disease incidence (15.5, 13.2 & 6.3%, respectively) and disease severity (9.4, 5.5 & 3.3%, respectively) were obtained due to treatment with fungicide Ridomil gold MZ 68 WG as watermelon seeds soaking and foliar spray followed by treatment of *N. muscorum* + *P. fluorescens* when applied as seed soaking and foliar spray. While the lowest efficient was *C. globosum* treatment when applied as foliar spray only.

Table (2): Effect of bioagents on disease incidence % of cladosporium leaf spot at the average of two successive growing seasons 2018/2019.

Treatments (T)	Disease incidence %								
	Mean of the two growing seasons								
	Application methods (M)								
	1 st spray			2 nd spray			3 rd spray		
	Seed soaking + foliar spray	Foliar spray	Mean (T)	Seed soaking + foliar spray	Foliar spray	Mean (T)	Seed soaking + foliar spray	Foliar spray	Mean (T)
<i>A. oryzae</i>	24.8	26.2	25.5	22.7	24.2	23.5	17.7	20.0	18.9
<i>B. subtilis</i>	26.1	28.9	27.5	22.8	25.3	24.1	19.2	20.7	20.0
<i>C. globosum</i>	30.0	32.7	31.4	26.5	29.5	28.0	21.6	24.0	22.8
<i>G. catenulatum</i>	27.7	29.5	28.6	23.7	26.9	25.3	19.9	21.8	20.9
<i>N. muscorum</i>	24.0	25.8	24.9	21.6	23.8	22.7	16.9	19.2	18.1
<i>P. fluorescens</i>	25.5	28.3	26.9	22.7	25.3	24.0	18.6	20.8	19.7
<i>T. harzianum</i>	27.1	29.1	28.1	23.6	25.7	24.7	19.8	21.8	20.8
<i>A. oryzae</i> + <i>B. subtilis</i>	21.7	23.4	22.5	17.1	19.0	18.1	14.3	15.7	15.0
<i>A. oryzae</i> + <i>P. fluorescens</i>	19.8	21.2	20.5	16.7	18.6	17.7	13.7	16.2	15.0
<i>A. oryzae</i> + <i>T. harzianum</i>	23.1	25.3	24.2	19.0	22.6	19.7	15.4	17.7	16.5
<i>N. muscorum</i> + <i>B. subtilis</i>	19.6	20.6	20.1	16.1	17.6	16.9	13.7	15.2	14.5
<i>N. muscorum</i> + <i>P. fluorescens</i>	18.7	19.7	19.2	14.5	16.3	15.4	10.9	14.1	13.5
<i>N. muscorum</i> + <i>T. harzianum</i>	20.0	21.4	20.7	16.5	19.9	18.2	14.5	15.4	15.0
Ridomil gold MZ 68 WG	15.5	18.9	17.2	13.2	15.5	14.5	6.3	11.2	8.8
Control	36.6	35.1	35.9	53.5	48.0	50.8	61.3	60.9	61.1
Mean (M)	24.0	25.7	---	22.0	23.9	---	18.9	21.0	---
L.S.D. at 0.05	T = 1.0 M = 0.4 TM = 1.4			T = 1.1 M = 0.4 TM = 1.6			T = 1.5 M = 0.6 TM = 2.2		

Table (3): Effect of bioagents on disease severity % of cladosporium leaf spot at the average of two successive growing seasons 2018/2019.

