Efficacy of Compost and Some Biocontrol Agents in Controlling Cucumber White Mould Disease under Protected House Conditions

Gamal A. Ahmed [1], Abeer H. Makhlouf [2], Mohamed E. Selim[2]

ABSTRACT

Five different *Trichoderma* isolates and three different bacterial genera *(Serratia marcescens, Bacillus subtilis, Pseudomonas fluorescens*) were used in the present study either separately or combined with compost to evaluate their biocontrol potential against *Sclerotinia sclerotiorum* either *in vitro* or *in vivo*. The results showed that all tested bioagents significantly affected the radial growth of the pathogen under laboratory conditions. The highest reduction (61.85%) was detected with isolates *T. asperellum* followed by *T. album* which inhibited the radial growth by 60.74%. The combination between *T. viride* + *T. asperellum* + *T. album* was the most effective in reducing disease severity during both seasons and increased yield up to 2.5 kg/plant. The influence of the tested biocontrol fungi and bacteria on essential lysis enzymes activity *i.e.* chitinase and Beta 1,3- glucanase was investigated. The results showed that both tested fungal and bacterial agents alone or combined with compost affected the activity of both enzymes positively. Similar findings were observed with aspect to three different oxidative enzymes activity *i.e.* peroxidase (PO), polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL). The profiling of peroxidase activity was induced in treated cucumber plants. Moreover, the results revealed that treating cucumber plants with the investigated bioagents expressed new protein bands.

Key words: *Trichoderma* – biological control – cucumber – white mould

INTRODUCTION

Cucumber (*Cucumis sativus*) is among the most widely grown vegetables all over the world (Paris *et al*, 2011). *Sclerotinia sclerotiorum* (Lib.) de Bary is a worldwide pathogen that can infect more than 400 plant species. This pathogen causes severe yield losses of many economic crops (Lu, 2003). Traditional management techniques such as crop rotation and cultural practice are not enough alone to stop the disease damage due to the host wide range and the ability of the pathogen to survive for a long time in the soil as sclerotia (Purdy 1979, Bolan and Hall 1994 and Elkahoui, *et al*, 2014). The low cost and eco-friendly application of biological control methods are gaining a high attention among all methods used to control plant diseases in general and Sclerotinia diseases particularly. Biocontrol depending on using either antagonistic fungi *i.e. Trichoderma hamatum, Gliocladium virens, Trichoderma viride* or bacteria, *Pseudomonas fluorescens,* found to be important in recent integrated pest management techniques that, can reduce the radial growth of the *Sclerotinia sclerotiorum* respectively (Abhiniti *et al*, 2011and EL-Kafrawy 2008). Soil treatment with *T. hamatum, G. virens or B. subtilis* gave the maximum protection against the fungal infection followed by *T. viride* and *P. fluorescens.* Moreover, this treatment improved plant height and increased both flowers number and fruit yield. The biocontrol agents tested were nearly as effective as the fungicide Topsin. M. *Helmy; 2016 also found that, Streptomyces sp.*, followed by *P. flourescens* and *Bacillus subtilis* significantly reduced mycelium growth of *Sclerotinia sclerotiorum*. Moreover, they significantly reduced the severity of the disease on cucumber plants as well as they enhanced the morphological and physiological parameters of treated plants compared with the control. Control of root rot and Sclerotinia diseases using biofungicides have been investigated in many types of researches (Li GQ, *et al*., 2003, Domenech, *et al*., 2006, Abdullah, *et al*., 2008, Berry, *et al*., 2010, Zeng, *et al*., 2012, and Rodriguez, *et al.*, 2015). Oxidative enzymes such as peroxidase and polyphenol oxidase enhance the formation of lignin, while other oxidative phenols contribute in formation of defense barriers for reinforcing the cell structure (Avdiushko, *et al*., 1993). Chitinase and β-1, 3 glucanase enzymes play a significant role in plant defense against fungi by hydrolyse their cell wall (Tian, *et al*., 2006 and Barilli, *et al*., 2010). Ahmed, *et al.,* (2017) found that, integration between *T. album* + *B. subtilis* + *Ps. fluorescens* and compost was the most effective treatment for reducing tomato Sclerotinia rot disease incidence and disease severity. As well as, this treatment increased the fruit weight per plant. Moreover, all treatments increased the phenols and flavonoids content in tomato plants. The addition of 10% compost to the soil significantly decreased the incidence of many diseases on different crops *i.e.*

DOI: 10.21608/asejaiqjsae.2021.178433

[1]Plant Pathol. Dept., Fac. Agric., Moshtohor, Benha University. Egypt.
[2]Agricultural botany Dept., Fac. Of Agric., Menoufiya University. Egypt.
Corresponding Author: G. A. Ahmed, Benha University,
Faculty of Agriculture - Moshtohor, Toukh, Kalyoubia, 13736, Egypt
E-mail: gamal.mohamed@fagr.bu.edu.eg

Received May 10, 2021, Accepted, June 13, 2021.

Aphanomyces root rot of peas; Rhizoctonia root rot of bean, Sclerotinia drop of lettuce, Fusarium wilt of cucumber and phytophthora crown rot of pepper (Lumsden, *et al*., 1983).

The objective of current work is the controlling of cucumber Sclerotinia disease by the compatibility of compost in combination with bioagents in the greenhouse and protected house conditions in addition to estimating some biochemical parameters as a response of treating the plants.

MATERIAL AND METHODS

1- Isolation and identification of the causal organism

Diseased samples of cucumber plants showing *Sclerotinia* rot symptoms were collected from Qalyubia governorate (Moshtohor) and subjected to isolation trials. *Sclerotinia* was isolated from the lesions that appeared on diseased plants. The infected tissues were cut into small pieces, surface sterilized with sodium hypochlorite (0.5%) for 2-3 minutes, washed for several times with sterilized distilled water, dried between sterilized filter papers and transferred directly to the PDA medium in in the plate 9 cm. The plates were incubated for 1-2 days at 22±2ºC. The mycelium grown from the lesion pieces were transferred to potato dextrose agar (PDA) slants. The fungus was purified by the hyphal tip technique (Brown, 1924). The purified fungal isolates were identified according to Singh, (1982). PDA slants from the fungus were kept in refrigerator at 4ºC for further experiments.

