Potential impacts of bioagents to improve Strawberry to plant disease resistance

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Abstract

The current study was conducted to investigate the ability of two fungal strains Trichodermaharzianum (T1), T.viride (T2) and two bacterial strains, Bacillus subtilis (B1), Streptomyces griseus (S1) to antagonize Macrophomina phaseolina the causal agent of Strawberry (Fragaria ananossa Duchesne) root rot disease. Induction of plant defense to the pathogen was also studied. Disease symptoms, disease index and phytochemical indicators of plant resistance were recorded.

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All experiments were carried out at Techogreen Agricultural Company, Ismailia, Egypt.

Maximum growth inhibition of M. phaseolina was achieved by T. harzianum(2.16 mm)followed by T.viride (T2)(2.66mm) and B. subtilis (3 mm), meanwhile, S. griseus recorded the lowest antagonistic effect (3.16 mm).

T. viride as well as, B. subtilis showed highly significant reduction in percentage of disease infection 10 %, followed by Streptomyces griseus and T. harzianum (20 %), compared to 70% of infected control. Application of all tested inducers caused highly significant increases in chlorophyll A and chlorophyll B as well as carotenoids. The positive responses of the tested elicitors were extended to increase total phenol and activities of antioxidant enzymes (superoxide dismutase, Peroxidase, Polyphenol oxidase and catalase). At the same time, the results showed that strawberry infected plants treated with elicitors showed variation in number, molecular weight of protein bands as well as variation in number, relative mobility and density of enzymes bands of polypeptide peroxidase and polyphenol oxidase.

Key words: *Strawberry plant– Macrophomina phaseolina– Trichoderma harzianum- Bacillus subtilis– Antioxidant enzymes-Disease symptoms-Disease index.*

1. Introduction

Strawberry (*Fragari ananassa* Duchesne) is a member of Rosaceae family which is considered as essential plant that provides basic fruit. Strawberries have been grown worldwide since fruits are nutritious and rich in vitamin C, flavonoids, ellagic acid and autocianidin **(Suvalaxmi*et***

***al.*, 2015).** Phytochemicals and antioxidants present in strawberry fruits decrease the hazard of cardiovascular disease and tumorogenesis **(Hannum, 2004).** Crown and root rots are important diseases of commercial strawberry farms. Several fungi have been reported as causal agents for strawberry crown and root rots. Strawberry plants reported to be infected by soil-borne pathogens causing root and crown rots**(Fang *et al.*, 2012**). Black root rot is a complex disease caused by one or more of fungal pathogens, including Fusarium oxysporum (**Juber et al., 2014**), Macrophomina phaseolina(**Hutton etal., 2013**), Phytophthora spp. (**Mingzhu, 2011**) and Rhizoctonia spp. (**Fang et al., 2013**). This complex disease is responsible for host, deterioration and blackening of the main root system, decline of vigorous and decreased productivity and consequently considerable reduction in yield(**Abdel-Sattaret al; 2008**).

Disease inhibition by using biotic elicitors is the sustained terms of interactions among the host, the pathogen and the biotic elicitors originating from the microbial community around the plant and the physical environment **(Barea *et al.*, 2004).** Applications of biological control using microorganisms possess antagonistic activities has a potential for the management of many plant diseases, use of antagonist mixtures may also provide improved disease control over the use of single organisms **(Moretti *et al.*, 2008) .** The induction of systemic resistance using plant growth microorganisms (PGPM) is also a form of biological control **(Schouten *et al.*, 2004).** Use of *T. harzianum* and *T. viride* bioagents as seedling dressing to control strawberry root rot and to increase yield components has long been tried (**Sullivan, 2004**), This synergistic dual effect was attributed to production of growth regulators (**Karlidag *et al.*, 2012**) in addition to the chemical effects of antioxidant which play a clear role in improving plant