Treatments (T)	Disease severity %								
	Mean of the two growing seasons								
	Application methods (M)								
	1 st spray			1 st spray			1 st spray		
	Seed soaking + foliar spray	Foliar spray	Mean (T)	Seed soaking + foliar spray	Foliar spray	Mean (T)	Seed soaking + foliar spray	Foliar spray	Mean (T)
<i>A. oryzae</i>	15.9	19.6	17.8	14.4	16.0	15.2	10.0	11.8	10.9
<i>B. subtilis</i>	19.0	21.4	20.2	17.7	19.2	18.5	13.1	15.8	14.5
<i>C. globosum</i>	24.7	28.0	26.4	22.9	25.5	24.2	20.7	23.0	21.9
<i>G. catenulatum</i>	23.4	27.5	25.5	20.0	23.0	21.5	18.5	21.8	20.2
<i>N. muscorum</i>	15.7	18.0	16.9	13.1	15.1	14.1	9.9	11.5	10.7
<i>P. fluorescens</i>	17.5	20.5	19.0	15.2	17.9	16.6	12.2	13.7	13.0
<i>T. harzianum</i>	21.5	23.8	22.7	18.3	21.7	20.0	15.6	18.3	17.0
<i>A. oryzae</i> + <i>B. subtilis</i>	12.3	16.4	14.4	9.3	11.9	10.6	7.2	9.9	8.5
<i>A. oryzae</i> + <i>P. fluorescens</i>	10.3	14.9	12.6	8.5	10.4	9.5	5.4	8.3	6.9
<i>A. oryzae</i> + <i>T. harzianum</i>	13.1	16.6	14.9	11.1	12.6	11.8	7.7	10.7	9.2
<i>N. muscorum</i> + <i>B. subtilis</i>	10.0	14.7	12.4	7.5	10.0	8.8	4.2	6.2	5.2
<i>N. muscorum</i> + <i>P. fluorescens</i>	9.4	14.8	12.1	7.2	8.3	7.8	4.2	5.4	4.8
<i>N. muscorum</i> + <i>T. harzianum</i>	11.4	15.3	13.4	8.3	10.6	9.5	5.0	8.1	6.6
Ridomil gold MZ 68 WG	9.4	10.1	9.8	5.5	7.9	6.7	3.3	4.5	3.9
Control	29.7	29.4	29.6	38.8	35.3	37.1	45.7	48.3	47.0
Mean (M)	16.2	19.4	---	14.5	16.4	---	12.2	14.5	---
L.S.D. at 0.05	T = 1.0 M = 0.4 TM = 1.4			T = 1.4 M = 0.5 TM = 2.0			T = 1.6 M = 0.6 TM = 2.2		

