P-ISSN: 3009 – 7487
E-ISSN: 3009 – 7886

JOURNAL OF PLANT AND FOOD SCIENCES December, 2023

Biological control of powdery mildew on chamomile plants

Mergawy, M.M.

Central lab. of Organic Agriculture, Agricultural Research Center (ARC), Giza, Egypt.
Madianamr18@yahoo.com https://orcid.org/0000-0002-4555-6219
***Corresponding author:** Mergawy, M.M.

Received: 10 November 2023; in revised form: 19 November 2023 / Accepted: 3 December 2023 / Published: 30 December 2023
Doi 10.21608/JPFS.2023.259281.1004

ABSTRACT

Chamomile is considered one of the most medicinal and aromatic plants in Egypt and the world. Powdery mildew is among the most important diseases which attack chamomile in the different cultivation areas. Powdery mildew started to appear during November with very low disease severity. The peaks of disease incidence and severity were noticed during February and March. The fungal pathogen of this disease as was identified (*Sphaerotheca fuliginea*). Regarding bio control agents, the highest effect in controlling powdery mildew was recorded when Blight stop or *T. hamatum* was used. Indicate that the lowest percentages of powdery mildew incidence or severity were recorded when *T. hamatum* at concentration 20 ml/liter of water, or Blight stop at 10 ml / liter of water was used. These low percentages in disease severities were correlated with the highest yield in plant inflorescences and obtained yield reached 0.833 and 0.845 tons/feddan when *T. hamatum* at concentration 20ml/liter of water, or Blight stop at recommended dose, respectively. Also, the highest plant height (cm), number of branches/plant, fresh and dry 100 inflorescences were obtained when *T. hamatum* was used, the highest essential oil content was recorded when *T. hamatum* or Blight stop were used. On the other hand, powdery mildew disease incidence led to increase free, conjugated and total phenols. Also, powdery mildew led to increase in reduced, non -reduced and total sugar contents. These results indicate the possibility of using *T. hamatum* or Blight stop as bio control agents against powdery mildew disease of Chamomile plants.

Key words: Chamomile, powdery mildew, *Sphaerotheca fuliginea*, *Trichoderma hamatum* and Blight stop.

INTRODUCTION

Chamomile (*Matricaria chamomilla* L.) is considered one of the most medicinal and aromatic plants in Egypt. Chamomile belongs to the Asteraceae family and is a plant grown mainly for its dry inflorescence. Chamomile contains an essential oil that is used in medicinal industries and cosmetic products. The essential oil is recognized by being as heavy viscous liquid, contains many active compounds such as bisabolol, bisabolol oxide A, bisabolol oxide B, Beta, trans farnesene, bisabolon oxide and chamazulene. The essential oil of chamomile is recognized through the blue color due to chamazulene which is blue (Piccaglia and Marotti, 1993 and Reda *et al.*, 1999). This oil is extensively used in perfumery, cosmetics, and aromatherapy, and in food industry (Singh *et al.*, 2011). Chamomile Plants are attacked by many diseases such as powdery mildew, downy mildew, root rot and wilt (Hilal and Abd-EL-Moity, 1994, Hilal *et al.*, 1998, El-Morsy, & Shalaby, 2013 and Mergawy, 2016). Powdery mildew is considered one of the most important diseases that attack chamomile in various cultivated areas. This disease was detected in Egypt for the first time in 1970 and the pathogen of this disease was identified as *Sphaerotheca fuliginea* (EL-Zarka, 1970, Hilal *et al.*, 1998 and Mergawy, 2016). The use of chemical control against powdery mildew disease led to rejection of the shipments, due to the presence of chemical toxic residues in chamomile products. Biological control, when properly implemented, is safe, with no residues in final product, cheaper and longer lasting. The present work was designed to identification the pathogen of powdery mildew as morphological characterization and evaluate the efficiency of some products of bioagents against powdery mildew of chamomile under field conditions as well as their effect on the growth parameters.

MATERIALS AND METHODES

Occurrence and severity of chamomile powdery mildew:

Chamomile powdery mildew disease incidence was surveyed throughout different locations at Fayoum governorate during 2020/2021 growing season. This survey was repeated, every month during November 2020 to March 2021, just to illustrate spread of these diseases during growing season. The obtained survey data were arranged and tabulated as percentages of severity and disease incidence according to scale developed by the author and formula developed by Whitney *et al*. (1983). Percentage of powdery mildew diseases incidence were determined according to the following formula: -

$$\% \text{ Disease incidence} = \frac{\text{number of diseased plants}}{\text{Total no. of plants}} \times 100$$

% Disease severity was calculated using scale developed by the author (Table, 1) and equation developed and modified by Whitney *et al*. 1983 as follows:

$$\textit{Disease}\text{ Severity }\% = \frac{\sum(\text{rating no.} \times \text{no. of plants in each rating})}{\textit{total}\text{ no. of plants} \times \text{highest rating}} \times 100$$

Table (1): Scale of disease severities for powdery mildew.

