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Bio-Control of Chamomile Powdery Mildew Using Cyanobacteria and Some Antagonistic Microorganisms

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Field experiments were carried out under natural infection in the Experimental Farm of Sids Agricultural Research Station, Agric. Res. Center, Beni-Sweif governorate to evaluate the potentiality of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and cyanobacteria (*Spirulina platensis*) alone or in mixture to control powdery mildew on chamomile and its productivity using two application methods: soaking the seedling roots before transplanting + foliar spray and foliar spray only. Generally, all the tested bioagents were effective in reduction the disease incidence and severity leading to a significant increase in fresh and dry weights of the blossoms as well as essential oil percent and oil yield compared with the control treatment. Soaking the chamomile seedling roots and spraying the leaves with suspensions for bioagents tested were the most effective in reducing the disease incidence and severity than spraying the leaves by the suspensions only. Mixing the cyanobacteria (*S. platensis*) with the fungal and bacterial antagonistics was more effective compared to each treatment separately. The highest efficacy was obtained from plots received mixed treatment of *S. platensis* + *P. fluorescens* in addition to the fungicide Topas 100 EC. Furthermore, all the treatments showed significant increases in the defence-related enzymes, peroxidase (PO) and polyphenoloxidase (PPO) as compared with the untreated control. *Spirulina platensis* significantly enhanced the growth of *T. harzianum* and did not show any inhibitory effect against bacterial growth as well as significantly increased the number of bacterial colony forming units (cfu) of *B. subtilis* and *P. fluorescens* which significantly improved the microbial status in the rhizosphere of chamomile plants.

Keywords: Powdery mildew, cyanobacteria, bioagents, defence-related enzymes, peroxidase, polyphenoloxidase, productivity and chamomile.

German chamomile (*Matricaria chamomilla* L.) belonging to the Asteraceae family is one of the most commonly used medicinal plants all over the world (Mohammad, 2011). Nowadays, it is highly favored and widely used in the folk and traditional medicine (Srivastava *et al.*, 2010 and Gosztola, 2012). One of the main

reasons for the pharmaceutical characters of chamomile is related to different classes of active constituents, including the essential oil (Szoke *et al*. 2004). The plant contains 0.24-1.9 % essential oil, from which over 120 secondary metabolites have been identified, such as chamazulene, (−)-α-bisabolol, apigenin and luteolin and many of these are pharmacologically active. Some of these compounds in the essential oil and the extracts are also used in perfumery and flavoring. The flower extract and essential oil possess anti-inflammatory, spasmolytic, carminative, antiseptic, sedative and ulcer protecting properties (Newall *et al.*, 1996; Smitherman *et al.*, 2005; Zaidi *et al.*, 2012; Šavikin *et al.*, 2013 and Zucchi *et al.*, 2013).

Powdery mildew caused by *Erysiphe cichoracearum* is considered one of the most important diseases attacking chamomile plant in different cultivated areas in Egypt. Chamomile powdery mildew frequently occurs in the open field, estimated percentages of infection reach 20-80% (El-Morsy and Shalaby, 2013). In severe infection, diseased plant seems as covered with a layer of talk powder and this causes great damage especially for flowers which are the important part of the plant, so the disease has negative effect on the quantity and quality of inflorescences yield, so all the possible procedures should be considered to manage it. The use of fungicides against plant diseases causes several problems such as carcinogenicity, development of fungicidal resistance populations of the pathogen and phytotoxicity as well as adverse effects of environmental balance (Pimentel *et al.*, 1992 and Chen *et al.*, 2007). Recently, increasing concerns for the production of chemical-free medicinal and aromatic plants has been the main goal of many researchers to find out environmentally safe strategies to control plant diseases in order to ensure the high quality and safety of the product. Thus, it is urgent to apply alternative safe efficient methods against plant diseases. Biological control received most of the attention because of their multiple modes of action to protect plants and their potential to be incorporated in integrated programs of management (Shoda, 2000 and Paulitz & Bélanger, 2001). Powdery mildew has been successfully controlled by using algal, fungal and bacterial antagonists as reported by a number of researchers under greenhouse and field trials (Singh *et al*., 2000; El-Gamal, 2003; Deore *et al.*, 2004 and Hegazi & El-Kot, 2010). *Bacillus subtilis*, *P. fluorescens* and *T. harzianum* are a promising biocontrol agents provide protection or prevention against plant pathogens by competition for nutrients and space, antibiosis, production of lytic enzymes and induced host resistance through increased activity of many enzymes such as peroxidase and polyphenoloxidase which play a defense role against invading pathogens (Kohl and Fokkema, 1998; Hegazi & El-Kot, 2010 and Roberti *et al.*, 2016). In addition, they can produce some compounds which may act as plant growth promoters (Compant *et al.*, 2005). Algae are one of the most potential biocontrol agents that have been studied for the control of plant pathogenic fungi (Hewedy *et al*., 2000). Cyanobacteria (blue-green algae) are able to produce a large variety of active secondary metabolites such as antibiotics, antifungal compounds and toxic activity and fix nitrogen (Skulberg, 2000). Abedin and Taha (2008) found

that *S. platensis* had antifungal activity towards the plant pathogenic fungi, increased growth parameters and yield in plants (Shalaby and El Ramady, 2014 and Jufri *et al.*, 2016). The plant growth and essential oil content are highly affected by plant microbiome.