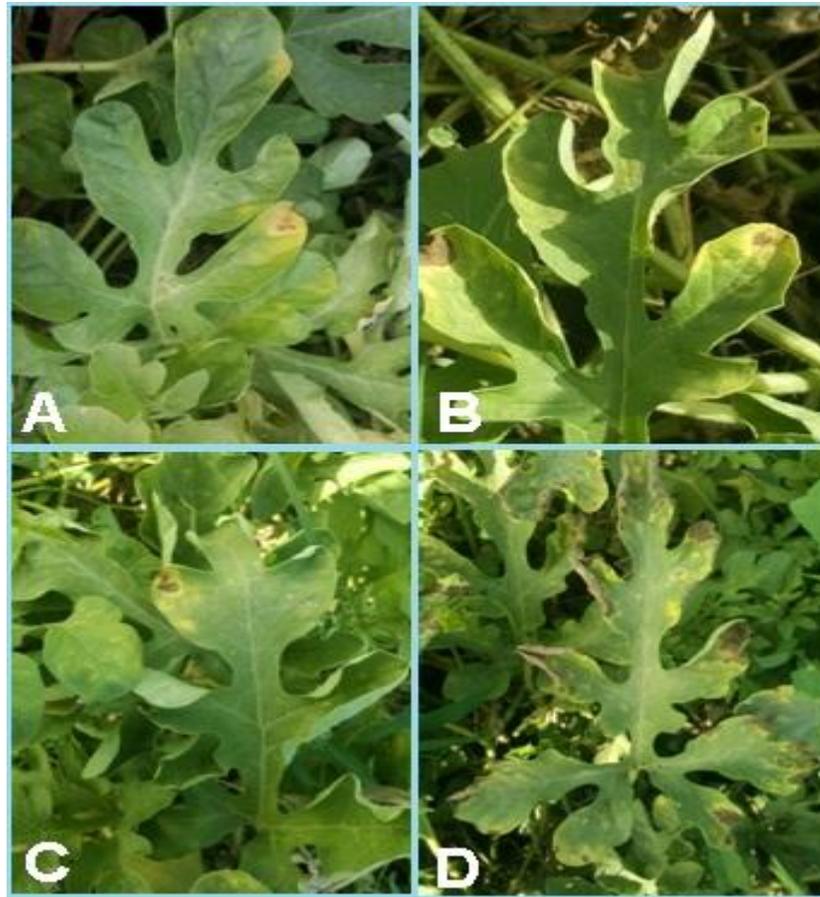


Fig. 1: Effect of some bioagents on cladosporium leaf spot, A; *N. muscorum* + *P. fluorescens*, B; *N. muscorum* + *B. subtilis* and C; *N. muscorum* + *T. harzianum* compared with control D

Effect of some biocontrol agents on some plant growth parameters:

Data presented in Tables, (4 and 5) show a general improvement in watermelon plants. Plant parameters (plant length (m), number of branches/plant, fruits number/plant and fruits weight/plant (kg)) were significantly increased, due to treatment with the tested bioagents when used either each alone or in combination compared to the untreated control. Combined treatment between *N. muscorum* and *P. fluorescens* gave the highest values in these parameters followed by the combined treatment between *N. muscorum* and *B. subtilis* without significant differences between them. The corresponding mean values were 4.7 & 4.5 m, respectively for

plant length; 17.0 & 15.7, respectively for branches number/plant; 3.7 & 3.7, respectively for fruits number/plant and 25.7 & 23.2 kg, respectively for fruits weight/plant. Moderate values were recorded with *A. oryzae* + *P. fluorescens* treatment followed by *N. muscorum* + *T. harzianum* treatment without significant differences between them. The lowest values were recorded with *C. globosum* treatment which recorded 2.7 m, 8.1, 2.4 and 15.6 kg, respectively. Generally, watermelon seed soaking and spraying the leaves with the tested bioagents caused a significant promotion to plant growth and increased the yield than the spraying method only.

Table (4): Effect of some biocontrol agents on some plant growth parameters at the average of two successive growing seasons 2018/2019.

Treatments (T)	Mean of the two growing seasons					
	Application methods (M)					
	Plant length (m)			Branches number/plant		
	Seed soaking + foliar spray	Foliar spray	Mean (T)	Seed soaking + foliar spray	Foliar spray	Mean (T)
<i>A. oryzae</i>	4.0	3.8	3.9	9.8	9.6	9.7
<i>B. subtilis</i>	3.5	3.3	3.4	12.8	12.3	12.6
<i>C. globosum</i>	2.8	2.4	2.6	9.2	6.9	8.1
<i>G. catenulatum</i>	2.8	2.6	2.7	9.6	9.3	9.5
<i>N. muscorum</i>	4.0	3.9	4.0	12.4	12.2	12.3
<i>P. fluorescens</i>	3.7	3.4	3.6	12.2	9.6	10.9
<i>T. harzianum</i>	3.7	3.2	3.5	9.8	9.6	9.7
<i>A. oryzae</i> + <i>B. subtilis</i>	4.4	4.0	4.2	12.9	12.7	12.8
<i>A. oryzae</i> + <i>P. fluorescens</i>	4.6	4.0	4.3	15.6	15.1	15.4
<i>A. oryzae</i> + <i>T. harzianum</i>	4.1	3.9	4.0	12.6	12.1	12.4
<i>N. muscorum</i> + <i>B. subtilis</i>	4.7	4.3	4.5	15.7	15.6	15.7
<i>N. muscorum</i> + <i>P. fluorescens</i>	4.8	4.6	4.7	18.1	15.9	17.0
<i>N. muscorum</i> + <i>T. harzianum</i>	4.5	3.9	4.2	15.1	15.0	15.1
Ridomil gold MZ 68 WG	2.6	2.6	2.6	9.0	9.0	9.0
Control	1.6	1.6	1.6	9.0	9.0	9.0
Mean (M)	3.7	3.4	---	12.3	11.6	---
L.S.D. at 0.05	T=0.4 M= 0.1 TM= 0.5			T=0.5 M= 0.2 TM= 0.7		