Grade	% of disease severity
0	No powdery mildew colonies observed
1	1 – 10% of leaf area covered with mildew
2	11-20% of leaf area covered with mildew
3	21-30% of leaf area covered with mildew
4	31-40% of leaf area covered with mildew
5	41-50% of leaf plant area covered with mildew
6	51-60% of leaf area covered with mildew
7	61-70% of leaf area covered with mildew
8	71-80% of leaf area covered with mildew
9	81-90% of leaf area covered with mildew
10	91-100% of leaf area covered with mildew

Identification of the pathogen cause powdery mildew:

To identify the causal fungus of powdery mildew, microscopic preparations were made by placing epidermal strips from the infected chamomile plants (bearing the fungal conidiophores and conidia) on glass slide, then examined using light microscope. Pathogen was identified according to morphological characteristics according to key developed by (EL-

Zarka, 1970 and Hilal *et al.*, 1998) for powdery mildew. The same samples were examined by the staff of the Mycology and Plant Disease Survey Research, Plant Pathol. Res. Inst, ARC to confirm our identification.

Preparation of biocontrol agents:

The following three different biological preparations were used in these studies:

1- *Trichoderma hamatum* was isolated from Ismailia governorate. The sequences of the isolate were submitted to NCBI Gen Bank and gave an accession number (MT111894).
2- *Bacillus subtilis* was isolated from Beheira governorate. The sequences of the isolates were submitted to NCBI Gen Bank and gave an accession number (MT110640).
3- *Streptomyces rochei* was isolated from Fayoum governorate. The sequences of the isolates were submitted to NCBI Gen Bank and gave an accession number (OP164572).

Trichoderma hamatum isolate was grown on Liquid Gliotoxin Fermentation Medium (G.F.M) (Brian and Hemming, 1945) under complete darkness condition, just to stimulate secondary metabolites production (Abd El-Moity and Shatla, 1981). After incubation for 9 days, culture was prepared as suspension and number of cfu was adjusted to be 30 ×106cfu/ ml.

Bacillus subtilis isolate was grown in bottles contains Nutrient Glucose Broth (NGB) (Schaad, 1980). Bottles contain medium plus *B. subtilis* were incubated for 3 days. Number of bacterial cells was adjusted to be 30×106cfu /ml.

Streptomyces rochei isolate was grown on Starch Nitrate Agar (SNA) medium [soluble starch (20g), K_2HPO_4 (1g), KNO_3 (2g), $MgSO_4.7H_2O$ (0.5g), NaCl (0.5g), $CaCo_3$ (3g), Agar (20g), $FeSO_4.7H_2O$ (0.001g) and distilled water (1000 ml).

Biological agents (*T. hamatum, B. subtilis* and *Str. rochei*) were produced at Central lab. of Organic Agriculture, Agricultural Research Center (ARC), Giza, Egypt).

Field experiments:

All field experiments were carried out at farm located at Makhlof farm in Aboxa village, Abshaway county, Fayoum governorate. Soil in this farm belongs to light clay soil type, with pH value 7.6. Irrigation water comes from Nile River. Field experiments were carried out during two successive seasons 2021 ⁄ 2022 and 2022 ⁄ 2023. All experiments were designed in complete randomized blocks (CRB). Four replicates were used for each treatment. Each replicate was 3x 4m. Each replicate contains 4 rows each row 4 m length. Total number of plants⁄ replicate was 60 plants. Plants received recommended fertilizer N P K (90-50-100) from approved sources as listed in annex in (I) Eu.889 ⁄2008. Plants were examined periodically and collected data were statistically analyzed according to the standard procedures including general liner model (GLM) available in SAS (1996). Significantly treatment differences were compared using Duncan's Multiple- Range test. The following experiments were carried out:

Effects of three concentrations of bioagents on chamomile powdery mildew incidence in comparison with the commercial biocide "Blight stop"(contain: mixture of *T. harzianum* and *B. subtilis*) at rate 10ml /L water.

To determine the most effective concentration, of different biocontrol agents under test, in comparison with effect of the commercial biocide (Blight stop), produced by Central Lab of Organic Agric. Agricultural Research Center. They were used at 5, 10 and 20 ml ⁄ liter of water from stock suspension (30x106 cfu ⁄ ml.). Each concentration was used for four replicates, each contains 60 plants. Each treatment (spray) was repeated 4 times with 15 days interval. Percentage of diseases incidence and severity were determined according to the previously formulas.

The following parameters were determined .

1- Weight of fresh and dry of chamomile inflorescence yield ⁄ feddan.
2- -Some agronomical characteristics in treated plants, *i.e* plant height, number of branches, number of inflorescences ⁄ plant, fresh and dry weight of 100 inflorescences.
3- Some chemical components in treated plants such as free, conjugated and total phenols, reduced, non- reduced and total sugars. Data were collected and statistically analyzed as mentioned before.

Chemical analysis:

To correlate between appearance time of powdery mildew disease and chemical content in diseased and healthy plants as well as effect of different treatments on chemical components, these experiments were carried out.