2- Laboratory experiments

Effect of antagonistic fungi on the growth of *Sclerotinia sclerotiorum in vitro*

Two discs (Ø 5 mm) of 4 days old plain agar culture of antagonistic fungi; *Tricoderma harzianum, T. viride*, *T. asperellum, T. album* and *T. lignorum* (these isolates were obtained from plant pathology Dept., Fac. of Agric., Benha Univ. Egypt) and *S. sclerotiorum* were inoculated simultaneously each opposite the other 1 cm apart from the plate edge in individual plates (Ø 9 cm) contained 10 mL PDA medium. In the control treatment, each plate was inoculated with 1 disc of mycelial growth of a given isolate of *S. sclerotiorum*. Three plates in the middle were used for each treatment. All dishes were incubated at 22 ±2°C for 10 days. The percentage of the fungal growth reduction (X) was calculated by using the following formula suggested (Abd-El-Moity, 1985).

X = G1- G2 / G1 x 100

Where: X= fungal growth reduction.

G1= linear growth of the pathogen inoculated alone.

G2= linear growth of the pathogen inoculated against the antagonistic fungus.

Effect of antagonistic bacteria on growth of *S. sclerotiorum in vitro*

Studying the effect of antagonistic bacteria isolates (*Serratia marcescens, Bacillus subtilis and Pseudomonas fluorescens)* on the growth of *S. sclerotiorum* were conducted as follow; individual plates (Ø 9 cm) contained PDA medium were streaked at one side (1cm apart from the plate edge) with a loop full of the antagonistic bacteria (48 hrs- old) grown on nutrient broth medium (NB) and incubated for 24 hrs at 22°C then the same plate was inoculated at the opposite side 1cm apart from the plate edge with 5mm disc of 4-days-old plain agar culture of *S. sclerotiorum*. All plates were incubated at 22±2°C for 5 days (Maurhofer *et al*., 1995).

3-Greenhouse experiments

The inoculum of *S. sclerotiorum* was grown for two weeks on sand barley medium (3:1, w:w and 40% water). Inoculum of *S. sclerotiorum* fungus was added to the potting soil at the rate of 3.0% w/w, mixed thoroughly with the soil surface of each pot then watered and left for one week to ensure even distribution of the inoculum**.**

Effect of treating cucumber seedlings with some antagonistic on incidence with Sclerotinia mould disease

In this experiment, healthy cucumber seedlings of Barracuda hybrid f1 20- days-old (from Qaha nurseries, Qalyubia governorate) were inoculated. In each hole containing a cucumber plant, 30 ml of individual spore suspension ($3x10^{10}$ spore/ mL) of *Trichoderma* isolates and bacterial suspension (10^8cells / mL) were drenched at the collar level. After one week the plants were transplanted into pots (30 cm) (Benchabane *et al.,* 2000). After two months of inoculation and treatment, the disease severity was assessed using 0-5 scale where:

0 = no symptom, 1= 0-25% of root browning, 2 =26-50% of root browning, 3 =51-75% of root browning, 4 =76-100% of root browning, and 5 = plant death according to Abdeljalil *et al.,* (2016)**.**

Disease Severity % = Σ (a x b) / N x K x 100

Where: a = Number of infected plants in each category.

b = Numerical value of each category.

N = Total number of examined plants.

K = The highest degree of infection category.

4. Experiments of commercial protected house

In two experiments (during 2017 and 2018) healthy cucumber transplants of Barracuda hybrid f1 were individually treated with *T. viride*, *T. asperellum, T.*

album, T. viride, + T. asperellum, T. viride + T. album, T. asperellum + T. album, T. viride + T. asperellum+ T. album, Ps. fluorescens, B. subtilis and *Ps. fluorescens + B. subtilis* as pervious in greenhouse. Transplants were planted in pots (30 cm in diameter) amended with mixture of soil and compost (was obtained from El-Nile Company, Giza, Egypt) at 5% or without compost. Soil was infested with inoculum of *S. sclerotiorum* fungus at the rate of 3.0% w/w. Untreated transplants were used as a control. One transplant/pot and five replicates for each treatment were used. Disease Severity was recorded as mentioned before and average weight of fruits (kg)/plant was recorded.

5. Determining of enzyme activities:

Leaves sample of cucumber plants cv. Barracuda hybrid that treated with different treatments under the study in greenhouse were taken 30 days after transplanting. Leaf samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant layer was used to determine enzyme activities (Tuzun *et al.,* 1989).

Determination of Peroxidase (PO):

Peroxidase activity was determined according to the method described by Allam and Hollis (1972) and Abdelsalam *et al.* (2020). Peroxidase activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

Determination of Polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by Matta and Dimond (1963). Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/min.

Determination of phenylalanine ammonia lyase (PAL):

The activity of PAL was determined according to the method described by Dickerson *et al.,* (1984). PAL activity was expressed as µmol trans-cinnamic acid min^{-1} g^{-1} protein.

Determination of chitinase

Determination of the activity of chitinase was carried out according to the method of Boller and Mauch, (1988). Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/g fresh weight tissue/60 minutes.

Determination of β-1,3-Glucanase:

Determination of the activity of the β-1,3-glucanase was carried out according to the method of Sun *et al.,* 2006. β 1-,3-glucanase was expressed as mM glucose

6-Activity gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Eighty microliters (80 µL of protein) of leaves sample were subjected to SDS-polyacrylamide gel electrophoresis was performed in 12 % acrylamide slab gels following the system of Laemmli (1970) to identify their protein profiles. Gels were photographed scanned, analyzed using Gel Doc VILBER LOURMAT system.

Peroxidase.

Activity gel electrophoresis of peroxidase was carried out to study the expression pattern of different isoforms of PO with various treatments. For native anionic polyacrylamide gel electrophoresis according to the method of Sindhu *et al*., (1984).

7. Statistical analysis

Statistical analysis of experiments was done as given by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

RESULTS

1- Effect of some bioagents on the growth of *S. sclerotiorum* under *in vitro* conditions

It is clear from the data in **Table 1** and **Fig. 1** that all applied biological fungi reduced growth of *S. sclerotiorum*. *Trichoderma asperellum* was the most effective antagonistic fungus that inhibited the radial growth of the pathogen giving (61.85%) followed by *T. album* which inhibited the radial growth by 60.74%. However, *T. harzianum* and *T. lignorum* reduced the growth of *S. sclerotiorum* by 54.44 and 53.71%, respectively.