The interaction between the tested bioagents and their application methods had significant effect on the aforementioned parameters. The maximum values were obtained from plots received watermelon seeds soaked in a mixture of *N. muscorum* + *P. fluorescens* and spraying the leaves with their suspensions followed by *N. muscorum* + *B. subtilis*, *A. oryzae* + *P. fluorescens* treatments without significant differences among them except in fruits weight/plant. While the lowest values of plant height (2.6 m), branches number/plant (6.9), fruits number/plant (2.2) and fruits weight/plant (15.1 kg) were obtained from plots received *C. globosum* as foliar spray only compared to the other tested bioagents treatments.

Table (5): Effect of some biocontrol agents on fruits number/plant and fruits weight/plant at the average of two successive growing seasons 2018/2019.

Treatments (T)	Mean of the two growing seasons					
	Application methods (M)					
	Fruits number/plant			Fruits weight/plant		
	Seed soaking + foliar spray	Foliar spray	Mean (T)	Seed soaking + foliar spray	Foliar spray	Mean (T)
<i>A. oryzae</i>	2.5	2.2	2.4	15.7	15.2	15.5
<i>B. subtilis</i>	2.8	2.5	2.7	18.5	18.0	18.3
<i>C. globosum</i>	2.5	2.2	2.4	15.7	15.1	15.4
<i>G. catenulatum</i>	2.7	2.3	2.5	18.1	15.4	16.8
<i>N. muscorum</i>	2.9	2.7	2.8	18.7	15.8	17.3
<i>P. fluorescens</i>	2.9	2.7	2.8	18.0	15.5	16.8
<i>T. harzianum</i>	2.8	2.6	2.7	18.3	18.2	18.3
<i>A. oryzae</i> + <i>B. subtilis</i>	3.6	3.0	3.3	21.1	18.7	19.9
<i>A. oryzae</i> + <i>P. fluorescens</i>	3.7	3.5	3.6	21.3	21.1	21.2
<i>A. oryzae</i> + <i>T. harzianum</i>	2.8	2.7	2.7	18.7	18.5	18.6
<i>N. muscorum</i> + <i>B. subtilis</i>	3.8	3.6	3.7	24.5	21.9	23.2
<i>N. muscorum</i> + <i>P. fluorescens</i>	3.8	3.7	3.7	27.2	24.4	25.7
<i>N. muscorum</i> + <i>T. harzianum</i>	3.4	3.2	3.3	21.3	21.1	21.2
Ridomil gold MZ 68 WG	2.6	2.6	2.6	12.9	12.9	12.9
Control	2.0	2.0	2.0	12.9	12.9	12.9
Mean (M)	3.0	2.8	---	18.9	17.6	---
L.S.D. at 0.05	T=0.4 M=0.1 TM=0.6			T=0.6 M=0.2 TM=0.9		

Evaluation of total phenols:

Data presented in Table, 6 show the effect of the tested bioagents on the total phenols in the treated watermelon plants compared to the untreated control. Generally, phenolic content in watermelon leaves revealed a reverse relationship with the degree of infection by the pathogen. When the mixture of bioagents was applied, the phenolic content of leaves was higher than using each treatment alone. Maximum increase was detected with *N. muscorum* mixed with *P. fluorescens* treatment, being 18.977 followed by treatment of *N. muscorum* mixed with *B. subtilis*, being 18.080 without significant differences between them. The lowest phenolic content was detected in watermelon plants treated with *C. globosum* followed by *G. Catenulatum* treatment without significant differences. The corresponding mean values were 0.992 and 1.313, respectively. Soaking the watermelon seeds and spraying the leaves with bioagents suspensions significantly increased the phenolic content compared to spraying the leaves by any suspension only.

Table (6): Total phenols in watermelon plants as affected by different bioagents tested.