physiology and metabolism (**Hernandez *et al.*, 2011**). *Trichoderma asperellum*, Bacillus megaterium and B.laterosporus showed in vitro significant inhibition exceeded 36% in radial growth of M. phaseolina and F.solani. T.asperellum was also the most effective for reduction of crown and root rot caused by *F. solani* (up to 100% in greenhouse and up to 81% under field conditions), high plant protection and disease reduction were combined with increased amount of total phenols and proteins in addition to, increased amount of total sugars and chlorophyll was reported (**Pastrana *et al.*, 2016**) .The **p**lant growth promoting rhizo-bacteria (PGPR) have another mechanisms including hormonal and nutritional balance, induced resistance against plant pathogens, and enhance nutrient uptake of solubilized nutrients by plants, in addition, PGPR shows synergistic and antagonistic interactions with microorganisms within the rhizosphere which indirectly enhance the growth of plant (**Vejan *et al.*, 2016**). *Macrophomina phaseolina* is considered among the most difficult diseases to control (**Pastrana *et al.*, 2016**). Thus, the objectives of this study were to evaluate the abilities of two fungal strains, namely Trichoderma harzianum (T1), T. viride (T2) and two bacterial strains, Bacillus subtilis (B1), Streptomyces griseus (S1) to antagonize M. phaseolina the causal agent of Strawberry (Fragaria ananossa Duchesne) root rot disease and their potential to Induce plant defense machinery against the pathogen.

2. Materials and Methods:

2.1. Plant material and growing conditions:

Strawberry transplants **(**Fortuna cultivar) were obtained from agricultural research center (ARC), ministry of agriculture, Giza,

Egypt. All strawberry transplants received the same fertilizers and irrigation regime

2.2. **Source, isolation of pathogen and antagonistic bioagents.**

According to survey studies; the most frequent and aggressive pathogens affecting strawberry plant was *M. phaseolina* , hence, selected for this studies. *M. phaseolina* isolate of high virulence and four antagonistic moicrooganisms, namely *T. harzianum* (T1) *T. viride* (T2) *B. subtilis* (B1), *S. griseus* (S1) were kindly obtained from by Prof. Abdel Moiety, T. H., Central Lab. of Organic Agriculture, Plant Pathology Research Institute, Agr. Res. Center, Giza, Egypt. These antagonists were isolated from soil samples of various farm fields at Agriculture Research Centre (ARC) Giza Egyp and tested for their efficacy against the selected pathogenic fungus *M. phaseolina* by soil plate methods, using potato dextrose agar (PDA) as described by (**Dhingra and Sinclair, (1995)**. Isolates were maintained on PDA slants and stored at 4°C. . *B. subtilis* (B1), *S. griseus* (S1) showed high antifungal activities as well as maximum hydrolysis zone values of gelatinase, protease and chitinase activities (**Bahloul, 2013**). Bacterial species were identified by Bio-log Technique, inocula suspensions were approximately adjusted to 10^9 CFU/ml culture (colony forming unit).

2.3. **Field experiments:**

Applied elicitors were added at one week prior to infection with M. phaseolina. The field trials were conducted at the Experimental garden of Techogreen Agricultural, Ismailia, Egypt in 2019. Seedlings were planted in 6 groups as following; :(1) plants without any treatments were referred as healthy control, (2) plants infected with M. phaseolina as infected control, (3) plants treated with T. harzianum (T1) then infected with M.

phaseolina, (4) plants treated with T. viride (T2) then infected with M. phaseolina,(5) plants treated with B. subtilis(B1) then infected with M. phaseolina,(6) plants treated with S. griseus (S1) then infected with M. phaseolina. Disease development was recorded 15 days after inoculation. Disease severity was recorded. The Plant samples were collected for biochemical indicators for resistance analysis when the plants were four months old.

2.4. **Disease symptoms and Disease index:**

Disease symptoms were assessed 60 days after inoculation and the disease index was evaluated according to **(Demir *et al*.,2006)** with slight modifications using score consisting of five classes: 0 (no symptoms), 1 (slight yellow of lower leaves), 2 (moderate yellow plant), 3 (wilted plant with browning of vascular bands), 4 (plants severely stunted and destroyed). Percent Disease index (PDI) was calculated using the five-grade scale according to the formula: PDI= $(1n_1+ 2n_2 + 3n_3 + 4n_4)100/4n_t$.Where n_1-n_4 the number of plants in the indicated classes, and N_t total number of plants tested.