The present study was undertaken to evaluate the potential of *B. subtilis*, *P. fluorescens*, *T. harzianum* and cyanobacteria (*Spirulina platensis*) alone or in mixture to control powdery mildew on chamomile and its effect on the productivity using two application methods: seedling roots soaking and/or foliar spray under field conditions.

Materials and Methods

Bioagents used in experiments:

In experiments; four bio agents namely *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and cyanobacteria (*Spirulina platensis*) were kindly obtained from Department of Microbiology, Soil, Water & Environment Res. Inst., ARC, Giza, Egypt and used alone or in mixtures to evaluate their potential on *E. cichoracearum* spore germination, the effect of cyanobacteria (*S. platensis*) on the growth of the tested fungal and bacterial bioagents *in vitro* conditions as well as to control powdery mildew on chamomile and its effect on the productivity using two application methods: seedling roots soaking and/or foliar spray under field conditions.

Bacterial suspension was multiplied by cultivating *B. subtilis* in nutrient broth medium, while *P. fluorescens* in 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 28±2ºC for 48 h. *T. harzianum* was grown in *Trichoderma*-selective medium broth (TSM) (Elad *et al*., 1981), amended with 300 mg/l streptomycin and 50 µg ml^{-1} rose Bengal in conical flask, incubated at 25^{o}C for 15 days. A cyanobacteria (*S. platensis*) was grown in Zarrouk medium for 1 month (Zarrouk, 1966) and applied at the rate of 50L/fed.

In vitro assay:

Effect of bioagents on conidial germination:

Drops of the tested bioagent suspension either alone or in mixture were placed on glass slides and conidia of *E. cichoracearum* were directly lifted with the help of small paint brush from heavily infected chamomile leaves. The slides were then placed in moist chambers prepared by placing two moist filter papers in the inner surfaces of a Petri plate. Conidia immersed in distilled water as well as fungicide suspension (Topas 100 EC) were served as the control. Three replications were made for each treatment. The slides were incubated at 25±2°C for 24 h and the percent of germination was calculated under a light microscope (Abd-Alla, 2012).

Effect of S. platensis on T. harzianum growth:

Dry *S. platensis* (0.03 g) was aseptically added to 100 ml conical flasks containing 95 ml sterilized PDA medium before solidifying and rotated gently to ensure equal distribution. Sterilized distilled water was added to flask to bring the total volume to 100 ml. Five milliliters of sterilized distilled water were added aseptically to the control flasks. The supplemented media were poured into sterilized Petri dishes (9 cm diam.) approximately three plates, each containing 20 ml of medium and then inoculated in the centre with a 5-mm diameter mycelial disc of *T. harzianum* taken from the margin of 5-10 day old culture. The plates were incubated at 25^{o}C in the dark. Fungal growth was recorded after the growth of any treatment reached the edge of the plate and the results expressed as the percentage of reduction or increase of radial growth in relation to the control.

Effect of S. platensis on bacterial growth:

Zone of growth inhibition technique was adopted according to Allen (1961). Conical flasks each containing 150 ml of warm sterilized nutrient or King B agar medium were artificially seeded before solidification with 5 ml of a 48 h old nutrient or King B broth of the tested bacteria, then poured into Petri dishes (three replicates). Wells (5 mm-diam.) were made up into solidified medium using sterilized cork borer. Equal volume (0.1 ml) of the tested cyanobacteria was poured into each well. The same technique was also followed for control (without treatment). All Petri dishes were incubated at 25^{o}C for 72 h then examined. Clear zone diameter of bacterial growth inhibition was measured and the inhibited growth area was measured.

The same plate count technique of Allen (1961) was used to determine the lethal dose of the tested cyanobacteria on bacteria. Amount of 0.03 g of the tested cyanobacteria was added to conical flasks containing sterilized nutrient or King B broth medium. Prepared flasks were individually inoculated with 1.0 ml of the bacterial growth on the same medium for 48 h then incubated for 72 h at 25^{o}C. Serial dilutions (10^{-1} to 10^{-7}), of each bacterial growth, were prepared. A volume of 1.0 ml of final dilution of bacteria was poured into each Petri dish with 20 ml of the corresponding agar medium. Petri dishes were then swirled gently to ensure an even distribution of bacteria into the medium. Three plates were used as replicates for each particular treatment and control as well. All plates were incubated at 25^{o}C for 48 h then examined. Bacterial colonies were counted and the number of colony forming unites (cfu) per 1.0 ml of bacterial suspension was calculated.

Field experiments:

A two-year field experiment was achieved under natural infection in the Experimental Farm of Sids Agricultural Research Station, Agric. Res. Center, Beni-Sweif governorate during the two growing seasons of 2017 and 2018 to evaluate the potential of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and cyanobacteria (*Spirulina platensis*) alone or in mixture to control powdery

mildew on chamomile and its effect on the productivity using two application methods: seedling roots soaking and/or foliar spray. 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 experimental plot was 6×3.5 m^2.