Treatments (T)	Total phenol		
	Seed soaking + foliar spray	Foliar spray	Mean (T)
<i>A. oryzae</i>	6.414	3.629	5.022
<i>B. subtilis</i>	5.606	2.116	3.861
<i>C. globosum</i>	1.111	0.872	0.992
<i>G. catenulatum</i>	1.715	0.911	1.313
<i>N. muscorum</i>	7.609	4.408	6.009
<i>P. fluorescens</i>	6.062	2.527	4.295
<i>T. harzianum</i>	4.411	2.076	3.244
<i>A. oryzae</i> + <i>B. subtilis</i>	13.453	7.866	10.660
<i>A. oryzae</i> + <i>P. fluorescens</i>	19.801	10.909	15.366
<i>A. oryzae</i> + <i>T. harzianum</i>	12.591	7.710	10.151
<i>N. muscorum</i> + <i>B. subtilis</i>	25.230	10.930	18.080
<i>N. muscorum</i> + <i>P. fluorescens</i>	26.934	11.020	18.977
<i>N. muscorum</i> + <i>T. harzianum</i>	16.776	9.033	12.905
Ridomil gold MZ 68 WG	2.042	2.037	2.040
Control	0.007	0.009	0.008
Mean (M)	9.984	5.070	---
L.S.D. at 0.05	T=1.653	M=0.604	TM=2.338

The interaction between bioagents treatments tested and their application methods had significant effect on total phenol. *N. muscorum* mixed with *P. fluorescens* showed the highest total phenol (26.934) followed by *N. muscorum* + *B. subtilis* (25.230) when applied as seed soaking and foliar spray treatment with significant differences between them while the lowest value was noticed when watermelon plants were sprayed with *C. globosum* treatment.

Evaluation of growth hormones:

Data presented in Table, 7 show the capabilities of the tested bioagents to produce phytohormones. It is worthy to note that; *N. muscorum* produced higher abscisic acid (ABA) than the other bioagents tested being, 5.23 µg/ml. *A. oryzae* exhibited moderate performance (1.75 µg/ml). While the lowest value obtained with *T. harzianum* (0.33µg/ml) compared with 0.55µg/ml in *B. subtilis* treatment and 0.62 µg/ml in *P. fluorescens* treatment which lies in the same statistical group. In the case of Gibberellic acid (GA3), *A. oryzae* produced the highest amount (88.39 µg/ml) followed by *N. muscorum* (28.59 µg/ml) with significant differences between them. *P. fluorescens* significantly produced moderate gibberellic acid (3.02µg/ml) higher than *B. subtilis* (1.92 µg/ml)) and *T. harzianum* (1.50µg/ml) which statistically lies in the same group. On the other hand, *N. muscorum* produced indole acetic acid (IAA) higher than *A. oryzae*, being, 18.23 and 11.18µg/ml respectively with significant differences between them. While *T. harzianum* produced lower indole acetic acid (IAA) than *B. subtilis* and *P. fluorescens*.

Table (7): Production of plant growth promoters by the tested bioagents.

Bioagents	Abscisic acid (ABA) (µg/ml)	Gibberellic acid (GA3) (µg/ml)	Indole acetic acid (IAA) (µg/ml)
<i>A. oryzae</i>	1.75	88.40	11.18
<i>B. subtilis</i>	0.55	1.92	7.03
<i>N. muscorum</i>	5.23	28.59	18.23
<i>P. fluorescens</i>	0.62	3.02	7.15
<i>T. harzianum</i>	0.33	1.50	6.79
L.S.D. at 0.05	0.23	0.51	0.34

Discussion

Many experiments have been undertaken to identify alternate management mechanism of mechanisms of plant pathogens instead of fungicides, as the fungicide have environmental pollution and may contribute to the production of fungicide-resistant strains (Soria *et al.*, 2012). In the present study, all the tested bioagents applied as seed treatment with foliar spray or as foliar spray only significantly reduced losses caused by cladosporium leaf spot on watermelon and increased its productivity compared to the control. Seed treatment of watermelon and spraying the leaves with bioagents suspensions was the most effective in reducing the disease