2.5. **Analysis of plant metabolic and biochemical resistance indicators:**

Determination of pigments: The method used for the quantitative determination of pigments was according to **Vernon and Selly, (1966).** Determination of phenolic compounds (mg/100g of dry wt) was carried out according to **Daniel and George (1972).** Peroxidase activity was assayed according to (**Srivastava, 1987**). The activity of polyphenol **oxidase** enzyme was determined according to the method adopted by **Matta and Dimond (1963).** Protein fingerprint was analyzed using Sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS - PAGE) according to **Studier (1973)**. Native-polyacrylamide gel

electrophoresis (Native-PAGE) was conducted to identify isozyme variations among the studied plants using two isozyme systems according to **Stegmann *et al*., (1985)**. Peroxidase (Px) isozyme was determined according to the method of **(Brown, 1978).** Polyphenoloxidase (PPO) isozyme was determined according to the method of **Baazi*et al*., (1994).**

2.6. **Statistical analyses.**

Experimental data were subjected to one-way analysis of variance (ANOVA) and the differences between means were separated using Duncan's multiple rang test and the (L.S.D) at 5% level of probability using Co-state software **(Snedecor and Cochran, 1982)**.

3. **Results:**

Effect of antagonistic bioagents on the linear growth of ***M. phaeseolina.***

Results in Table (1) showed variations in antagonism among all the antagonistic bioagents. Maximum growth inhibition of tested pathogen was achieved by *T. harzianum* (2.16 mm) followed by *T. viride* (T2) (2.66 mm) and B. *subtilis* (3 mm). The lowest antagonistic effect (3.16 mm) was recorded for *S. griseus* treatment.

3.1. **Effect of elicitors on disease assessment of infected strawberry plants.**

Application of tested elicitors highly reduced the incidence of disease symptoms caused by *M. phaseolina* compared to untreated inoculated infected control plants table (1). Disease progress was highly affected in plants treated with bioagents. Significant high reduction of disease infection (10%) was recorded for plants treated with *T. viride, B. subtilis* followed by (20%) for plants treated with *S. griseus, T. harzianum* compared to (70%) of control plants infected with *M. phaseolina.*

Table 1 Control of root rot disease caused by M. phaseolina using tested inducers

Treatment	Disease index DI (%)	Infection (%) (DI/4Nt) ×100
Pathogen	70	58
M+T1	20	8
M+B1	10	2
M+T2	10	6
M+S1	20	14

DI= Disease index

Pathogen= *M. phaseolina* M+T1 = *M. phaseolina +T.harzianum* T1 M+B1 = *M. phaseolina +B.subtitles* *(B1)* M+T2 = *M. phaseolina +T. viride T2* M+S1 = *M. phaseolina + S. griseus.*

3.2. **Physiological and metabolic changes**:

Results showed highly significant reduction in photosynthetic pigments of strawberry plants infected with *M. phaseolina*. All applied elicitors showed considerable increase in chlorophyll (a) content of infected strawberry plants compared to the infected control (untreated). The highest significant increase in chlorophyll (a) contents was reported in infected strawberry plants treated with *S. griseus*, *T. viride* and *B. subtitles*, respectively, while the lowest was reported for *T. harzianum* treatment. On the other hand, significant difference in chlorophyll (b) content was recorded in strawberry plant shoots treated with *S. griseus*, *T. harzianum*, compared to all other treatments including the infected control strawberry plants. .Marked increase in carotenoides of the infected plants treated with *T. harzianum* and *B. subtilis* was reported compared to control treatment (Table 2).

Significant increase in total phenol contents as well as antioxidant enzyme activities (superoxide dismutase, Peroxidase,

Polyphenol oxidase and catalase) was reported in strawberry shoots infected with *M. phaseolina*. The highest increase in total phenols was reported for *S. griseus* ,*T.harzianum* and *T.viride*. The lowest increase in total phenols was observed in plnats treated with *B. subtilis.* (Table 3)

Application of *S. griseus, T. harzianum* and *T. viride* significantly increased SOD activity of shoots respectively, while B. subtilis was the least effective in SOD increased activity. These observations increased were found to be statistically significant (with one exception, M. phaseolina-infected plants treated with applied inducers showed significant increase in peroxidase activity). Nevertheless, non-significant decrease in peroxidase activity was observed in plants treated with *B. subtilis. S. griseus, T. viride and T. harzianum* respectively (Table 3).