Chamomile seedling roots were soaked in the suspensions of each of *Trichoderma* isolate ($1x10^6$ conidia/ml), *B. subtilis*, *P. fluorescens* (3×10^8 cfu) and *S. platensis* for 2 h before transplanting. The Chamomile seedlings were obtained from Sids Agricultural Research Station, Agric. Res. Center, Beni-Sweif governorate

In each season, the soil was mechanically plowed and planked twice. During the preparation for cultivation, calcium super-phosphate (15.5 % P_2O_5) as a source of phosphorus was added at the rate of 200 kg/fed. The treated and untreated seedlings, 15 cm length were transplanted at 30 cm spacing between plants on September, 15^{th} 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 sulphate (20.6 % N), at the rate of 400 kg/fed. (recommended rate) as follow: the first one (100 kg/fed.) was added after one month from transplanting and the remainder amounts were added after each harvest. Potassium sulfate (48 % K_2O) as a source of potassium was added at the rate of 75 kg/fed. The plants (40 days after transplanting) were sprayed every 10 days, always performed early in the morning, with the tested bioagents, 1% Tween 20 as well as the fungicide Topas 100 EC (Penconazole), Syngenta Co. at the rate of 25 cm^3/100L water before the appearance of first symptoms until run off. Monitoring and scouting the plants weekly for the appearance of powdery mildew and disease incidence and severity were estimated as follow:

Disease incidence:

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:

Disease severity was measured using a scale of (0-4) according to Whitney *et al.* (1983), in which 0, 1, 2, 3, and 4 approximated 0, 25, 50, 75 and 100 %, respectively, of the matured leaf area covered by mildew. Percentage of disease severity was recorded according to the following equation:

$$\text{Disease severity \%} = [\sum (n \times c)]/ (N \times C) \times 100$$

Whereas: n = Number of infected leaves, c = Category number, N = Total number of examined leaves and C = The highest category number of infection.

The blossoms of the chamomile plant were harvested after 3 months from transplanting. The harvest was carried out every month and 4 harvests were collected during the season. The fresh and dry weights of blossoms were evaluated in all the treatments.

To determine the percentage of essential oil, 100g dry blossoms representing each replicate were taken from the four harvests then subjected to steam distillation and determined according to Guenther (1961) and oil yield (kg/fed.) was determined.

Peroxidase activity was determined using the method described in the Worthington enzyme manual (Worthington, 1971). Polyphenoloxidase activity was measured following the method described by (Esterbaner *et al.*, 1977). Analysis of enzymes was carried out at the Mycology and Disease Survey Res. Dept., Plant Pathol. Res. Inst., ARC.

Plant hormones (abscisic acid, gibberellic acid and indole acetic acid) were determined according to the method described by Shindy and Smith (1975). Carbon dioxide (CO_2) evolution was determined according to Gaur *et al*. (1971) at the Soil, Water & Environment Res. Inst., ARC.

Statistical analysis:

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

Results

Results in Table, 1 show that reduction in spore germination percent was significantly noticed with all bioagents tested each alone or in mixture and the highest reduction was exhibited in case of fungicide Topas 100 EC and *S. platensis* + *P. fluorescens* treatments without significant differences between them, being 94.5 % reduction in spore germination followed by *S. platensis* + *B. subtilis* and *S. platensis* + *T. harzianum* treatments with averages of 92.6 and 89.4 %, respectively. Moreover, moderate reduction was observed with the bioagents *S. platensis*, *P. fluorescens* and *B. subtilis* each alone. The bioagent *T. harzianum* showed the lowest inhibitory effect towards spore germination.

Data presented in Table, 2 show the effect of *S. platensis* on the viability of both bacteria and growth of *T. harzianum*. *S. platensis* significantly enhanced the growth of *T. harzianum* and increased the colony diameter by 136.8 % compared to the untreated control. On the other hand, *S. platensis* did not show any inhibitory effect against bacterial growth (Fig. 1). The numbers of bacterial colony forming units (cfu) were significantly increased by 67.0 % for *B. subtilis* and 83.5 % for *P.*

fluorescens when these bacteria were grown on each respective broth medium amended with *S. platensis.*

Table 1: Effect of different bioagents on spore germination of *Erysiphe cichoracearum in vitro*.

Treatments	Germination %	Reduction* %
B. subtilis	9.6	69.0
P. fluorescens	7.0	77.4
S. platensis	5.6	81.9
T. harzianum	12.3	60.3
S. platensis + *B. subtilis*	2.3	92.6
S. platensis + *P. fluorescens*	1.7	94.5
S. platensis + *T. harzianum*	3.3	89.4
Topas 100 EC	1.7	94.5
Control	31.0	---
L.S.D. at 0.05	1.6	---

* Reduction % related to the control.

Table 2: Effect of *S. platensis* on linear growth of *T. harzianum* and viability of *B. subtilis* and *P. fluorescens in vitro.*

Treatments	Mycelial linear growth		Total counts of viable cells			
	T. harzianum		*B. subtilis*		*P. fluorescens*	
	Colony diameter (mm)	Increase* (%)	Total counts (10^7 cfu/ml)	Increase* (%)	Total counts (10^7 cfu/ml)	Increase* (%)
S. platensis	90.0	136.8	811.5	67.0	790.3	83.5
Control	38.0	---	485.8	---	430.6	---
L.S.D. at 0.05	2.3	---	77.0	---	79.5	---

* Increase % related to the control.