Chemical changes in chamomile plants due to powdery mildew infection:

Chamomile plants (60-day-old) show slight powdery mildew symptoms was collected. Another group of healthy plants, at the same old and from the same farm, were also collected. Chamomile samples, either healthy or diseased, were subjected different chemical analysis (free, conjugated and total phenols in addition to reduced, non-reduced and total sugars)

Effect of different biological or chemical treatments on chemical components of treated plants compere with control treatment:

Samples of chamomile plants, treated with different biological or approved chemical treatments were collected from different replicates. Collected samples of treated plants, from replicates of each treatment, were mixed thoroughly and then one sample for each treatment was used to determine: free, conjugated and total phenols (Bray and Thorpe, 1954), reduced, non-reduced and total sugars (A.O.A.C.,1995). Obtained values were tabulated and compared with each other using different biological or chemical treatments.

Determination of essential oil content:

Fifty grams of dry flowers, were collected from each treatment to determine percentages of oil in different treatments. (Anonymus, 1968). These methods used hydro-distillation for dry chamomile flowers. The hydro-distillation process was taken place for 2.5-3 hrs to be sure that all oil already extracted, and no further increase in oil was observed. After 3-4 hrs, the obtained oil was measured for each treatment. Obtained data were tabulated and compared with other parameters**.**

RESULTS AND DISCUSSION

1-Disease survey:

Symptoms of powdery mildew of chamomile plant (*Matricaria chamomilla* L.) were observed during disease survey of November to March at growing season 2020/2021 in several fields at Fayoum governorate. Symptoms firstly appeared on the lower parts of plant as white powdery areas, then increase in size, and coalesce to cover the stems and leaves (Fig.1). The survey was carried out during different months of growing season (November – March) to determine the most critical period for disease spreading. Data in tables (2) indicate that during November all examined fields in Fayoum governorate showed different percentages of powdery mildew, Percentage of powdery mildew ranged from 8% in Abshaway to 19.66% in Yousef El -Seddik. When disease severity was considered, obtained data show that very slight differences were recorded and percentage of disease severity ranged from 3.33 in Abshaway to 5.33 in Etsa. Data also show that powdery mildew disease incidence was gradually increased during growing season. The maximum record either for disease incidence or severity for powdery mildew disease, under study, was noticed during March. The highest powdery mildew was recorded in Etsa and 99 % disease incidence was recorded, whereas the highest powdery mildew disease severity was recorded in El- Fayoum with 63.76%. Obtained data indicate that either powdery mildew spread in all tested chamomile cultivated area. Powdery mildew started to appear during November with very low disease severity. The peaks of disease incidence and severity were noticed during February and March this is due to powdery mildew do prefer low sugar content in plant leaves and inflorescences during February and March, the old lower leaves already transfer most of sugar to inflorescences consequently it became susceptible for disease. The most favorable temperature for powdery mildew infection and disease development is 20-300 this range is available during this period (Zaher, 1970, Cheah *et al.*, 1996, Awad, 2004, Pap *et al.*, 2013, El-Morsy & Shalaby, 2013 and Mergawy, 2016).

Table (2): Percentages of disease incidence and severity of powdery mildew disease for Chamomile plants grown in different cultivated areas in Fayoum governorate during 2020/2021 season.

Location	% Disease incidence and severity									
	November		December		January		February		March	
	*	**	*	**	*	**	*	**	*	**
Yousef El – Seddik	19.66	4.66	20.83	7.16	77.33	24.33	92.33	51.00	96.00	**58.33**
Abshaway	8.00	3.33	16.66	7.1 0	47.33	19.00	67.66	37.66	91.00	**51.33**
Sinnoures	10 .00	3.33	15.33	5 .00	37.00	22.00	68.66	47.00	86.66	**55.00**
Etsa	13.00	5.33	17.00	7.00	32.66	22.33	71 .00	53.66	99.00	**61.00**
El- Fayoum	9.33	3.33	19 .00	6.66	54.00	22.00	61.66	42.66	94.66	**63.76**

*% Disease incidence. ** % Disease severity.