The results of the present work (Table 3) revealed that, all applied elicitors *significantly increased PPO activity compared to infected control. It was found that S.griseus,T.harzianum and T.viride increased PPO and CAT activities, followed by B. subtilis* with the least effect on increased PPO and CAT activities.

Table 2 Effect of elicitors on photosynthetic pigments of infected strawberry plants:

Treatment	Chlorophyll (a)	Chlorophyll (b)	Carotenoids
control	0.67 b	1.08 a	0.09 d
Pathogen	0.51 e	0.51 d	0.63 a
M+T1	0.42 f	0.623 c	0.41 b
M+B1	0.55 d	0.52 d	0.28 c
M+T2	0.59 c	0.53 cd	0.125 d
M+S1	0.84 a	0.82 b	0.094 d
LSD at % 5	0.02	0.09	0.04

Table 3 Effect of Bio-elicitors on the total soluble protein contents and Phenolic compounds of strawberry plant infected with M. phaseolina

Treatment	Phenols(mg/100g)dry weight	superoxide dismutase SOD (unit/g)fresh weight/ hour	Peroxidase POD (unit/g)fresh weight/ hour	Polyphenol oxidase PPO (unit/g)fresh weight/ hour	Catalase CAT (unit/g)fresh weight/ hour
control	0.26 c	0.73 e	0.047 c	0.73 e	0.14 c
Pathogen	0.26 c	0.92 d	0.064 c	0.92 d	0.16 c
M+T1	0.34 ab	1.24 c	0.147 b	1.24 c	0.44 ab
M+B1	0.26 c	0.95 d	0.05 c	0.95 d	0.19 c
M+T2	0.32 b	1.35 b	0.16 ab	1.35 b	0.48 ab
M+S1	0.35 a	1.56 a	0.20 a	1.56 a	0.62 a
LSD at % 5	0.035	0.06	0.05	0.06	0.16

Control= healthy, Pathogen = M. phaseolina M+T1 = M. phaseolina +T. harzianum T1 M+B1 = M. phaseolina +B. subtilis (B1) M+T2 = M. phaseolina+T. viride T2 M+S1 = M. phaseolina +S. griseus S1.

Variants possessing different letters are statistically significant $P<0.05$.

3.3. Expressed protein as a response to induction of Systemic Resistance:

Induction of systemic resistance

Strawberry plants treated with biotic inducers and inoculated with *M. phaseolina* showed variation in number and molecular weight of protein bands. The variability analysis among tested inducers developed 87 protein bands with molecular weight range 16-180 KDa Fig(1) and Table (4) , there were was one band absent in (control), and (*B. subtitles*) and (*T. viride*) compared with all other samples at 123 KDa respectively. On the other hand, all bands were present in all samples.

Table 4 Protein fractions in leaf of Macrophomina phaseolina infected strawberry plants treated with biotic inducers using SDS-PAGE.

Band No.	M.W KDa	control	Pathogen	M+T1	M+B1	M+T2	M+S1
1	180	1	1	1	1	1	1
2	123	1	0	0	1	1	0
3	120	1	1	1	1	1	1
4	96	1	1	1	1	1	1
5	82	1	1	1	1	1	1
6	75	1	1	1	1	1	1
7	72	1	1	1	1	1	1
8	70	1	1	1	1	1	1
9	63	1	1	1	1	1	1
10	62	1	1	1	1	1	1
11	34	1	1	1	1	1	1
12	28	1	1	1	1	1	1
13	19	1	1	1	1	1	1
14	18	1	1	1	1	1	1
15	16	1	1	1	1	1	1
Total		15	14	14	15	15	14

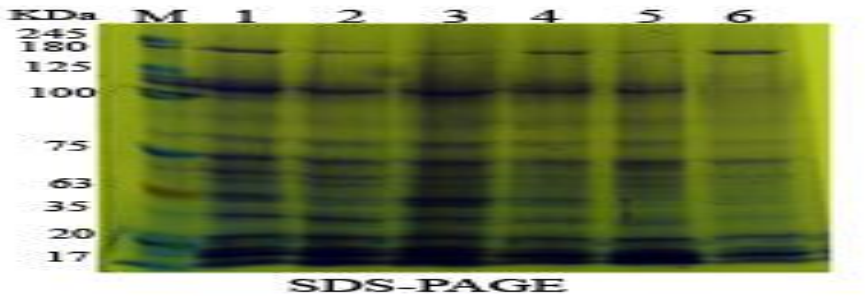

Figure 1 Protein fractions of strawberry plants treated with biotic inducers using SDS-PAGE M: Marker. Control = healthy.