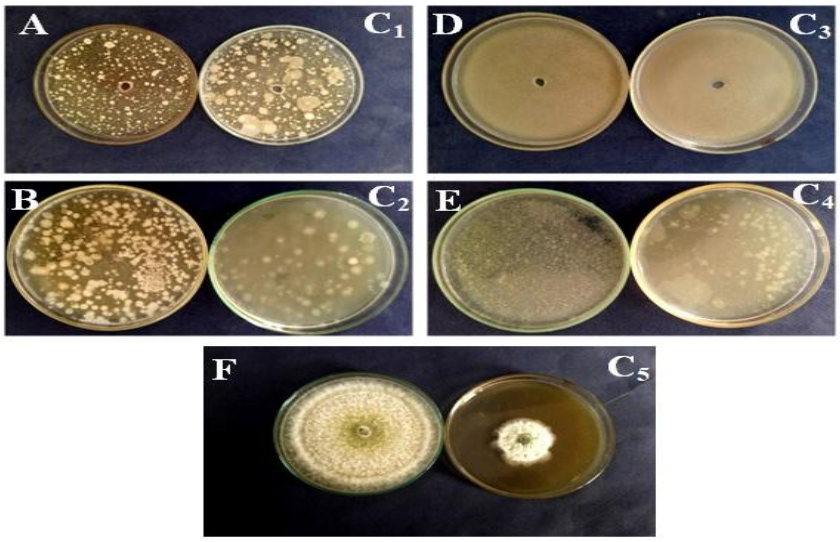

Fig. 1: Effect of *S. platensis* on inhibition zone (A), Total counts of viable cells (B) of *B. subtilis*; inhibition zone (D), Total counts of viable cells (E) of *P. fluorescens* and growth of *T. harzianum* (F) compared with the untreated control (C_1, C_2 for *B. subtilis*; C_3, C_4 for *P. fluorescens*; C_5 for *T. harzianum*).

Data presented in Tables, 3 and 4 demonstrate that all the tested bioagents significantly reduced disease incidence and delayed the progress of chamomile powdery mildew when used individually or as mixtures compared with the untreated control during the two growing seasons under field conditions. In season 2017, the lowest disease incidence and severity, being 21.1 and 9.0 %, respectively were noticed in plots received the mixed treatment of *S. platensis* + *P. fluorescens* which statistically on a par with the fungicide Topas 100 EC (18.2 and 6.9 %, respectively). Treatment *B. subtilis* mixed with cyanobacteria (*S. platensis*) came in the second rank followed by *S. platensis* + *T. harzianum* treatment. The corresponding mean values of powdery mildew incidence in season 2017 were 35.4 and 45.9 % and disease severity, 21.1 and 26.6 %, respectively. Moderate disease incidence and severity were observed in plots received *S. platensis* and *P. fluorescens* applied each alone followed by *B. subtilis* treatments, being 54.3, 57.5 and 71.9 %, respectively for disease incidence and 32.3, 35.2 and 39.6 %, respectively for disease severity during the season of 2017. Meanwhile, application of *T. harzianum* gave the highest disease severity compared with the bioagents tested. The same trend was noticed in the second growing season. In general, the effectiveness of the tested bioagents significantly varied according to the application method. Soaking the chamomile seedling roots and spraying the leaves with

bioagents suspensions was the most effective application method in reducing the disease incidence and severity than spraying the leaves by suspensions only.

The interaction between the tested bioagents and their application methods was significant during the two successive growing seasons. Application of the fungicide as seedling treatment and foliar spray was the most efficient treatment, maintaining powdery mildew incidence and severity at especially low levels (16.5 and 5.3 %, respectively) in the first growing season (2017) followed by treatment of soaking seedling roots and foliar spray with *S. platensis* + *P. fluorescens* (18.7 and 6.9 %, respectively) without significant differences between them. Meanwhile, the lowest efficient was *T. harzianum* treatment when applied as foliar spray only. The same trend was observed in the second growing season (2018).

Table 3: Effect of some biocontrol agents and the fungicide Topas 100 EC on the incidence of powdery mildew on chamomile grown under field conditions during the two successive growing seasons 2017 and 2018.

Treatments (T)	Disease incidence %					
	Application methods (M)					
	Season of 2017			Season of 2018		
	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)
B. subtilis	67.8	76.0	71.9	62.6	71.3	67.0
P. fluorescens	53.4	61.6	57.5	49.0	53.5	51.3
S. platensis	50.2	58.3	54.3	42.5	50.0	46.3
T. harzianum	82.9	95.7	89.3	81.3	92.0	86.7
S. platensis + *B. subtilis*	31.0	39.8	35.4	25.2	35.4	30.3
S. platensis + *P. fluorescens*	18.7	23.4	21.1	11.4	18.6	15.0
S. platensis + *T. harzianum*	40.5	51.2	45.9	33.6	48.6	41.1
Topas 100 EC	16.5	19.8	18.2	7.7	15.2	11.5
Control	100.0	100.0	100.0	100.0	100.0	100.0
Mean (M)	51.2	58.4	---	45.9	53.8	---
L.S.D. at 0.05	T= 3.5 M= 1.7 TM= 5.0			T= 2.9 M= 1.4 TM= 4.2		

Table 4: Effect of some biocontrol agents and the fungicide Topas 100 EC on the severity of powdery mildew on chamomile grown under field conditions during the two successive growing seasons 2017 and 2018.

Treatments (T)	Disease severity %					
	Application methods (M)					
	Season of 2017			Season of 2018		
	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)
B. subtilis	37.1	42.0	39.6	35.3	40.5	37.9
P. fluorescens	32.6	37.8	35.2	30.0	37.0	33.5
S. platensis	30.0	34.6	32.3	27.6	32.1	29.9
T. harzianum	54.5	64.1	59.3	53.3	63.3	58.3
S. platensis + *B. subtilis*	17.8	24.3	21.1	14.3	22.3	18.3
S. platensis + *P. fluorescens*	6.9	11.1	9.0	3.2	9.7	6.5
S. platensis + *T. harzianum*	23.5	29.7	26.6	20.5	27.2	23.9
Topas 100 EC	5.3	8.5	6.9	2.6	5.9	4.3
Control	87.0	88.6	87.8	87.6	87.6	87.6
Mean (M)	32.7	37.9	---	30.5	36.2	---
L.S.D. at 0.05	T= 2.2 M= 1.0 TM= 3.1			T= 2.4 M= 1.1 TM= 3.4		