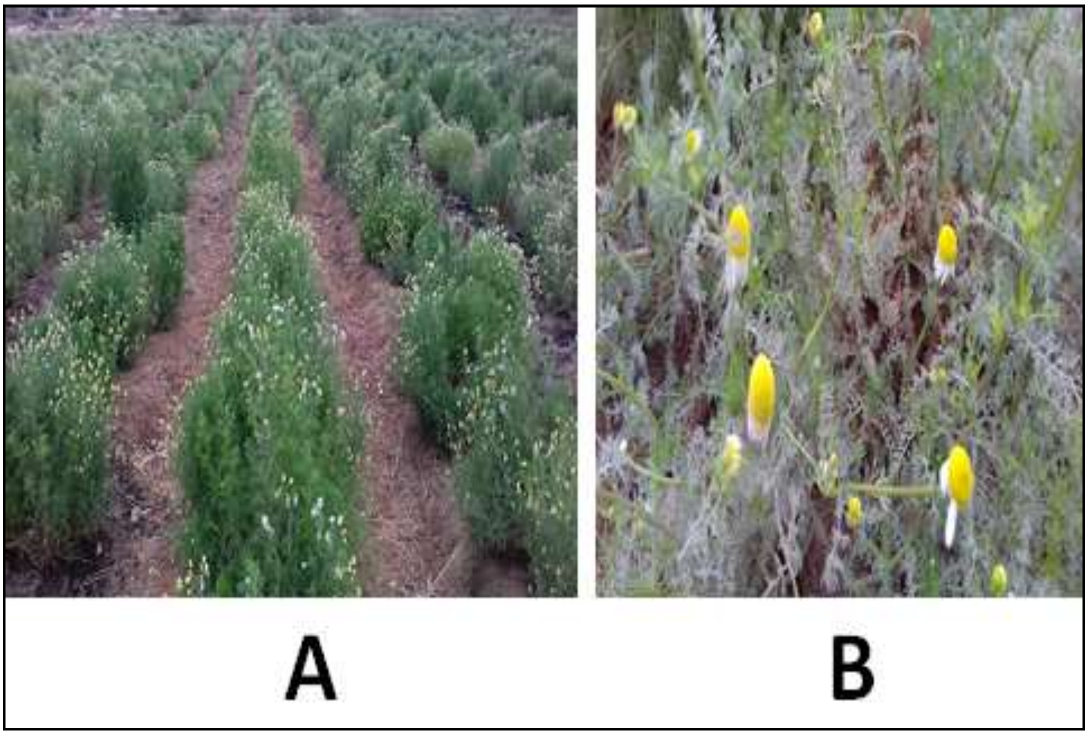

Fig. (1): Chamomile plants naturally infected by powdery mildew disease.
A: Healthy control. B: Diseased plants.

2-Identification of the causal pathogen of powdery mildew:

In symptomatic Chamomile plants, microscopic examinations of prepared slides (Fig. 2) reveal that the causal pathogen of Chamomile powdery mildew was identified according to its morphological and diagnostically criteria, as *Spherotheca fuliginea* (Schlech ex Fr.) poll. These criteria, were: the markedly swollen conidiospores contains about 4-3 spores. Also, conidia are oval to barrel shape, the measure of the conidia ranged between 26-43 x17-25 µm. These results are in harmony with those reported by (EL-Zarka, 1970, Hilal *et al.*, 1998 and Mergawy, 2016).

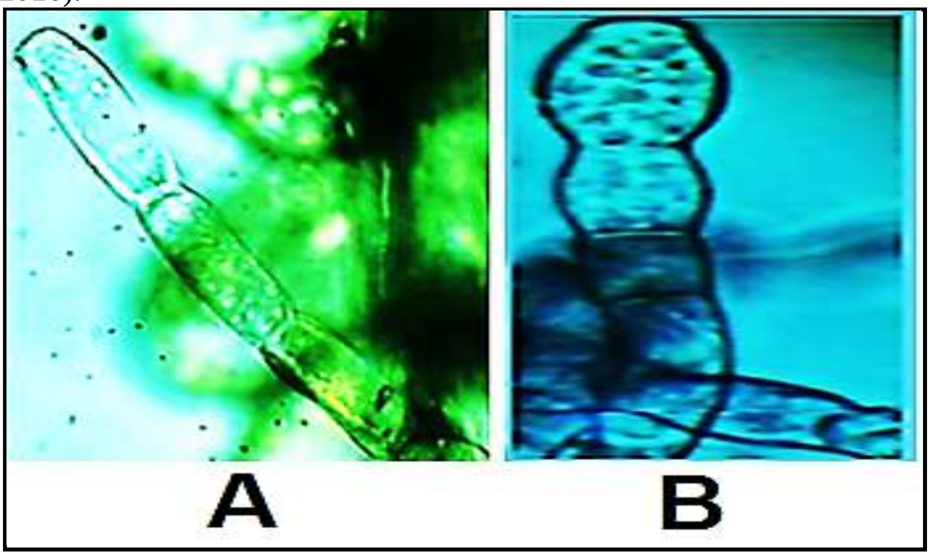

Fig. 2 (A&B): Conidiophores, Mycelium and Conidia of *Spherotheca fuliginea*.