Table 1. Effect of some bioagents on the growth of *S. sclerotiorum in vitro*

Treatment	Mycelial growth (mm)	Efficacy %
T. harzianum	41.00	54.44
T. viride	37.67	58.15
T. asperellum	34.33	61.85
T. album	35.33	60.74
T. lignorum	41.67	53.71
Control	90	00.00
L.S.D 0.05	2.85	

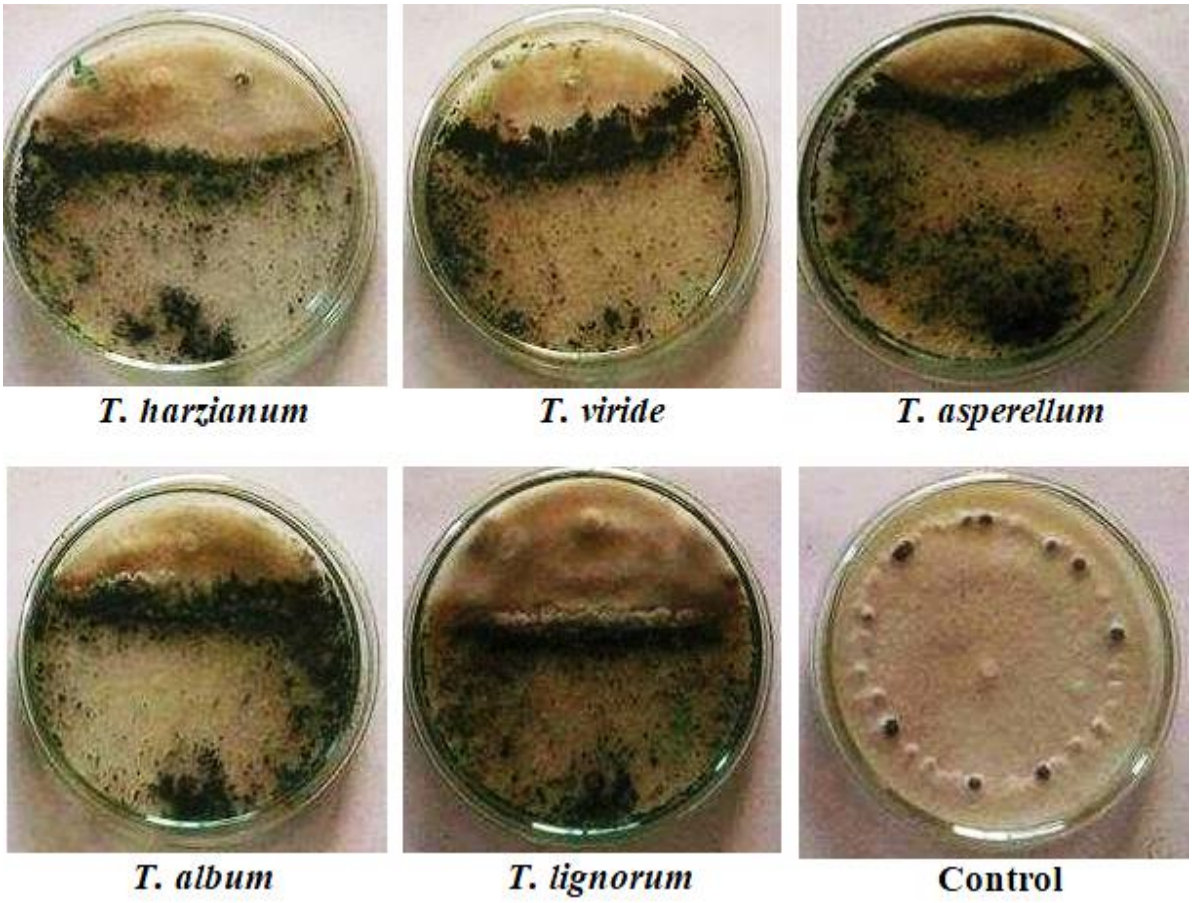

Fig. 1. Effect of some bioagents on the growth of *S. sclerotiorum* under *in vitro* conditions

2. Effect of some antagonistic bacteria on the growth of *S. sclerotiorum in vitro*

Data in Table 2 show that all tested antagonistic bacteria reduced linear growth of *S. sclerotiorum*. *Pseudomonas fluorescens* was The most effective antagonistic bacteria which reduced the linear growth of the pathogen by 22.22%

Table 2. Effect of some antagonistic bacteria on the growth of *S. sclerotiorum in vitro*

Antagonist	Mycelial growth (mm)	Efficacy %
Serratia marcescens	73.67	18.14
Bacillus subtilis	71.00	21.11
Ps. fluorescens	70.00	22.22
Control	90	0.00
L.S.D 0.05 =	4.03	

3. Effect of biological agents and compost alone or in combination on white mould severity of cucumber plants under greenhouse conditions

Data in Table 3 show that adding vegetarian compost to soil increased the activity of all antagonistic agent treatments than the individual treatment.

In this respect, *T. album* and *Ps. fluorescens* in compost-free soil reduced the disease severity by 85.26 and 83.13 % respectively. *Trichoderma asperellum* and *T. viride* reduced the disease severity by 82.94 and 77.52 % respectively in the same soil (compost free).

As for soil amended with compost results show that the highest reduction in disease severity was recorded by *T. album* and *T. asperellum* which recorded 94.19 and 93.02% respectively. Whereas, compost only reduced disease severity by 62.03 %.

Table 3. Effect of biological agents and compost alone or in combination on white mould severity of cucumber plants under greenhouse conditions

Treatment	Without compost		With compost	
	Disease severity %	% of Reduction	Disease severity %	% of Reduction
T. harzianum	15.11	73.64	6.67	88.37
T. viride	12.89	77.52	5.78	89.92
T. asperellum	9.78	82.94	4.00	93.02
T. album	8.45	85.26	3.33	94.19
T. lignorum	15.11	73.64	6.22	89.15
Ps. fluorescens	9.67	83.13	4.45	92.24
B. subtilis	17.33	69.77	6.89	87.98
Serratia marcescen	18.44	67.84	7.11	87.60
Compost	-----	-----	21.77	62.03
Control	57.33	0.00	57.33	0.00
L.S.D 0.05 =	Treatment 4.87	Compost 2.30	Interaction 5.97	

4- Effect of biological agents and compost alone or in combination on white mould severity of cucumber plants under protected house conditions

The data in Table 4 reveal that all tested treatments at both seasons (2017 and 2018) significantly reduced the percentage of white mould severity compared with the control. Adding compost to soil increased the efficacy of all treatments.