incidence and severity than spraying the leaves by the suspensions only. Mixing the cyanobacteria *Nostoc muscorum* or *Anabaena oryzae* with the fungal and bacterial antagonistic was more effective compared to each treatment separately. Among them, mixed treatment, of cyanobacterium (*N. muscorum*) and the bacteria (*P. fluorescens*) showed the highest efficacy in this concern followed by *N. muscorum* + *B. subtilis* and *A. oryzae* + *P. fluorescens* treatments. These results are in agreement with those reported by Abdel-Hafez *et al.* (2015), who reported that application of culture filtrates of *N. muscorum* reduced purple blotch disease severity of onion under greenhouse conditions. *N. muscorum* contained high concentrations of phenolic compounds (145.0 mg l^{-1}) and alkaloids (378.12 mg l^{-1}) which are employed as natural defense mechanisms against pathogenic fungi (Zeeshan *et al.*, 2010). Abdel-Hafez *et al.* (2015) showed that the most prevalent component in culture filtrates of *N. muscorum* was β -ionone. Indol alkaloid norharmane and α -isomethyl ionone were the second most prevalent in *N. muscorum* which may confer antimicrobial effects on the culture filtrates. *Anabaena* sp. produced numerous secondary metabolites, toxins and other compounds as lipopeptides, amino acids, fatty acids. Among the lipopeptides, some of them have demonstrated cytotoxic, antitumoral, antiviral, antibiotic activity and some effects as herbicides and antifungal against pathogens (Patterson *et al.*, 1994). Gnanamanickam *et al.* (1992) observed that seed treatment followed by two sprayings with *P. fluorescens* effectively controlled rice sheath blight disease and leaf spot of groundnut (Meena and Marimuthu, 2012). Paul and Sharma (2006) reported the production of two antibiotics - pyoluteorin and pyrrolnitrin by *P. fluorescens*, which inhibit the growth of *Phytophthora capsici*, the pathogen of root rot of black pepper. However, Bargabus *et al.* (2004) reported that the main mode of action of *P. fluorescens* includes induce systemic resistance in addition to the production of siderophores which contributed to suppression of pathogens (Mathiyazhagan *et al.*, 2004). In this study, treatment of watermelon seeds with the tested bioagents induced significant protection against *Cladosporium cucumerinum*, the causal agent of watermelon leaf spot that supports the suggestion of systemic resistance and excluding the possibility of direct antagonism because of the spatial separation between rhizobacteria in the rhizosphere and pathogens in the phylloplane.

Bacillus produces siderophores in order to adhere to the surface of non-biological biofilm formation, which makes the *Bacillus* secretes broad-spectrum resistance compounds to a certain extent and increases the spectrum resistance of plants (Höfte and Bakker, 2007). Asaka and Shoda, (1996) reported that *B. subtilis* secreted antibiotics iturin A and surfactin for controlling plant diseases and enhancing the expression of the chitinase promoter (Shali *et al.*, 2017). The high chitinase activity might have resulted in lyses of invading fungal pathogens (Fernando *et al.*, 2007). The present study demonstrated that phenolic contents of watermelon leaves of treated plants with the tested bioagents were higher than that of control. When the mixture of bioagents was applied, the phenolic content of

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leaves was higher than the using each treatment alone. Comparison between the degree of infection by the pathogen and the corresponding phenolic content in watermelon leaves revealed a reverse relationship, i.e. when the phenolic content is high, the degree of infection is low. The phenolic compounds contribute to enhance the mechanical strength of host cell wall and inhibit the fungal growth; they are acting as fungi toxic. *Trichoderma* species have been recognized as biocontrol agents against several pathogens (Perazzolli *et al.*, 2008 and Shores *et al.*, 2010). The biocontrol ability of *Trichoderma* species is based on different mechanisms such as interference with pathogen's pathogenicity enzymes (Kapat *et al.*, 1998), direct interaction with the pathogen through antibiosis or parasitism (Elad & Freeman, 2002 and Howell, 2003) and activation of plant disease resistance (Korolev *et al.*, 2008).