Pathogen= M. phaseolina M+T1 = M. phaseolina +T. harzianum T1 M+B1 = M. phaseolina +B. subtilis (B1) M+T2 = M. phaseolina + T. viride

T2 M+S1 = M. phaseolina + S. griseus S1. 0 =Absence of band and 1 = presence of band

3.4. **Oxidative enzymes:**

Peroxidase isozyme: Results showed variations in number, relative mobility, and density of polypeptide bands in infected and healthy plants treated with inducers. Peroxidase isozyme profile in Fig (2) and Tab (5) represented five peroxidase isozymes with differences in banding density, px1 were different in density in (control) and (S. griseus) with low density and all other samples exhibit moderate density at with relative mobility 0.10. In Px 2 , Px3, Px4 and Px5 with relative mobility 0.20, 0.30, 0.45 and 0.80. All samples were different in banding density.

Table (5): Disc-PAGE banding patterns of peroxidase isozymes in infected strawberry plants treated with biotic inducers**.**

Table 5 Disc-PAGE banding patterns of peroxidase isozymes in infected strawberry plants treated with biotic inducers.

Peroxidase Groups	Relative Mobility (R.M)	1	2	3	4	5	6
Px 1	0.10	1^-	1^+	1^+	1^+	1^+	1^-
Px2	0.20	1^+	1^-	1^-	1^+	1^-	1^-
Px3	0.30	1^+	1^+	1^+	1^+	1^{++}	1^+
Px4	0.45	1^-	1^+	1^+	1^+	1^{++}	1^+
Px5	0.80	1^-	1^+	1^-	1^-	1^-	1^-

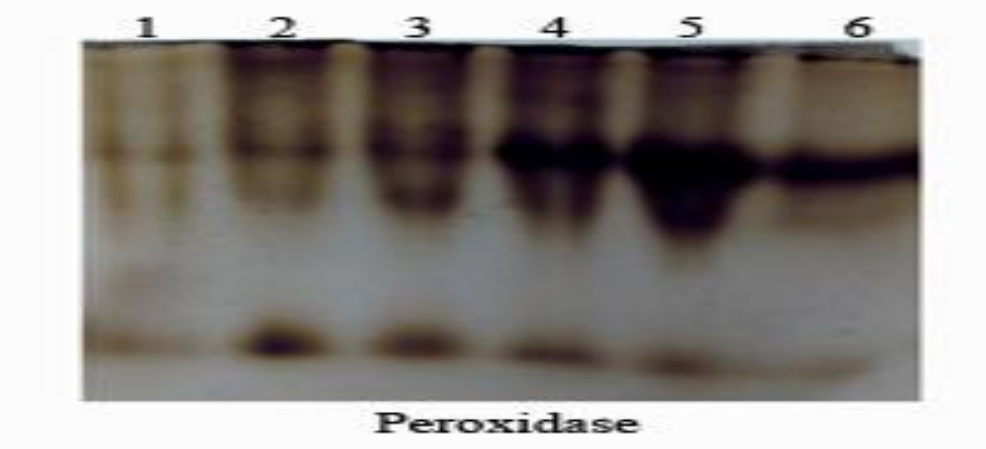

Figure 2 Pathogen= M. phaseolina M+T1 = M. phaseolina +T. harzianum

T1 M+B1 = M. phaseolina +B. subtilis (B1) M+T2 = M. phaseolina +T.

viride T2 M+S1 = M. phaseolina +S. griseus S1. 0 =Absence of band and 1 = presence of band

3.5. Polyphenol oxidase isozyme:

Applied inducers caused variations in infected and healthy controls in number, relative mobility and density of polypeptide bands. Fig (3) and Table (6) illustrated five polyphenyl Oxidase isozymes groups with differences in banding density, PPO1 represent low banding pattern density in (*T. harzianum*) while other samples showed moderate density with relative mobility 0.10. in PPO2. There were difference in density in (control) and (*T. harzianum*) with low density and all other samples were moderate density and relative mobility 0.20. On the other hand, PPO4 represented all samples were moderate in banding density except infected plants showed low density of bands, with relative mobility at 0.45. PPO2 and PPO5 illustrated differences in banding density between all six samples.