Results show a general improvement in both vegetative and blossoms yield of chamomile. Fresh and dry weights of chamomile blossoms (Tables, 5 and 6) as well as oil percent and oil yield were influenced by the tested bioagent (Tables, 7 and 8). In 2017 growing season, the combination between *S. platensis* and *P. fluorescens* showed the highest blossoms fresh and dry weights, oil percentage and oil yield, being 3.67 ton/fed., 731.1 kg/fed., 0.60 % and 4.4 kg/fed., respectively followed by plots received a mixture treatment of *S. platensis* + *B. subtilis*. On the other hand, the lowest blossoms fresh and dry weights, oil percentage and oil yield were obtained from plots received *T. harzianum*, being 2.89 ton/fed.; 557.1 kg/fed., 0.24 % and 1.3 kg/fed., respectively. The fresh and dry weight, oil % and oil yield values were 2.78 ton/fed.; 429.3 kg/fed., 0.21 % and 0.9 kg/fed., respectively in control

plants. The same trend was also true for 2018 growing season. Overall, the improvement in both vegetative and blossoms yield of chamomile was significant regarding roots soaking + spraying the leaves with the tested bioagents method over foliar spray method only.

Table 5: Effect of some biocontrol agents and the fungicide Topas 100 EC on fresh weight of chamomile blossoms (ton/fed.) grown under field conditions during the two successive growing seasons 2017 and 2018.

Treatments (T)	Fresh weight of blossoms (ton/fed.)					
	Application methods (M)					
	Season of 2017			Season of 2018		
	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)
B. subtilis	3.18	2.95	3.07	3.29	3.06	3.18
P. fluorescens	3.20	3.00	3.10	3.35	3.10	3.23
S. platensis	3.45	3.27	3.36	3.69	3.36	3.53
T. harzianum	3.03	2.75	2.89	3.08	2.89	2.99
S. platensis + *B. subtilis*	3.69	3.47	3.58	3.85	3.49	3.67
S. platensis + *P. fluorescens*	3.82	3.52	3.67	3.95	3.58	3.77
S. platensis + *T. harzianum*	3.54	3.30	3.42	3.81	3.35	3.58
Topas 100 EC	3.16	2.84	3.00	3.12	2.90	3.01
Control	2.81	2.74	2.78	2.53	2.64	2.59
Mean (M)	3.32	3.09	---	3.41	3.15	---
L.S.D. at 0.05	T= 0.17 M= 0.08 TM= 0.24			T= 0.21 M= 0.10 TM= 0.29		

The interaction between bioagents tested and application methods had significant effect on fresh and dry weights of chamomile blossoms as well as oil percentage and oil yields. In 2017 growing season, the maximum values of these parameters were obtained from plots received chamomile seedling roots previously soaked in a mixture of *S. platensis* + *P. fluorescens* and spraying the leaves with their suspensions (3.82 ton/fed.; 779.3 kg/fed., 0.62 % and 4.8 kg/fed., respectively),

while the lowest blossoms fresh and dry weights, oil % and oil yields (2.75 ton/fed.; 534.9 kg/fed., 0.22 % and 1.2 kg/fed., respectively) was obtained from plots received *T. harzianum* as foliar spray only. The same trend was observed in the second season (2018).

Table 6: Effect of some biocontrol agents and the fungicide Topas 100 EC on dry weight of chamomile blossoms (kg/fed.) grown under field conditions during the two successive growing seasons 2017 and 2018.

Treatments (T)	Dry weight of blossoms (kg/fed.)					
	Application methods (M)					
	Season of 2017			Season of 2018		
	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)
B. subtilis	643.4	588.5	616.0	653.0	603.7	628.4
P. fluorescens	655.6	591.0	623.3	665.9	611.8	638.9
S. platensis	703.8	624.4	664.1	715.9	631.8	673.9
T. harzianum	579.3	534.9	557.1	589.6	541.5	565.6
S. platensis + *B. subtilis*	749.1	635.4	692.3	770.0	695.6	732.8
S. platensis + *P. fluorescens*	779.3	682.9	731.1	790.3	716.0	753.2
S. platensis + *T. harzianum*	718.6	643.8	681.2	756.8	644.3	700.6
Topas 100 EC	590.5	544.4	567.5	620.7	554.6	587.7
Control	428.6	430.0	429.3	444.5	428.6	436.6
Mean (M)	649.8	586.1	---	667.4	603.1	---
L.S.D. at 0.05	T= 13.5 M= 6.4 TM= 19.1			T= 11.2 M= 5.3 TM= 15.9		

Table 7: Effect of some biocontrol agents and the fungicide Topas 100 EC on oil % of chamomile blossoms grown under field conditions during the two successive growing seasons 2017 and 2018.