3-The effect of biological control agents on the incidence of powdery mildew in chamomile and the resulting crop under field conditions during 2021/2022 & 2022/2023 season.:

To study effects of different isolated biocontrol agents, at different concentrations, in comparison with effect of commercial biocide Blight stop, this experiment was carried out. The aim was to compare effect of these isolated bioagents with that of Blight stop regarding powdery mildew incidence (Table 3 A&B). Effects of their treatments on some agronomical characteristics (*i.e.* plant height, number of branches ⁄ plant, number of inflorescences and fresh or dry weight of 100 inflorescences (Table 4) were measured. Chemical components (*i.e.* oil content, free, conjugated and total phenols in addition to reduced, non-reduced and total sugars were determined. Data in Tables 3(A&B), 4(A&B) and 5(A&B) indicate that the lowest percentages of powdery mildew incidence or severity were recorded when *T. hamatum* at concentration 20ml/ liter of water, or Blight stop at 10 ml ⁄ liter of water was used. These low percentages in disease severities were correlated with the highest yield in plant inflorescences and obtained yield reached 0.833 and 0.845 tons/feddan during 2021/2022 season and 0.846 and 0.845 tons/feddan during 2022/2023 season when *T. hamatum* at concentration 20ml/liter of water, or Blight stop at recommended dose. (Table 3 A&B). The lowest effective bioagent and the lowest yield was correlated to highest disease severity and these were recorded when *Streptomyces rochei* was used at 5ml/liter of water (Table 3 A&B). The highest effective bio control treatment *T. hamatum* showed also highest values when some agronomical parameters were measured (Table 4 A&B). Data in Table 4 (A&B) show that the highest plant height (cm), number of branches ⁄ plant, fresh and dry 100 inflorescences were obtained when *T. hamatum* was used. On the contrary, the lowest values compare with any other biological treatment were recorded when suspension 5 ml/liter of water of *B. subtilis* was used. However, Blight stop "commercial biocide" occupied the second rank after *T. hamatum*. When chemical components were assessed, obtained data (Table 5 A&B) show that, each biological treatment led to increase in percentage of essential oil than the control and the highest essential oil content was recorded when *T. hamatum* or Blight stop were used. On the other hand, the lowest essential oil percent was recorded when *B. subtilis* was used at the rate of 5ml/ liter of water. Data also revealed that powdery mildew disease incidence led to increase free, conjugated and total phenols. Also, powdery mildew leads to increase in reduced, non -reduced and total sugar contents.

T. hamatum or commercial biocide: Blight stop show the highest effect in controlling powdery mildew. This can be explained in the light of facts that *Trichoderma* sp. works through different mode of actions, *i.e.*, mycoparasitism (Abd El-Moity and Shatla, 1981) antifungal substance production (Abd El-Moity, 1976 & 1981. Elad and Kapat, 1999) *Trichoderma* sp. also works throw grow very fast and occupies the court of infection and act as mechanical barrier to prevent other pathogens to be in contact with host tissues (Abd El-Moity, 1981 and Cook, 1988). On the other hand, obtained data indicate that negative relations between percentage of disease incidence and obtained yield were detected. The highest yields were obtained when *Trichoderma* spp., either the isolated isolate or the isolates are found in commercial biocide Blight stop, were used. This can explain in work of many investigators who stated that in addition of action of *T. harzianum* isolates in depression disease incidence and severity it also produced growth regulators which improve plant growth consequently its yield (Ali, Ayat, 2013 and Gebily, Doha, 2015). Plant height, number of branches, number of inflorescences in addition to fresh and dry weight of 100 inflorescences show significant increases in plants received *Trichoderma hamatum* either through spraying isolated *T. hamatum* or through using Blight stop which contains *T. harzianum* and *B. subtilis*. These increases in agronomical characteristics can be due to the growth regulators produced by *T. hamatum*. In addition to these growth regulators, this fungus also protect plants against other diseases, consequently improve performance of plant in photosynthesis which lead to increase yield (Elad *et al.*, 1980 and Lamb and Rosskopf, 2002). When chemical components were determined in plants received different treatments, obtained data indicated that the highest percentages of essential oil were found in plants received *T. hamatum*. Free phenols also were determined in treated plants. Data revealed that the highest phenol contents, compare with control or any other treatments, were obtained when isolated *T. hamatum* or *B. subtilis* were used. This can be explained in the light of work of (Mahmoud *et al.* 1995).

Table (3-A): The effect of using of biological control agents on the occurrence of chamomile powdery mildew and the resulting yield under field conditions during 2021/2022 season.

Treatments	Concentrations	Powdery mildew disease		Fresh weight inflorescences per feddan (ton)	Dry weight inflorescences per feddan (ton)
		Disease incidence (%)	Disease severity (%)		
Trichoderma hamatum	5ml /L water	49.00 d*	9.33 d	3.85 d	0.770 d
	10ml/L water	41.00 e	7.33 e	3.911 c	0.782 c
	20ml/L water	19.00 g	4.33 f	4.16 b	0.833 b
Bacillus subtilis	5ml/L water	56.00 c	12.00 c	3.72 f	0.745 f
	10ml/L water	47.33 d	9.33 d	3.84 d	0.763 d
	20ml/L water	40.00 e	7.66 e	3.919 c	0.783 d
Streptomyces rochei	5ml/L water	61.00 b	15.00 b	3.75 f	0.750 ef
	10ml/L water	48.00 d	11.00 c	3.80 e	0.760 de
	20 m/L water	26.00 f	7.66 e	3.91 c	0.783 c
Blight stop **	10ml/L water	12.00h	3.33f	4.22a	0.845a
Control	Water only	100.00 a	60.66 a	2.58 g	0.519g
LSD at 0.05		1.7	1.5	0.3	0.01

* Values with the same letter, in each column, are not significantly different.
** contain: mixture of *T. harzianum* and *B. subtilis*.