Table 6 Disc-PAGE banding patterns of peroxidase isozymes in infected strawberry plants treated with biotic inducers.

Poly Phenyl Oxidase Groups	Relative Mobility (R.M)	1	2	3	4	5	6
PPO1	0.10	1^{+}	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}
PPO2	0.20	1^{-}	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}
PPO3	0.30	1^{+}	1^{+}	1^{+}	1^{+}	1^{++}	1^{++}
PPO4	0.45	1^{+}	1^{-}	1^{+}	1^{+}	1^{+}	1^{+}
PPO5	0.80	1^{+}	1^{+}	1^{+}	1^{++}	1^{++}	1^{++}

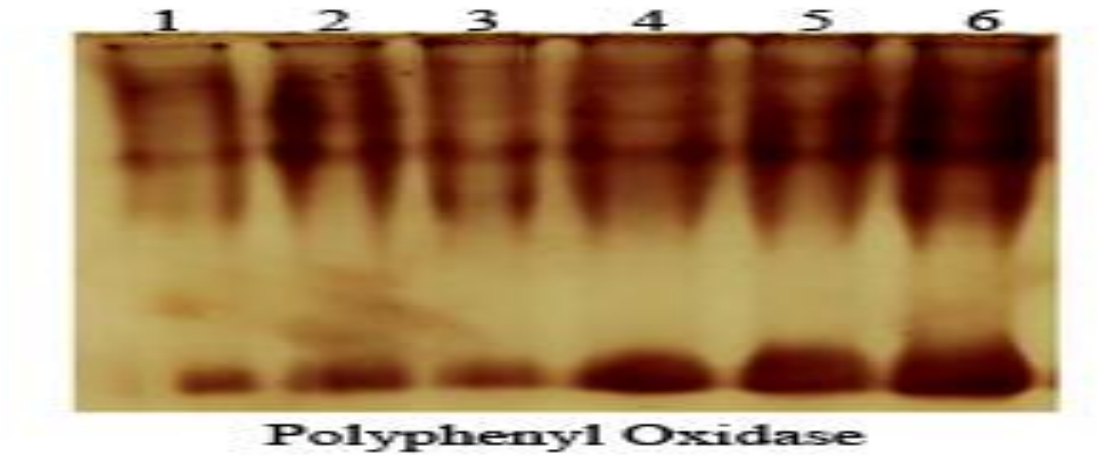

Figure 3 Pathogen= M. phaseolina M+T1 = M. phaseolina +T. harzianum T1 M+B1 = M. phaseolina +B. subtilis (B1) M+T2 = M. phaseolina +T. viride T2 M+S1 = M. phaseolina +S. griseus S1. 0 =Absence of band and 1 = presence of band

4. Discussion:

The objectives of this study were induction of systemic resistance in strawberry plants against *M. phaseolina*, the causal agent of root rot disease**.** *M. phaseolina* is a soil-borne fungus which invades vascular

system of the plant. Use of eco-friendly biotic elicitors prior to the infection stage of the plant is recommended to avoid the entire change of soil microflora associated with use of pesticide.. For this purpose, some plant growth promoting rhizobacteria (PGPR), two fungal isolates namely T. harzianum (T1) T. viride (T2) and two bacterial strains namely B. subtilis (B1), S. griseus (S1) were selected based on their ability to antagonize phytopathogens.. It has been found that all applied elicitors were effective in reducing disease index compared to the infected control. The percentage of disease index of strawberry plants treated with plant growth promoting microorganisims were reduced compared to control plants, which was similar to results obtained by (**Amusat *et al.*, 2008; Linderman, 2000**). Rhizobacterial strains and fungi used in the current study prviously showed to act as plant growth-promoting through stimulation of growth and induce systemic resistance (ISR) (Loon ,2007and Mandal and Ray, 2011).. Application of some *Bacillus* strains to the seedlings has been found effective for suppressing soil borne diseases, and successfully induced systemic resistance in the treated plants (**Kloepper *et al.*, 2004**and **Szczech and Shoda, 2007**). We have reported significant decrease in disease incidence and progress when plants were treated with *Trichoderma* isolates, our results are in agreements with the finding of Howell (2003) where application of *T. harzianum* as well as, *T. viride* showed highly significant reduction in percentage of disease infection compared to control plants infected with *Fusarium oxysporum*. These results were attributed to different mechanisms adopted by *Trichoderma* including myco-parasitism, production of antibiotic compounds, production of antifungal compounds, competition for space and nutrients (**Monteiro *et al.*, 2011** and **Balode, 2010**).