The combination of *Trichoderma viride* + *T. asperellum* + *T. album* was the most effective treatment (average 3.34%) in aspect to reducing disease severity during both seasons and increased yield to (2.5 kg/plant) followed by *T. viride* + *T. album* (average 3.67% and 2.38 kg/plant), *T. asperellum* + *T. album* (average 4% and 2.29 kg/plant) and *T. viride* + *T. asperellum* (average 4% and 2.27 kg/plant) compared with control (average 34.34% and 1.59 kg/plant). However, compost treatment alone was the least effective treatment at both seasons.

5- Effect of biological agents and compost alone or in combination on oxidative enzymes

Results presented in Table 5 indicate that all treatments increased the activity of peroxidase, polyphenol-oxidase and PAL enzymes compared with the untreated control. Generally, *Trichoderma viride*, *T. asperellum* and *T. harzianum* + Compost were superior for increasing the activity of peroxidase enzyme where they increased the activity of peroxidase by 213.70, 189.45 and 189.42 %, respectively. Meanwhile, *Serratia marcescens* + Compost was the lowest effective treatment, which increased the activity of peroxidase by 110.64%.

Alternately, *Ps. fluorescens* + Compost followed by *T. album* and *Bacillus subtilis* + Compost were the most effective treatments and increased the activity of polyphenoloxidase by 56.67, 50.63 and 44.17% respectively. Whereas, compost alone increased the activity of peroxidase by 16.25% and was the lowest effective one.

As for PAL, *Bacillus subtilis* + Compost, *T. album* +Compost and *Serratia marcescens* were the most effective treatments and increased the activity of PAL by 255.36, 157.21 and 155.36% respectively. *T. viride* +Compost was the lowest effective one.

Table 4. Effect of biological agents and compost alone or in combination on white mould and yield of cucumber plants under protected house conditions

Treatment	Disease severity %				Yield (kg/plant)				Mean			
	2017		2018		2017		2018		Disease severity %		Yield (kg/plant)	
	W-	W+	W-	W+	W-	W+	W-	W+	W-	W+	W-	W+
T. viride	12.00	8.67	14.67	12.00	1.85	2.00	1.75	1.90	13.34	10.34	1.80	1.95
T. asperellum	10.67	6.00	14.00	11.33	1.82	2.00	1.80	1.92	12.34	8.67	1.81	1.96
T. album	10.00	4.67	12.67	10.67	1.90	2.10	1.86	2.00	11.34	7.67	1.88	2.05
T. viride + T. asperellum	7.00	3.33	9.33	6.67	2.10	2.31	2.00	2.22	8.17	5.00	2.05	2.27
T. viride + T. album	6.67	2.67	8.67	5.33	2.20	2.43	2.10	2.33	7.67	4.00	2.15	2.38
T. asperellum + T. album	7.33	4.00	10.00	6.00	2.15	2.33	2.00	2.25	8.67	5.00	2.08	2.29
T. viride + T. asperellum + T. album	5.33	2.00	8.00	4.67	2.34	2.56	2.25	2.43	6.67	3.34	2.30	2.50
Ps. fluorescens	10.67	8.00	14.67	11.33	1.87	2.05	1.73	1.86	12.67	9.67	1.80	1.96
B. subtilis	11.33	9.33	16.00	12.67	1.83	1.96	1.74	1.90	13.67	11.00	1.79	1.93
Ps. fluorescens + B. subtilis	8.00	4.67	10.67	7.33	1.94	2.23	1.92	2.10	9.34	6.00	1.93	2.17
Compost	----	13.33	----	17.33	----	1.74	----	1.68	----	15.33	----	1.71
Control	32.67	----	36.00	----	1.62	----	1.55	----	34.34	----	1.59	----

W- = without compost W+ = with compost

LSD 0.05 =								
Treatment	1.56	0.67	1.85	0.80	0.28	0.12	0.25	.10
Interaction	2.21		2.28		0.68		0.56	

Effect of biological agents and compost alone or in combination on lysis enzymes activity

The effect of biological agents and compost alone or in combination on the activity of chitinase and β-1,3-glucanase is presented in Table 6. All the tested treatments increased chitinase activity. The highest activity of chitinase was induced by *Bacillus subtilis* and *Ps. fluorescens* + Compost (188.44 and 164.89% increase respectively), followed by *T. asperellum* 149.33% increase. The combination of *Trichoderma viride* + Compost was the lowest effective one, which increased the activity by 14.67%.

As for β-1,3-glucanase, the highest increase was recorded by *T. asperellum* + compost (193.75%) followed by *Ps. fluorescens* and *T. viride* (192.50 and 187.50%. respectively). Meanwhile *Serratia marcescens* + compost induced the lowest increase (8.44%).

Table 5. Effect of biological agents and compost alone or in combination on oxidative enzymes

Treatment	PO	PPO	PAL	Efficacy %		
				PO	PPO	PAL
T. harzianum	51.25	5.31	314.90	121.27	24.17	102.79
T. viride	61.07	7.07	257.12	213.70	40.42	65.58
T. asperellum	55.46	4.88	341.62	189.45	20.21	120.00
T. album	54.76	8.17	339.46	186.42	50.63	118.61
T. lignorum	42.82	7.09	273.73	134.89	40.63	76.28
Ps. fluorescens	46.68	6.41	366.18	151.55	34.38	135.82
Bacillus subtilis	48.44	6.01	395.79	159.13	30.63	154.89
Serratia marcescens	48.09	7.02	396.52	157.62	40.00	155.36
Compost	49.84	4.46	260.73	165.20	16.25	67.91
T. harzianum + *Compost*	55.46	4.79	208.01	189.42	19.38	33.96
T. viride + Compost	54.76	7.13	180.34	186.42	41.04	16.14
T. asperellum + Compost	52.65	4.50	371.24	177.33	16.88	139.08
T. album + Compost	44.93	5.31	399.40	143.98	24.14	157.21
T. lignorum + Compost	47.39	4.52	225.34	154.60	16.88	45.12
Ps. fluorescens + *Compost*	46.68	8.82	228.23	151.55	56.67	46.98
Bacillus subtilis + *Compost*	46.33	7.47	551.80	150.04	44.17	255.36
Serratia marcescens + Compost	37.21	4.50	283.84	110.64	16.67	82.79
Control	11.58	2.70	155.28	0.00	0.00	0.00