Table (3-B): The effect of using of biological control agents on the occurrence of chamomile powdery mildew and the resulting yield under field conditions during 2022/2023 season.

Treatments	Concentrations	Powdery mildew disease		Fresh weight inflorescences per feddan (ton)	Dry weight inflorescences per feddan (ton)
		Disease incidence (%)	Disease severity (%)		
T. hamatum	5ml /L water	56.63d*	15.00d	3.930e	0.786e
	10ml/L water	22.33 g	10.00f	4.108b	0.821b
	20ml/L water	17.00h	5.66g	4.233a	0.846a
B. subtilis	5ml/L water	66.00b	17.00c	3.900e	0.780e
	10ml/L water	43.00e	12.00e	3.995d	o.799d
	20ml/L water	27.00f	9.00f	4.070c	0.814c
Str. rochei	5ml/L water	61.00c	19.00b	3.836f	0.767f
	10ml/L water	41.00e	14.00d	3.923e	0.784e
	20 m/L water	26.00f	8.66f	4.016d	0.803d
Blight stop **	10ml/L water	16.66h	5.00g	4.220a	0.845a
Control	Water only	100.00a	60.66a	2.580g	0.516g
LSD at 0.05		2.60	1.69	0.033	0.0067

* Values with the same letter, in each column, are not significantly different.
** contain: mixture of *T. harzianum* and *B. subtilis*.

Table (4-A): The effect of using different concentrations of biological control agents on some agricultural traits of chamomile plants under field conditions during 2021/2022 season.

Treatments	Concentrations	plant height (cm)	Number of branches/ plant	Number of inflorescences/ plant	Weight of 100 inflorescences fresh(g)	Weight of 100 inflorescences dry(g)
T. harzianum	**5ml/L water**	90.00 e*	16.66 e	2225.00 d	38.41 d	8.17 d
	10ml/L water	95.00 cd	18.00 cde	2315.00 c	40.35 bcd	8.82 bcd
	20ml/L water	112.33 a	21.00a	2610.00 a	40.43 abc	9.86 a
B. subtilis	**5ml/L water**	77.00 g	14.33 f	2108.33 f	38.36 d	8.32 d
	10ml/L water	87.00 e	17.00 de	2213.00 d	39.57 d	8.98 abcd
	20ml/L water	92.66 de	18.33 bc	2305.00c	42.48 a	9.18 abc
Str. rochei	**5ml/L water**	79.00 g	15.00 f	2184.66 e	38.46 d	8.40 cd
	10ml/L water	91.00 e	18.00cde	2218.33 d	40.36 cd	9.38 abc
	20ml/L water	96.66 c	19.33bc	2393.00 b	41.28 abc	9.82 ab
Blight stop**	**10ml/L water**	108.66b	20.33ab	2602.66ab	42.15ab	9.96ab
Control	**Water only**	61.00 h	10.33g	985.00 g	17.45 e	3.16 e
LSD at 5%	-	2.8	1.6	12.3	2.1	1.03

* Values with the same letter, in each column, are not significantly different.
** contain: mixture of *T. harzianum* and *B. subtilis*.

Table (4-B): The effect of using different concentrations of biological control agents on some agricultural traits of chamomile plants under field conditions during 2022/2023 season.

Treatments	Concentrations	plant height (cm)	Number of branches ⁄ plant	Number of inflorescences ⁄ plant	Weight of 100 inflorescences fresh(g)	Weight of 100 inflorescences dry(g)
T. harzianum	5ml/L water	106.00c *	17.00d	2403.33e	36.84cd	7.900cde
	10ml/L water	111.00b	20.00b	2623.33b	39.24b	8.130bcd
	20ml/L water	115.00a	22.00a	2720.00a	39.93b	9.020ab
B. subtilis	5ml/L water	96.66e	15.00ef	2214.00f	35.57d	7.370de
	10ml/L water	102.33d	19.00bc	2388.33e	37.89bc	7.840cde
	20ml/L water	109.33b	20.00b	2443.33d	39.86b	8.440bc
Str. rochei	5ml/L water	88.00g	14.66f	2408.66e	35.21d	7.140e
	10ml/L water	91.33f	16.66de	2500.00c	36.43cd	7.930cde
	20ml/L water	102.66d	17.66cd	2611.00b	38.03bc	8.300bcd
Blight stop**	10ml/L water	108.00b	20.33ab	2602.66b	42.15a	9.690a
Control	Water only	61.00 h	10.33g	985.00 g	17.45 d	3.157 f
LSD at 5%	-	2.652	1.74	23.13	2.09	0.976

* Values with the same letter, in each column, are not significantly different.
** contain: mixture of *T. harzianum* and *B. subtilis*.