Treatments (T)	oil %					
	Application methods (M)					
	Season of 2017			Season of 2018		
	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)
B. subtilis	0.30	0.26	0.28	0.31	0.27	0.29
P. fluorescens	0.32	0.30	0.31	0.32	0.32	0.32
S. platensis	0.34	0.32	0.33	0.36	0.31	0.34
T. harzianum	0.25	0.22	0.24	0.24	0.22	0.23
S. platensis + *B. subtilis*	0.58	0.40	0.49	0.57	0.42	0.50
S. platensis + *P. fluorescens*	0.62	0.58	0.60	0.62	0.57	0.60
S. platensis + *T. harzianum*	0.36	0.34	0.35	0.37	0.34	0.36
Topas 100 EC	0.28	0.25	0.27	0.29	0.27	0.28
Control	0.21	0.20	0.21	0.22	0.22	0.22
Mean (M)	0.36	0.32	---	0.37	0.33	---
L.S.D. at 0.05	T= 0.01	M= 0.01	TM= 0.02	T= 0.01	M= 0.01	TM= 0.02

Table 8: Effect of some biocontrol agents and the fungicide Topas 100 EC on oil yield (kg/fed.) of chamomile grown under field conditions during the two successive growing seasons 2017 and 2018.

Treatments (T)	Oil yield (kg/fed)					
	Application methods (M)					
	Season of 2017			Season of 2018		
	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)
B. subtilis	1.9	1.5	1.7	2.0	1.6	1.8
P. fluorescens	2.1	1.8	2.0	2.1	2.0	2.1
S. platensis	2.4	2.0	2.2	2.6	2.0	2.3
T. harzianum	1.4	1.2	1.3	1.4	1.2	1.3
S. platensis + *B. subtilis*	4.3	2.5	3.4	4.4	2.9	3.7
S. platensis + *P. fluorescens*	4.8	4.0	4.4	4.9	4.1	4.5
S. platensis + *T. harzianum*	2.6	2.2	2.4	2.8	2.2	2.5
Topas 100 EC	1.7	1.4	1.6	1.8	1.5	1.7
Control	0.9	0.9	0.9	1.0	0.9	1.0
Mean (M)	2.5	1.9	---	2.6	2.0	---
L.S.D. at 0.05	T= 0.2 M= 0.1 TM= 0.3			T= 0.3 M= 0.1 TM= 0.4		

Data presented in Table, 9 show the effect of the tested bioagents on the activity of defense related enzymes in the treated chamomile plants compared with the control. Overall, all tested bioagents and fungicide Topas 100 EC significantly increased the activity of defense related enzymes. Mixing the fungal and bacterial antagonistic agents with the cyanobacteria (*S. platensis*) resulted excess in the activity for both peroxidase and polyphenoloxidase enzymes compared to the treatment separately. Maximum increase in peroxidase and polyphenoloxidase activities was detected due to using *S. platensis* mixed with *P. fluorescens*, being 1.528 and 0.144, respectively followed by treatments of *S. platensis* mixed with *B. subtilis* and *T. harzianum*. The lowest activity of these enzymes was detected in chamomile plants treated with *T. harzianum*. Soaking the chamomile seedling roots and spraying the leaves with bioagents suspensions significantly increased the activities of peroxidase and polyphenoloxidase compared to spraying the leaves by

any suspension only. Bioagents tested and application method interaction was found to be significant. Soaking the chamomile seedling roots in a mixture of *S. platensis* + *P. fluorescens* and spraying the leaves with their suspensions showed the highest peroxidase and polyphenoloxidase activities (1.536 and 0.195, respectively) while plants sprayed with *T. harzianum* only showed the lowest activities, being 0.585 and 0.051, respectively.

Table 9: Enzyme activities in chamomile plants treated by different bioagents.

Treatments (T)	Enzymatic activities					
	Application methods (M)					
	Peroxidase			Polyphenoloxidase		
	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)	Soaking seedling roots + foliar spray	Foliar spray	Mean (T)
B. subtilis	0.994	0.794	0.894	0.056	0.054	0.055
P. fluorescens	1.117	0.736	0.927	0.061	0.059	0.060
S. platensis	1.235	0.814	1.025	0.068	0.069	0.069
T. harzianum	0.610	0.585	0.598	0.052	0.051	0.052
S. platensis + *B. subtilis*	1.462	0.853	1.158	0.111	0.071	0.091
S. platensis + *P. fluorescens*	1.536	1.520	1.528	0.195	0.093	0.144
S. platensis + *T. harzianum*	1.238	0.830	1.034	0.084	0.073	0.079
Topas 100 EC	0.848	0.715	0.782	0.059	0.045	0.052
Control	0.392	0.285	0.339	0.043	0.033	0.038
Mean (M)	1.048	0.792	---	0.081	0.061	---
L.S.D. at 0.05	T= 0.06 M= 0.03 TM= 0.08			T= 0.005 M= 0.003 TM= 0.008		

Data presented in Table, 10 show the capabilities of the tested bioagents to produce phytohormones. In this regard, *S. platensis* produced gibberellic acid (GA3) and indole acetic acid (IAA) much higher than the other bioagents tested followed by *P. fluorescens*, *B. subtilis* and *T. harzianum*. On the other hand, *P. fluorescens* produced abscisic acid (ABA) higher than *B. subtilis* and *T. harzianum* while *S. platensis* showed no production of abscisic acid.

Table 10: 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)
B. subtilis	0.55	1.92	0.70
P. fluorescens	0.63	3.02	0.72
S. platensis	0.000	76.74	35.00
T. harzianum	0.34	1.50	0.68
L.S.D. at 0.05	0.05	0.18	0.08

It is clear that all treatments tested significantly improved the microbial status in the rhizosphere of chamomile plants as expressed as CO_2 evolution (Table, 11). Mixing the cyanobacteria (*S. platensis*) with the fungal and bacterial antagonistic agents significantly increased the CO_2 production compared to the treatment separately. In this regard, mixing *S. platensis* with *P. fluorescens* exhibited the superiority in rhizosphere microbial activity among all biological treatments which gave maximum value (737.0 mg/100g soil) followed by treatments of *S. platensis* mixed with *B. subtilis* and *T. harzianum* which produced 682.0 mg/100 g soil. While chemical fungicide treatment was the least among all treatments.