On the other hand results of this study showed a general improvement in both vegetative and fruits yield of watermelon due to treatment with the tested bioagents related to the control. The plant growth promoting rhizobacteria (PGPR) *B. subtilis*, *P. fluorescens* and the fungus (*T. harzianum*) in addition to cyanobacteria (*N. muscorum*, *A. oryzae*) produced indole acetic acid and gibberellic acid that play a key role in improving growth of many plants when applied as biofertilizers, these findings are in agreement with those reported by Aref (2011). Haiquan *et al.* (2013) mentioned that the mechanisms of PGPR mediated enhancement of plant growth and yields in many crops are as follows: ability to produce 1-aminocyclopropane-1-carboxylates (ACC) deaminase to reduce the level of ethylene in roots of the developed plants and thereby increasing the root length and growth; ability to produce hormones like indole acetic acid, gibberellic acid and cytokinins; asymbiotic nitrogen fixation; solubilization of mineral phosphates and mineralization of other nutrients and control of phytopathogenic microorganisms. Asad and Vafa (2011) found that PGPR can increase P availability to plants through solubilizing insoluble phosphates and this improved P nutrition, which in turn increases the biological nitrogen fixation and availability of other nutrients for plants. El-Sherif *et al.* (2013) found that cyanobacteria can be incorporated into soil as organic matter and also as a source of enzymes as they produce acid and alkaline extracellular phosphatases that are active in solution or located in the peri-plasmatic space of the cell wall. Both biomass and exopolysaccharides incorporated into soil induce a growth promotion of other microorganisms and increase the activity of soil enzymes that participate in the liberation of nutrients required by plants. Harman (2000) suggested that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by improving seed vigor and growth (Mastouri *et al.*, 2012), promote seedling growth and change root structure and increase root activity in seedlings, possibly via the production or control of plant hormones (Kleifield and Chet, 1992 and Hermosa *et al.*, 2012) and decomposing, recycling and utilizing soil nutrients (Shores *et al.*, 2010 and Harman, 2011), improve the ability of plant photosynthesis

(Huang *et al.*, 2011) which consequently increased fruit yield (Shen *et al.*, 2015 and Xiong *et al.*, 2017).

Conclusions

Therefore, it is recommended to use the seeds treatment and foliar spraying of leaves with *N. muscorum* or *A. oryzae* mixed with *P. fluorescens* as safe biocontrol agents compared with the fungicide for controlling watermelon leaf spot and increasing its productivity under field conditions.

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تأثير بعض الكائنات الحيوية على فطر كلادوسبوريوم
ككمارينوم المسبب لتبقع الاوراق الكلاوسبوريومي: تحد
جديد في إنتاج البطيخ في مصر.

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مصر

٢- معهد بحوث الاراضى والمياة والبيئة ، مركز البحوث الزراعية ،
الجيزة ، مصر

تم استخدام سبع عزلات من الكائنات الحيوية ، ترايكودرما هاريزيانوم
وسيدوموناس فلوريسنس وباسلس ساتلس و انابينا اوريذا و نوستوك
ماسكورمو كيتوميوم جلوبوزمو جليوكلاديوم كاتينولاتم لمكافحة مرض تبقع
الاوراق الكلاوسبوريوم فى البطيخ. نجح نوستوك ماسكورم المخلوط مع
سيدوموناس فلوريسنس فى تقليل النمو الفطرى للمرض و كذلك إنبات
الجراثيم الكونيدية للفطر كلادوسبوريوم ككمارينوم. وقد نجح استخدام
الكائنات الحيوية من تقليل و خفض حدوث الإصابة بمرض تبقع الاوراق
الكلاوسبوريومي بشكل كبير، وتنبت تقدم المرض عند استخدامها بشكل
فردى أو كخليط تحت ظروف الإصابة الحقلية. ساعدت المعاملات الحيوية
بشكل عام على تحسين نباتات البطيخ وزادت بشكل كبير من ارتفاع النبات
وعدد الافرع وأعداد الثمار ووزن الثمار سواء ما تم معالجتها بالمعاملات
الحيوية المختبرة إما بصورة فردية أو فى خليط مقارنة بالكنترول.