6. Effect of treating cucumber plants with some bioagents on PAGE of protein.

The results of SDS (PAGE) presented in Table 7 and demonstrated in Fig. 2 showed that fifteen protein bands with molecular weights ranging from 234.433 to 34.93 kDa are contained in cucumber plants. New protein bands are expressed as a result of treating cucumber plants with the bioagents. One band with 37.23 kDa was appeared in plants treated with *Ps. fluorescens*, while absent in other treated plants. Moreover, the band with 159.517 kDa appeared in plants treated with *T. viride* and absent in other treated plants. Also, a band with 34.93 kDa appeared in plants cultivated in the soil amended with compost while absent in all treated plants.

Table 6. Effect of biological agents and compost alone or in combination on lysis enzymes activity

Treatment	Chitinase	Beta 1,3-glucanase	Efficacy %	
			Chitinase	Beta 1,3- glucanase
T. harzianum	5.37	6.36	138.67	98.75
T. viride	4.20	9.20	86.67	187.50
T. asperellum	5.61	4.90	149.33	53.13
T. album	3.71	6.91	64.89	115.94
T. lignorum	4.29	5.15	90.67	60.94
Ps. fluorescens	5.21	9.36	131.56	192.50
Bacillus subtilis	6.49	6.59	188.44	105.94
Serratia marcescens	2.93	4.17	30.22	30.31
Compost	3.00	4.83	33.33	50.94
T. harzianum + *Compost*	3.62	5.52	60.89	72.50
T. viride + Compost	2.58	6.96	14.67	117.50
T. asperellum + Compost	4.95	9.40	120.00	193.75
T. album + Compost	3.63	4.83	61.33	50.94
T. lignorum + Compost	3.65	5.14	62.22	60.63
Ps. fluorescens + *Compost*	5.96	8.52	164.89	166.25
Bacillus subtilis + *Compost*	4.67	5.03	107.56	57.19
Serratia marcescens + Compost	3.15	3.47	40.00	8.44
Control	2.25	3.20	0.00	0.00

Table 7. Molecular weights of fractionated protein profiles of cucumber leaves treated with selected treatments

Band No	M.W KDa	*T. viride*	*T. asperellum*	*T. album*	*Bacillus subtilis*	*Ps. fluorescens*	Compost	Control
1	234.433	+	+	+	+	+	+	+
2	159.517	+	-	-	-	-	-	-
3	155.793	-	+	+	+	+	+	+
4	133.304	-	+	+	+	+	+	+
5	118.734	-	-	+	-	-	-	-
6	112.191	+	+	+	+	+	+	+
7	91.783	-	-	-	-	-	-	-
8	85.101	+	+	-	+	+	+	+
9	54.844	+	+	+	+	+	+	+
10	50.017	+	+	+	+	+	+	+
11	46.267	+	+	+	+	+	+	+
12	41.996	-	-	-	-	-	+	+
13	41.699	+	+	+	+	+	+	+
14	37.23	-	-	-	-	+	-	-
15	34.93	-	-	-	-	-	+	-
Total		8	9	9	9	10	11	10

+ = bands appeared - = bands disappeared

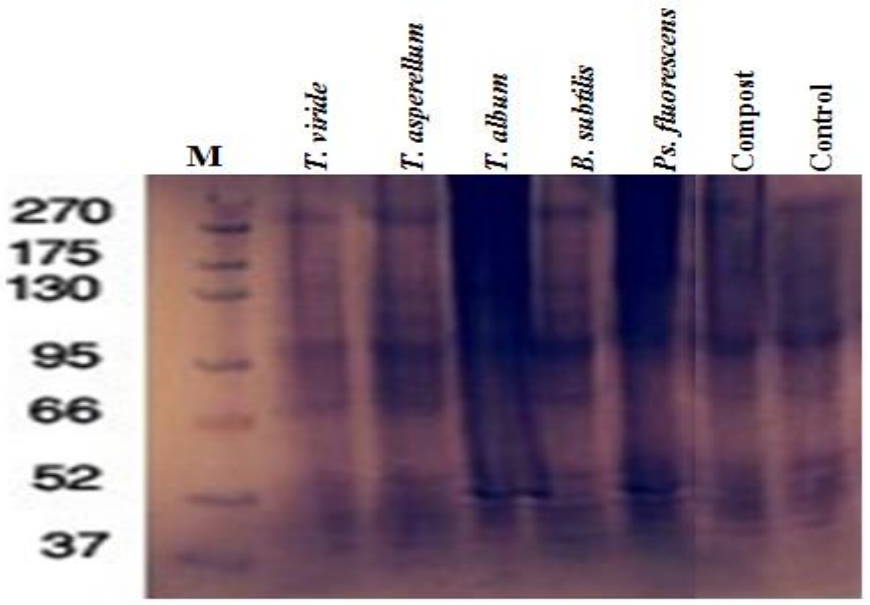

Fig. 2. Effect of treating cucumber plants with some bioagents on PAGE of protein Electrophoretic analysis of peroxidase isozymes

Table 8. Effect of bioagents and compost alone or in combination on Peroxidase isozymse activity

Peoxidase	Relative Mobility	*T. viride*	*T. asperellum*	*T. album*	*Bacillus subtilis*	*Ps. fluorescens*	Compost	Control
Px 1	0.55	1^{++}	1^{++}	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}
Px 2	0.65	1^{++}	1^{++}	1^{+}	1^{++}	1^{+}	1^{+}	1^{+}
Px 3	0.75	1^{++}	1^{++}	1^{++}	1^{++}	1^{+}	1^{++}	1^{++}
Px 4	0.85	1^{+}	1^{+}	1^{+}	1^{+}	$\mathbf{1}^{-}$	$\mathbf{1}^{-}$	$\mathbf{1}^{-}$

Peroxidase isozymes results of enzyme extract from cucumber plants treated with *T. viride, T. asperellum, T. album, Ps. fluorescens, B. subtilis* and Compost compared with control and planted in inoculated soil with *S. sclerotiorum* presented in Table 8 and Fig. 3 showed different PO patterns and induced the density of PO isozymes. Moreover, the increased density of the induced PO was found in *T. viride, T. asperellum* and *B. subtilis* compared with other treatments and control. Also, band 4 was low density in *Ps. fluorescens,* compost and control treatments.