Table 11: Evolution of CO_2 in the rhizosphere of chamomile plant.

Treatments	CO_2 Mean of the two growing seasons
B. subtilis	572.0
P. fluorescens	605.0
S. platensis	627.0
T. harzianum	517.0
S. platensis + *B. subtilis*	682.0
S. platensis + *P. fluorescens*	737.0
S. platensis + *T. harzianum*	682.0
Topas 100 EC	379.0
Control	412.5
L.S.D. at 0.05	26.9

Discussion

Application of biological control using antagonistic microorganisms has proved to be successful for controlling various plant diseases (O'Brien, 2017). Results of the present study demonstrated that all the tested bioagents significantly caused different degrees of suppression of the causal pathogen of chamomile powdery mildew compared to the control. Mixed treatment between the cyanobacteria (*S. platensis*) and the bacteria (*P. fluorescens*) showed the highest efficacy in this concern. This result is in line with the report of Hussien *et al.* (2009) who found that application of *S. platensis* filtrate at concentration of 30 and 40 % completely inhibited mycelium growth and spore production of *Cercospora beticola*. They

concluded that the antifungal activity of the algal culture filtrates has been attributed to the presence of bioactive compounds, *i.e.* total phenolic compounds, total saponins, and alkaloids in the algal culture filtrates. The inhibition mechanisms are related to the disruption of the cell membrane integrity in spores and newly formed germ tubes. Florescent *Pseudomonas* is able to suppress diseases by producing protease, glucanase enzymes, in addition to enhancement the induced systemic resistance (Ko *et al*., 2009). *Bacillus subtilis* can inhibit fungal pathogens directly through the production of antifungal compounds from the iturin and fengycin families of lipopeptides which are able to repress spore germination (Bélanger *et al*., 1998 and Romero *et al*., 2007). Raaijmakers *et al*. (2002) concluded that the presence of bacterial cells in close relation to visibly collapsed conidia and hyphae suggests the local secretion of antifungal substances at sufficient concentrations to induce structural damage, resulting in the concomitant inhibition of spore germination and vegetative growth. Besides the direct inhibition, it also can induce resistance to foliar pathogens when applied to the plant root (Ongena *et al.*, 2005a, b). Such induction of enhanced defensive capacity can be systemic as seed-treatment with bacteria at the time of seeding was shown to trigger protective effects on aboveground parts (Van Loon *et al.*, 1998). *Bacillus* sp. has a great ability to colonize the rhizosphere of plants due to several physiological properties including its multilayered cell wall, formation of stress resistant endospore and antibiotic secretion provide these species with high advantage to survive for long periods of time under various environmental conditions. Application of *T. harzianum* T39 to the soil instead of spray reduced the powdery mildew coverage on the leaves by 75 to 90 % (Elad *et al.*, 1998). The mode of action was mainly the induced resistance. Disease reduction was accompanied with a gradual increase in peroxidase and polyphenoloxidase activity during the experimental period. In the present study, soaking the chamomile seedling roots and spraying the leaves with the tested bioagent suspension significantly induced synthesis and accumulation of PO, PPO than spraying the leaves by the suspensions alone. Generally, mixing the bioagents especially when *S. platensis* was mixed with *P. fluorescens* resulted excess in the activity for both enzymes compared to any treatment separately. This result is in line with Anand *et al.* (2007) and El-Ghanam *et al.* (2015) who recorded that a maximum increase in peroxidase and polyphenoloxidase activity was detected with *S. platensis* and *P. fluorescens*, resulted in remarkable increase in disease reduction. Polyphenoloxidase is the main enzyme that can oxidize the phenolic substance to quinoid substance, which gives effect to sterilize or inhibit the reproduction of pathogen (Handelsman & Stabb, 1996 and Hammerschmidt, 1999). Peroxidase is a key enzyme in the biosynthesis of lignin and other oxidized phenols. It catalyzes the oxidation of hydroxy cinnamyl alcohols into free radical intermediates, which subsequently are coupled into lignin polymers. Besides, peroxidase itself was found to inhibit the spore germination and mycelial growth of certain fungi (Joseph *et al.*, 1998 and Anand *et al.*, 2007).