Decreased production of chlorophyll was reported in infected plant with *M. phaseolina* . presumably due to increased activity of chlorophyll degrading enzyme, chlorophyllase (**El-Shanhory *et al*., 2014** and **Farrag *et al*., 2017**). While, significant increase in the carotenoids were observed in infected plants (untreated). Results indicates that the harmful effect of *M. phaseolina infection* on photosynthetic pigments could be reduced via using of *T .harzianum, T. viride*, *B. subtilis* and *S. marcescens* inoculations, that can enrich the plant and soil with N_2 element. These findings are supported by **Abd El-Baky *et al*. (2010).**

Phenols play a significant role in the regulation of plant metabolic processes and over all plant growth as well as lignin synthesis **(Lewis and Yamamoto, 1990)**. In addition, phenols act as free radical as well as substrates for many antioxidant enzymes **(Martin- Tanguy, 2001)**. It is quite evident from the present study, that, the greatest value of total phenols was achieved by using *S. griseus*, *T. harzianum* and *T. viride*, followed by, *B. subtilis*, indicating induction of systemic acquire resistant (SAR). These are in accordance with **Sudhakar *et al*., (2007)**.

The results showed that antioxidant enzymes activity in plants infected with *M. phaseolina* and treated with bioagents were increased significantly.

Antioxidant enzymes activities (superoxide dismutase, Peroxidase, Polyphenol oxidase and catalase) of the tested strawberry plants were determined in shoots of healthy strawberry plants (un-infected). Concerning the effect of (*T.harzianum*, *B.subtitles*, *T.viride* and *S. griseus*), it was found that antioxidant enzymes activities were

increased significantly in all plants treated with inducers and inoculated with *M. phaseolina*, compared to control plants.

In this respect, enhanced PPO activities against disease and insect pests have been reported in several beneficial plants–microbe interactions **(Harish *et al.*, 2009 and Farrag *et al*., 2017).**At the same time, Quantitative proteins of induced strawberry plants infected with *M. phaseolina* were determined using SDS-PAGE, the results indicated that, 87 banding patterns of protein profile with molecular weight ranging from 16-180 KDa , there were one band absent in (control), and (*B. subtitles*) and (*T. viride*) compared to all other samples at 123 KDa respectively. On the other hand, all bands were present in all samples. It has been suggested that, the induced proteins may help to limit spread or multiplication of pathogen **(Chen *et al*., 2006; El-Dougdoug *et al*., 2014; Attia 2014 and Farrag *et al*., 2017).**

Several investigators have indicated that induced resistance in plants was associated with a great increase in chitinase activities. The results showed that the presence of molecular weight**,** 34 and 28 KD (PR3 – Chitinase) was found in all plants treated with biotic inducers. These induced proteins have been defined as pathogenesis related proteins, they implicated in plant defense because of their anti-pathogenic activities (**Van-Loon *et al*., 1994 , Abd-Elgawad and Kabeil, 2010 and Walter *et al.*, 2007).** Variation in isozyme reveals the information in biochemistry entity of resistant genes to physiological changes, genetic characteristics and development of different organisms **(El-Dougdoug *et al*., 2014 and Sharaf *et al*., 2016).** In this study, clear difference existed not only in enzymatic activity but also in enzymatic composition between tested plants. All samples were moderate density in banding patterns compared to low density in (control) and plants inoculated with

T.harzianum with relative mobility 0.20. On the other hand, PPO4 represented all samples were moderate in density except the infected plants showed low density of bands with relative mobility at 0.45. PPO3 and PPO5 illustrated differences in banding density between all six samples. In general, activities of isozymes in plants treated with all applied elicitors were higher than that in control plants, which might be the potential factor for induction of SAR against *M. phaseolina* according to previous findings.

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