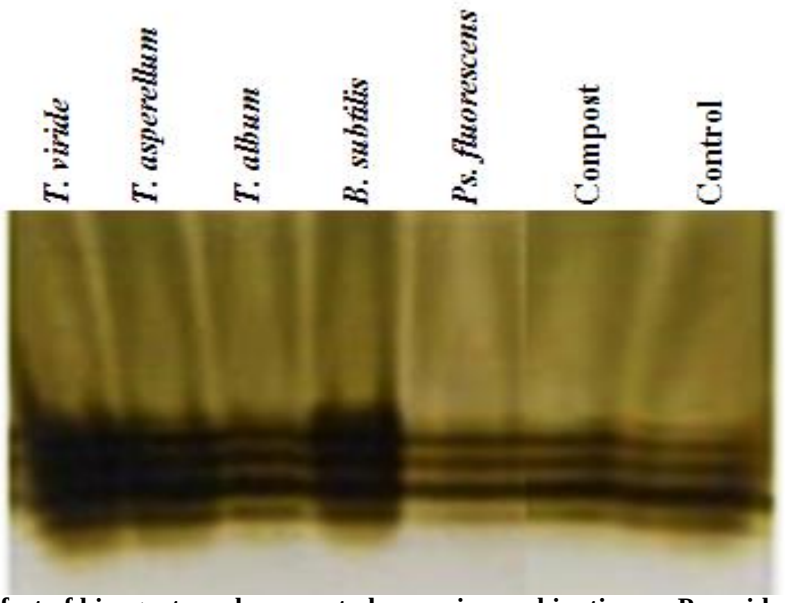

Fig. 3. Effect of bioagents and compost alone or in combination on Peroxidase isozymse

DISCUSSION

Trichoderma asperellum was the most effective antagonistic fungus and *Ps. fluorescens* was the best antagonistic bacteria inhibiting the radial growth of *S. sclerotiorum in vitro*. These results agree with those of Abdullah *et al.*, (2008), Amer *et al.*, (2010), Baharlouei *et al.*, (2011) and Saraf *et al.*, *(*2014). *Trichoderma album* was the most antagonistic fungus in inhibiting mycelial growth of *S. sclerotiorum* followed by *Trichoderma lignorum. Bacillus subtili*s was the best antagonistic bacteria in reducing growth of *S. sclerotiorum* followed by *Pseudomonas fluorescens*. Ahmed *et al., (*2017) reported that under greengouse conditions, integrated treatments of bioagents with compost were more effective than individual treatment. In the soil amended with compost, the highest decrease in disease severity was recorded by *T. album* and *T. asperellum* followed by *Ps. fluorescens*. Under protected house conditions all tested treatments at both seasons of 2017-2018, significantly decreased the percentage of white mould severity compared to the control. Adding compost to the soil increased the efficacy of all treatments. *Trichoderma viride* + *T. asperellum* + *T. album* was the most effective treatment resulted in reducing disease severity during both seasons followed by *T. viride* + *T. album* and *T. asperellum* + *T. album*. Compost individually was the least effective in both seasons. The current results are in harmony with those obtained by Ahmed *et al., (*2017). Under field conditions, adding compost to the soil before transplanting decreased the percentage of *S. sclerotiorum* infection and increased the yield. Integration of *T. album* + *B. subtilis* + *Ps. fluorescens* and compost was the most effective treatment and decreased disease incidence and severity of *S. sclerotioum* and also increased fruit weight per plant. The effect of organic amendments, suggests that both chemical and biological components of compost-amended soils can contribute to disease suppression (Abbasi *et al.*, 2002; Metcalf *et al*., 2004; Elkobrosy *et al.*, 2020 and Youssef *et al.*, 2020). Addition of 10% compost to soil significantly decreased diseases such as Aphanomyces root rot of peas; Rhizoctonia root rot of bean, cotton, and radish, Sclcrotinia drop of lettuce, Fusarium wilt of cucumber and Phytophthora crown rot of pepper (Lumsden *et al.*, 1983).

All treatments increased the activity of peroxidase, polyphenol-oxidase and PAL enzymes in comparison with the untreated control. *Trichoderma viride, T. asperellum* and *T. harzianum* + Compost were the superior for increasing the activity of the peroxidase enzyme respectively. Meanwhile, *Ps. fluorescens* + compost followed by *T. album and Bacillus subtilis* + compost were the most effective treatments and recorded the highest activity of polyphenoloxidase respectively. On the other hand, *Bacillus subtilis* + compost, *T. album* + compost and *Serratia marcescens were the most effective in increasing the activity of* PAL, respectively. The highest activity of chitinase was induced by *Bacillus subtilis* and *Ps. fluorescens* + Compost and *T. asperellum* respectively. Meanwhile, the highest increase of β-1,3-glucanase was recorded by

T. asperellum + Compost followed by *Ps. fluorescens* and *T. viride* respectively. The profiling of peroxidase isoenzymes was induced in treated cucumber plants. Results of SDS (PAGE) of protein showed that 15 protein bands with molecular weights ranging from 234.433 to 34.93 kDa were contained in cucumber plants. New protein bands were expressed as a result of treating cucumber plants with the bioagents. One band with 37.23 kDa was appeared in plants treated with *Ps. fluorescens*, while absent in other treated plants. Band with 159.517 kDa appeared in plants treated with *T. viride* but was absent in the other treated plants. A band with 34.93 kDa appeared in plants cultivated in soil amended with compost while absent in all treated plants. These results are in harmony with those recorded by Ahmed and El-Sisi (2020). Ahmed *et al., (*2017), found that**,** treating tomato plants with bioagents increased the activities of Peroxidase (PO), Polyphenoloxidase (PPO), Chitinase and β-1,3 glucanase enzymes in leaves of plants. Also, they reported that adding compost to the soil increased the effectiveness of bioagents. Ahmed, (2016) found that, treating bean seeds with bioagents caused considerable increase in the activities of peroxidase, polyphenol oxidase, chitinase and β-1,3-glucanase enzymes that play a significant role in plant defense mechanisms against pathogens infection. Results indicated that an increase in activity of the treated snap bean enzymatic activity of treated the snap bean plants. Oxidative enzymes such as peroxidase and polyphenol oxidase enhance formation of lignin, while other oxidative phenols contribute in formation of defence barriers for reinforcing the cell structure (Avdiushko *et al.,* 1993). Chitinase and β-1,3 glucanase enzymes play a significant role in plant defense against fungi by hydrolysing their cell wall (Barilli *et al.,* 2010).