Results of this study showed a general improvement in both vegetative and blossoms yield of chamomile as well as oil yield. These results are in the same direction with those of Aly *et al.* (2008) and Ali and Mostafa (2009). This increase could be attributed to the fact that *S. platensis* contains protein, 62 % amino acid, minerals and it contains also the whole spectrum of mixed natural carotene and xanthophyl phytopigments which are considered as a rich natural source of vitamin B-12, phytohormones and antioxidants (Kemka *et al.*, 2007). Chemical analysis of *S. platensis* as bio-stimulator revealed that it contains 6.7% N, 2.47% P and 2.14% K as well as adequate amounts of micro elements needed for plant nutrition (Aly and Esawy, 2008). In addition, the available form of ammonia necessary for plant growth can be supplied by most cyanobacteria which can fix the atmospheric nitrogen into an available form of ammonia and thus can be used as bio-fertilizer (Vaishampayan *et al.*, 2001). *Pseudomonas* spp. affect plant growth directly by producing and releasing secondary metabolites (plant growth regulators, phytohormones and biologically active substances), facilitating the availability and uptake of certain nutrients from the root environment. *Pseudomonas fluorescens* produces higher amounts of gibberellic acid (GA3) which is responsible for stem elongation and flowering process; mild amounts of indole acetic acid (IAA) which has an important role in the cell elongation and Abscesic acid (ABA) which has an important role in the adaptation of plants under stress conditions such as salinity, drought and low temperature (Bano *et al.*, 2016). This could explain the positive impact of *P. fluorescens* and algae on the fresh and dry blossom yield of chamomile. *Bacillus* sp. has been reported to have mechanisms to promote plant growth by phytohormones production, mineralization and mobilization of phosphorus, siderophore production (Gutierrez-Manero *et al.*, 2001; Whipps, 2001; Idris *et al.*, 2007 and Richardson *et al.*, 2009). The study demonstrated that all treatments tested significantly improved the microbial status in rhizosphere of chamomile plants. Mixing the cyanobacteria (*S. platensis*) with the fungal and bacterial antagonistic agents significantly increased the CO_2 production compared to any treatment separately. In this regard, mixing *S. platensis* with *P. fluorescens* exhibited the superiority in rhizosphere microbial activity among all biological treatments which gave maximum value of CO_2 production followed by treatments of *S. platensis* mixed with *B. subtilis* and *T. harzianum*. While chemical fungicide treatment was the least among all treatments. This could be attributed to the harmful effect of chemical fungicide to other living organisms and reduce useful soil microorganisms as reported by Khalifa *et al.* (1995) and Lewis *et al.*, (1996). This finding, is in agreement with those reported by Mahmoud *et al.* (2007) and El-Gabry *et al.* (2015). *Spirulina platensis* significantly enhanced the growth of *T. harzianum* and did not show any inhibitory effect against bacterial growth as well as significantly increased the numbers of bacterial colony forming units (cfu) of *B. subtilis* and *P. fluorescens* as noticed in the present study. This indicated that cyanobacteria (*S. platensis*) are capable of increasing the population of the fungal and bacterial antagonistic agents and hence improve the microbial status in rhizosphere and phylloplane of plants as

expressed by increasing the CO_2 production and consequently, increasing the bioagent capability of these antagonistic microorganisms. Our results coincide with those obtained with El-Mougy and Abdel-Kader (2013). However, strain of *P. fluorescens* which is the more active plant growth promoting rhizobacteria in their high energy of metabolism reflecting the high productivity of CO_2 in the rhizosphere. The production of CO_2 led to formation of carbonic acid that decreases the soil pH value at rhizosphere. This process had done by rhizosphere microorganism's which act to increase nutrient uptake and availability of the nutrient in the rhizosphere plant area which in turn supported higher plant growth and yield. These results are in agreement with those reported by Seied *et al.* (2013) who indicated that the high CO_2 production rates reflect the high energy of metabolism.

Conclusion

Cyanobacteria (*S. platensis*) and bacteria (*P. fluorescens*) showed a good potential to control powdery mildew of chamomile and improve the growth and yield of blossoms as well as oil yield. Therefore, it is recommended to use of soaking the seedling roots and foliar spraying with *S. platensis* mixed with *P. fluorescens* as safe biocontrol agents compared with the fungicide for controlling powdery mildew of chamomile and increasing its productivity under field conditions.

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المقاومة الحيوية لمرض البياض الدقيقى فى البابونج باستخدام الطحالب الخضراء المزرقه (Cyanobacteria) وبعض الكائنات الحية الدقيقة المضادة

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تم اجراء تجارب فى الحقل تحت ظروف العدوى الطبيعية بمرض البياض الدقيقى فى البابونج فى المزرعة البحثية بمحطة البحوث الزراعية بسدس-مركز البحوث الزراعية - محافظة بنى سويف من اجل تقييم فعالية بكتيريا *Bacillus subtilis* ، *Pseudomonas fluorescens* ، فطر *Trichoderma harzianum* وطحلب *Spirulina platensis* اما بمفردها او مختلطة فى مقاومة البياض الدقيقى وانتاجية نباتات البابونج بتطبيق طريقتين (نقع جذور الشتلات مع رش الاوراق أو رش الاوراق فقط). عموما، كل الكائنات الحية المختبرة كان لها تأثير ايجابى على الحد من نسبة الأصابة وشدة المرض مع زيادة معنوية فى الوزن الطازج والجاف للأزهار ونسبة ومحصول الزيت فى نهاية التجربة.ايضا وبالمقارنه بمعامله الكنترول وجد أن نقع جذور شتلات البابونج مع رش المجموع الخضرى بمعلق الكائنات الحية كان أكثر فعالية فى تقليل نسبة وشدة الأصابة مقارنة بمعاملة رش الأوراق فقط. لوحظ أن خلط طحلب *Spirulina platensis* مع البكتريا والفطر كان اكثر فعالية من استخدام المعاملات بمفردها. وكان أعلى تأثير فى القطعة التجريبيه التى تم معاملتها بخليط من *S. platensis + P. fluorescens* بالأضافة الى المبيد الفطري توباس. علاوة على ذلك, اظهرت كل المعاملات زيادة معنوية فى انزيمات المقاومة مثل انزيمات بيروكسيديز وبولى فينول أوكسيديز بالمقارنة مع نباتات البابونج الغير معاملة. ايضا وجد ان طحلب *Spirulina platensis* عمل على زيادة نمو فطر *Trichoderma harzianum* ونمو البكتريا المختبرة معنويا ولم يظهر اى تأثير مثبط لها وبالتالى تحسين الحالة الميكروبية فى محيط جذور نباتات البابونج.