Table (5-A): The effect of using different concentrations of biological control agents on the incidence of chamomile powdery mildew and some chemical components in plants treated under field conditions during 2021/2022 season.

Treatments	Concentrations	Reduction in disease incidence (%)	Essential oil content (ml/100g dry inflorescences	phenols			Sugars		
				Free	Conj.	Total	Reduced	Non Reduced	Total
T. hamatum	5ml /L	51.00 e*	0.36	108.78	44.48	153.26	17.26	7.70	24.96
	10ml /L	59.00 d	0.41	176.38	29.69	206.07	17.71	6.39	24.10
	20ml L	81.00 b	0.47	140.67	17.69	158.06	18.25	7.00	25.25
B. subtilis	5ml /L	44.00 f	0.34	144.67	62.03	206.70	19.67	6.51	26.18
	10ml /L	52.66 e	0.40	107.39	9.73	117.12	15.87	7.32	23.19
	20ml /L	60.00 d	0.41	120.28	27.17	147.45	14.55	7.10	21.65
Str. rochei	5ml /L	39.00 g	0.37	102.09	71.38	137.47	16.88	8.25	25.13
	10ml /L	52.00 e	0.42	120.79	32.47	153.26	15.82	6.37	22.19
	20ml /L	74.00 c	0.45	116.62	23.38	140.00	13.81	8.16	21.97
Blight stop**	10ml /L	88.00a	0.50	86.29	32.47	118.76	20.9	8.20	29.10
Control	Water only	0.00 h	0.26	41.56	44.48	86.04	11.26	5.37	16.63
LSD	-	1.718	-	-	-	-	-	-	-

* Values with the same letter, in each column, are not significantly different.
** contain: mixture of *T. harzianum* and *B. subtilis*.

Table (5-B): Effect of using different concentrations of biocontrol agents on chamomile powdery mildew incidence and some chemical components in treated plants, under field conditions during 2022/2023 season.

Treatments	Concentrations	Reduction in disease incidence (%)	Essential oil content (ml/100g dry inflorescences	Phenols (Mg/g)			Sugars (Mg/g)		
				Free	Cong.	Total	Reduced	Non Reduced	total
T. harzianum	5ml /L	47.00 fg*	0.36	106.75	25.66	132.41	26.09	2.096	28.18
	10ml /L	55.66 e	0.41	42.39	38.47	80.86	24.67	2.100	26.77
	20ml L	76.66 b	0.47	68.72	47.64	116.36	19.89	1.330	21.22
B. subtilis	5ml /L	39.00 h	0.34	92.95	80.02	172.97	25.23	4.000	29.23
	10ml /L	45.33 g	0.40	88.00	17.37	105.37	22.37	2.960	25.33
	20ml/ L	66.66 d	0.41	107.65	46.24	153.89	20.46	2.560	23.02
Str. rochei	5ml /L	33.00 i	0.37	93.84	40.21	134.05	24.61	3.530	28.14
	10ml /L	49.00 f	0.42	83.39	38.03	121.42	21.61	2.460	24.07
	20ml L	71.00 c	0.45	87.50	37.65	125.21	20.94	2.090	23.03
Blight stop**	10ml /L	83.33a	0.50	59.61	12.15	71.76	25.07	2.670	27.74
Control	-	0.00 j	0.26	97.82	281.23	379.05	11.85	8.110	19.96
LSD at 5%	-	2.376	-	-	-	-	-	-	-

* Values with the same letter, in each column, are not significantly different
** contain: mixture of *T. harzianum* and *B. subtilis*.

Conclusion :

Sphaerotheca fuliginea was identified as the pathogen of powdery mildew disease of infected chamomile plants collected from Fayoum governorate, Egypt, during 2020/2021 season, using morphological characterization. *Trichoderma hamatum, Bacillus subtilis, Streptomyces rochei* and Blight stop were evaluated for their efficacy against powdery mildew of chamomile. *T. hamatum* or Blight stop as bio control agents were the best treatments for the management of chamomile powdery mildew and improvement of plant growth, which indicated the possibility of their using as fungicidal alternatives.

REFERENCES

A.O.A.C. (1995). Official methods of analysis of the association of official agricultural chemists. 16th ed., Washington, D.C., USA Egyptian J. of Phytopathol. 37:1-8.

Abd El-Moity, T. H. (1976). Studies on the biological control of white rot of onion. M.Sc. Thesis, Fac. Agric, Minufiya Univ., pp. 121.

Abd El-Moity, T. H. (1981). Further studies on the biological control of white rot disease of onion. Ph.D. Thesis, Fac. Agric., Monoufia Univ, pp. 135.

Abd El-Moity, T. H., Shatla, M.N. (1981) Biological control of white rot disease of onion *Sclerotium cepivorum* by *Trichoderma harzianum* phytopathology Z., 100: 29-35.

Ali, Ayat, M. (2013). Control of strawberry fungal diseases under organic agriculture system. Ph.D. Thesis, Fac. of Agric., Cairo Univ., pp.123.