CONCLUSIONS

Based on obtained results from the current study, both tested bio control agents i.e. *Trichoderma* isolates and bacterial strains were able individuality or in combination to affect negatively the development of *Sclerotinia sclerotiuorum* fungus on cucumber plants and led to significant increase in yield. Compost addition enhanced the biocontrol activity of used biological control agents.

REFERENCES

Abbasi, P. A., J.Al-Dahmani, F.Sahin, H. A. J. Hoitink and S. A. Miller. 2002. Effect of compost amendments on disease severity and yield of tomato in conventional and organic production systems. Plant disease. 86(2):156-161.

Abdelsalam, N. R., H. M.Ali, M. Z.Salem and H. E. El-Wakil. 2020. Quantitative and Qualitative Genetic Studies of Some Acacia Species Grown in Egypt. Plants. 9(2): 243.

Abdeljalil, N.Ouhaibi-Ben, J.Vallance, J.Gerbore, P. Rey and M.Daami-Remadi. 2016. Bio-suppression of Sclerotinia stem rot of tomato and biostimulation of plant growth using tomato-associated rhizobacteria. J Plant Pathol Microbiol. 7(331):2.

Abd-El-Moity, T.H. 1985. Effect of single and mixture of *Trichoderma harzianum* isolates on controlling three different soils -borne pathogens. Egypt. J. Microbiol. 111-120.

Abdullah, M.T., Y.Nida, N.Y. Ali and P.Suleman. 2008. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens.* Crop Protection. 27:1354– 1359.

Abhiniti. M., A.Tripti and P.C. Trivedi. 2011. *In vitro* efficacy of various fungal and bacterial antagonists against *Rhizoctonia solani*, causal Agent of Disease in *Capsicum Annuum* L. International J. of Pharma and Bio Sci. 2 (3): 288-292.

Ahmed, G.A. 2016. Efficiency of some antioxidants and bioagents in controlling Rhizoctonia damping-off of snap bean. Middle East J. of Applied Sci. 6(4): 748-758.

Ahmed, G. A. and A. A. Elsisi. 2020. Efficacy of compost and some essential oils alone or in combination in controlling cucumber white mould disease under protected house conditions. J. of Plant Protection and Pathology, Mansoura Univ. 11 (6):291-297.

Ahmed, G.A., A.M.M.Mahdy, R.N. Fawzy and N.A. Gomaa. 2017. Integrated management of tomato sclerotinia rot disease by using the combined treatments between compost, bioagents and some commercial biocides. Benha J. of Applied Sci. 2 (1):9-22.

Allam, A.I. and J.P. Hollis. 1972. Sulfide inhibition of oxidase in rice roots. Phytopathology. 62: 634-639.

Amer, M.A. I.I.A. Abou elseoud, M.R. Rasmy and M.M. Khater. 2010. Effects of a mycorrhiza fungi, bacteria, and yeast as biological control of *Sclerotinia sclerotiorum* on the growth of common bean (*Phaseolus vulgaris* L.). Alexandria sci. exchange j. 31(4): 339-352.

Avdiushko, S.A., X.S. Ye and J. Kuc. 1993. Detection of several enzymatic activities in leaf prints of cucumber plants. Physiol. and Mol. Plant Pathol. 42: 441-454.

Baharlouei, A., G.R. Sharifi-Sirchi and G.H.S. Bonjar. 2011. Biological control of *Sclerotinia sclerotiorum* (oilseed rape isolate) by an effective antagonist Streptomyces. African J. of Biotechnology. 10(30): 5785-5794.

Barilli, E., E.Prats and D.Rubiales. 2010. Benzothiadiazole and BABA improve resistance to *Uromyces pisi* (Pers.) Wint. In *Pisum sativum* L. with an enhancement of enzymatic activities and total phenolic content. Eur. J. Plant Pathol. 128: 483-493.

Berry, C., W.G.D.Fernando, P.C. Loewen and T.R.Kievit. 2010. Lipopeptides are essential for Pseudomonas sp. DF41 biocontrol of *Sclerotinia Sclerotiorum*. Biol Control. 55: 211-218.

Bolan, G.J. and R. Hall. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. Can J Plant Pathol. 16: 93108.

Boller, T and F. Mauch. 1988. Chitinase from *Phaseolus vulguris*, leaves". Meth. Enzymol. 161: 479 – 484.

Brown, W. 1924. Two mycological methods: a method of isolating single strains of fungi by cutting out a hyphal tip. Ann Bot. 38: 402-404.

Dickerson, D.P., S.F.Pascholati, A.E.Hagerman, L.G.Butler and R.L. Nicholson. 1984. Phenylalanin ammonia-lyase and hydroxy cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium carbonum*. Physiol Plant Pathol. 25:111–123.

Domenech, J., M.S.Reddy, J.W.Kloepper, B.Ramos and J. Gutierrez-Manero. 2006. Combined application of the biological product LS213 with Bacillus, Pseudomonas or Chryseobacterium for growth promotion and biological control of soil-borne diseases in pepper and tomato. BioControl. 51: 245258.

Elkafrawy, A. 2008. Biological control of white rot of cucumber caused by *Sclerotinia sclerotiorum* under greenhouse conditions. J. of Agricultural Research. 86(2):427-439.

Elkahoui, S., N.Djebali, I.Karkouch, A.Hadj Ibrahim and L. Kalai. 2014. Mass spectrometry identification of antifungal lipopeptides from *Bacillus sp*. BCLRB2 against *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Appl Biochem Microbiol. 50: 161-165.

Elkobrosy, D. H., D. G.Aseel, N. R.Abdelsalam, M. A.El-Saedy, S. Shama and E. E. Hafez. 2020. the effect of cyst nematode (globodera rostochiensis) isolate ddh1 on gene expression in systemic leaves of potato plant. J. of microbiology, Biotechnology and Food Sci. 10(1):93-97.

Helmy, G.K. 2016. Screening Potential of Some Bacterial Species and *Trichoderma hrzianum* Against *Sclerotinia sclerotiorum* on Cucumber. J. Plant Prot. and Path., Mansoura Univ. 7 (12): 506-872,2016

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680-685.

Li, G.Q., H.C.Huang and S.N. Acharya. 2003. Antagonism and biocontrol potential of *Ulocladium atrum* on *Sclerotinia sclerotiorum*. Biol Control. 28: 11-18.

Lu, G. 2003. Engineering *Sclerotinia Sclerotiorum* resistance in oilseed crop. African J. of Biotechnolog. 2(12): 509-516.

Lumsden, R.D., J.A. Lewis and P.D. Millner. 1983. Effect of composted sewage sludge on several soilborne pathogens and diseases. Phytopathology. 73: 1543-1548.

Matta, A. and A.E. Dimond. 1963. Symptoms of Fusarium wilt in relation to quantity of Fungus and enzyme activity in tomato stems. Phytopathology. 53: 574-587.

Maurhofer, M., C.Keel, D.Haas and G.De´fago. 1995. Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. Plant Pathol. 44:40–50.

Metcalf, D.A., J.J.C. Dennis and C.R. Wilson. 2004. Effect of inoculum density of *Sclerotium cepivorum* on the ability of *Trichoderma koningii* to suppress white rot of onion. Plant Disease. 88: 287-291.

Paris, H.S., M. Daunay and J. Janick. 2011. Occidental diffusion of cucumber (*Cucumis sativus*) 500-1300 CE: two routes to Europe. Annals of Botany. 1:10.

Purdy, L.H. 1979. *Sclerotinia sclerotiorum*: History, diseases and symptomatology, host range, geographic distribution, and impact. Phytopathology. 69: 875880.

Rodriguez, M.A., C.Rothen, T.E.Lo, G.M.Cabrera and A.M. Godeas. 2015. Suppressive soil against *Sclerotinia sclerotiorum* as a source of potential biocontrol agents: selection and evaluation of *Clonostachys rosea* BAFC1646. Biocontrol Sci Technol. 25: 1388-1409.

Saraf, M., U. Pandya and A. Thakkar. 2014. Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. Microbiol. Res. 169: 18-29.

Sindhu, J.S., S.Ravi and J. L. Minocha. 1984. Peroxidase isozyme patterns in primary trisomics of pearl millet. Theoretical and Applied Genetics.68:179-182.

Singh, R.S. 1982. Plant Pathogens "the fungi" Oxford and IBH Publishing Co. New Delhi, Bombay Calcuta. pp: 443.

Snedecor, G.W. and W. G. Cochran. 1989. Statistical Methods, 8th Edition, Iowa State University Press. Ames.

Sun, H., J.Yang, C. Lin, X. Huang, R.Xing and K.Q. Zhang. 2006. Purification and properties of a β-1,3-glucanase from *Chaetomium* sp. that is involved in mycoparasitism. Biotechnology Letters. 28:131-135.

Tian, S., Y.Wan, G. Qin and Y.Xu. 2006. Induction of defense responses against Alternaria rot by different elicitors in harvested pear fruit. Applied Microbiol.Biotechnol. 70:729-734.

Tuzun, S., M.N.Rao, U.Vogli, C.L. Schardl and J.A. KU. 1989. Induced systemic resistance to blue mold, early induction and accumulation of B, 1, 3-gluconases chitinases and other pathogenesis –related proteins (b-proteins) in immunized tobacco. Phytopathology.79:979-983.

Youssef, N. H., A. A.Al-Huqail, H. M. Ali, N. R.Abdelsalam and M. A. Sabra. 2020. The role of Serendipita indica and Lactobacilli mixtures on mitigating mycotoxins and heavy metals' risks of contaminated sewage sludge and its composts. Scientific Reports. 10(1), 1-13.

Zeng, W., W.Kirk and J. Hao. 2012. Field management of Sclerotinia stem rot of soybean using biological control agents. Biological Control. 60(2): 141-147.

الملخص العربي

فعالية السماد العضوي (الكمبوست) وبعض عوامل المكافحة الحيوية في مكافحة مرض العفن الابيض في الخيار تحت ظروف البيوت المحمية

جمال عاشور أحمد، عبير حمدي مخلوف و محمد علوي سليم

تم استخدام خمسة أنواع مختلفة من فطر *Trichoderma* وثلاثة أجناس بكتيرية مختلفة هي (*Serratia marcescens*، *Bacillus subtilis*، *Pseudomona fluorescens*) إما بشكل منفصل أو مع السماد العضوي لتقييم إمكانات المكافحة الحيوية ضد فطر *Scelotenia sclerotiurum* سواء في المعمل أو الصوبة. أظهرت النتائج أن جميع العوامل الحيوية المختبرة أثرت بشكل كبير على نمو المسبب المرضي تحت الظروف المعملية. سجل أعلى انخفاض مع عزلة *T. asperellum* وكان (٦١.٨٥٪) يليه *T. Album* الذي أدى إلى تثبيط النمو بنسبة ٦٠.٧٤٪. كان *T. viride* + *T. asperellum* + *T. Album* الأكثر فاعلية في تقليل شدة المرض خلال الموسمين وزيادة الإنتاجية حتى ٢.٥ كجم / نبات. تم دراسة تأثير البكتيريا والفطريات المختبرة على نشاط إنزيمات التحلل الأساسية مثل الكيتينز وبيتا ١،٣–جلوكاينيز. أظهرت النتائج أن كلا من العوامل الفطرية والبكتيرية المختبرة بمفردها أو مجتمعة مع السماد العضوي أثرت بشكل إيجابي على نشاط الإنزيمين. وقد لوحظت نتائج مماثلة فيما يتعلق بنشاط ثلاثة إنزيمات مضاده للاكسده مختلفة مثل البيروكسيديز والبوليفينول أوكسيديز والـ PAL. كما تم زيادة نشاط مشابهات أنزيم البيروكسيديز في نباتات الخيار المعاملة. علاوة على ذلك ، أوضحت النتائج أن معاملة نبات الخيار بالعوامل الحيوية ادي الي ظهور حزم بروتينية جديدة.