Anonymus (1968). Determination of volatile oils in drugs. British Pharmacopoeia.

Awad, Nadia A. S. (2004). New approaches for controlling powdery mildew disease of cucumber. Ph. D. Thesis, Fac. Agric., Suez Canal Univ., Egypt, pp.168.

Bray, H.G., Thorpe, W.V. (1954). Analysis of phenolic compounds of interest in metabolism. Methods in Biochemistry Analysis, 1:27-51.

Brian, P.W., Hemming, H.G. (1945). Gliotoxin a fungistatic metabolic product of *Trichoderma viride*. Ann. Appl. Biol., 32:214-220.

Cheah, L.H., Page, B.B.C., Cox, J. K., Callaghan, M.O. (1996). Epidemiology of powdery mildew (*Sphaerotheca fuliginea*) of squash. Proceedings of the Forty Ninth New Zealand Plant Protection Conference, Nelson, New Zealand, 13(15):147 - 151.

Cook, R. J. (1988). Biological control and holistic plant healt care in agriculture. Amer. J. of Alternative Agric., 3:51–62.

Elad, Y., Kapat, A. (1999). The role of *Trichoderma harzianum* protease in bio-control of *Botrytis cinerea*. Eur. J. Plant Pathol., 105: 177-189.

Elad, Y., Chet, I., Katan, J. (1980). *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizocionia solani* .Phytopathol, 70:119-121.

El-Morsy, S.A., Shalaby, I.A. (2013). Control of chamomile powdery mildew disease using potassien, citrien and chitosan. Journal of Plant Protection and Pathology, 4(11), 985-997.

EL-Zarka, A.M. (1970). Appearance of powdery mildew disease on chamomile (*Matricaria chamomilla* L.) in UAR (Egypt) and methods of control. Proc. 2nd. Microbial Conf., Egypt, pp. 49.

Gebily, Doha, A. (2015). Studies on root rot disease of fennel under organic farming regulations. M.Sc. Thesis, Fac. Agric, Fayoum Univ., pp.121.

Hilal, A.A., Abd EL-Moity, T.H. (1994). Recent outbreaks of downy mildews on chamomile and rose plants. The first Conf of Ornamental Hort., (2):638-651.

Hilal, A.A., Harridy, I.M., Abo EL-Ela, A.M., Baiuomy, M.A.M., EL-Morsy, S.A. (1998). Studies on the commonly and newly occurring disease of seven medicinal and aromatic plants and yield losses in relation to some agricultural practices in Egypt. Egypt. J. Appl. Sci., 13(7):41-60.

Lamb, B., E. Rosskopf (2002). Biologically-based disease management products in Florida vegetable production. Vegetarian. Vegetable Crops Extension Publication. pp.218.

Mahmoud, Fatma A. F., Heweidy, M.A., Essmat, Nadia E.A., Ghoneim, Soheir S. H. (1995). A new technique to induce resistance in *faba bean* against chocolate spot disease. Menofia J. Agric. Res., 20(5):1741-1754.

Mergawy, M.M. (2016). Control of foliage diseases in chamomile (*Matricaria chamomile* L.) plants under organic agriculture regulations. Ph. D. Thesis, Fac. Agric. Fayoum Univ., pp. 107.

Pap, P., Rankovic, B., Masirevic, S. (2013). Effect of temperature, relative humidity and light on conidial germination of oak powdery mildew (*Microsphaera alphitoides* Griff. Et maubl.) under controlled conditions. Arch. Biol. Sci., Belgrade, 65(3):1069-1077.

Piccaglia, R., Marotti, M. (1993). Characterization of several aromatic plants grown in northern Italy. Flavour and Fagrance J., 8:115-122.

Reda, F., Tarraf, S., Abdel-Rahim, E. A., Afify, A. S., Ayads, H. S. (1999). Effect of low temperature on growth, some biochemical constituents and essential oil of Chamomile. ANNALS OF AGRICULTURAL SCIENCE-CAIRO-, 44(2), 741-760.

SAS (1996). SAS/ STAT user's Guide, version 6, 12th ED. Vol.2 SAS Institute Inc. Cary, North Carolina, 846pp.

Schaad. N.W. (1980). Laboratory guide for identification of plant pathogens. Baeten a Bacteriology Committee of American Phytopathological Society, St. Paul. Minnesota, p. 72.

Singh O., Khanam, Z., Misra, N., Srivastava, M.K. (2011). Chamomile (*Matricaria chamomilla* L.): An overview. Pharmacogn. Rev., 5(9):82-95.

Whitney, E.D., Lewellen, R.T., Skoyen, I.O. (1983). Reaction of sugar beet to powdery mildew: genetic variation, association among testing procedure and resistance breeding. Phytopathology, 73:182-185.

Zaher, E.A. (1970). Studies on the powdery mildew incited by *Leveillula taurica*. M. Sc. Thesis, Dept. Agric. Bot., Fac. Agric., Cairo Univ., Egypt. AL-Azhar J. Agric. Res., 20:217 -236.