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RESEARCH ARTICLE

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ROLE OF THE BIOCONTROL AGENTS, *TRICHODERMA VIRIDE* AND *BACILLUS SUBTILIS,* IN ELIMINATION OF THE DETERIORATIVE EFFECTS OF THE ROOT-ROT PATHOGENS, *FUSARIUM OXYSPORUM* AND *F. SOLANI,* ON SOME METABOLIC AND ENZYME ACTIVITIES OF CUCUMBER PLANTS

ABSTRACT:

The effect of two biocontrol agents, namely: *Trichoderma viride* TM15 and *Bacillus subtilis* BM10, on growth, protein, free amino acids, chlorophyll, carotenoids and phenolic compounds contents, oxidative and hydrolytic enzymes activities of cucumber plants (*Cucumis sativus* L.) grown in soil infested with either *Fusarium oxysporum* f.sp. *radicis cucumerinum* or *F. solani* f.sp. *cucurbitae* were investigated. The results indicated that both phytopathogens caused significant decreases in plant length, fresh and dry weight of infected plants. Treatment with the tested bioagents resulted in remarkable increases in all of these growth parameters. Meanwhile, protein and free amino acids contents were increased (P = 0.05) following infections as compared to untreated control. Application of the tested bioagents rebalanced the levels of protein and free amino acids. The present results clearly established the fact that the bioagents employed not only counteract the deteriorative effect of the pathogens on chlorophyll and carotenoids, but resulted also in noticeable increments in their contents. Infection with the pathogenic *Fusarium* isolates induced significant increases in phenolic compounds contents, phenol oxidase, peroxidase, catalase, invertase, amylase, cellulase and carboxymethyl cellulase activities of cucumber plants as compared to those of the healthy plants. Inoculation of soil with either *T. viride* or *B. subtilis* brought about the relative magnitudes of the abovementioned physiological parameters to be comparable to those of the healthy plants.

KEY WORDS: Cucumber, *Fusarium* spp., *Bacillus subtilis*, *Trichoderma viride*, Protein, pigments, phenols, enzymes.

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INTRODUCTION:

Root-rot disease caused by soil borne fungi is considered to be the most serious disease of cucumber plants (*Cucumis sativus* L.). The disease is wide spread and probably occur wherever cucumber plants are grown in greenhouses and open fields in Saudi Arabia; causing sever problems for producers. The available control measures commonly applied in this concern are: fumigation with methyl bromide and sterilization of soil with basmid or with calcium cyanide (Al-Yehia, 1997).

Nowadays, there is a global attempt to minimize the use of harmful substances, particularly chemical pesticides in agriculture. Biological control of soil borne phytopathogens has proven to be a good alternative to the use of chemical pesticides. Biological control has advantages over chemical control, because it is less likely to damage non-target organisms, and because resistance is lower to evolve (Adams, 1990). Bioagents are also preferred over chemical methods because they do not leave any residue of toxic substances; they are environment friendly and may be cheaper (Campbell *et al.*, 1976; Mohammadi and Lahdenpera, 1992; Mujeebur and Shahana, 2001; Mansour *et al*., 2008).

In a previous study by Al-Tuwaijiri (2008) an account has been given on the use of two highly antagonistic organisms, namely: *Trichoderma viride* TM15 and *Bacillus subtilis* BM10, for control of cucumber root-rot incited by *Fusarium oxysporum* f.sp. *radicis cucumerinum*, and *F. solani* f.sp. *cucurbitae*.

The present work was undertaken to investigate the possible effects of these biocontrol agents on growth, protein and total free amino acids contents, chlorophyll and carotenoids contents, phenolic compounds contents, oxidative and hydrolytic enzymes activities of cucumber plants grown in soil infested with *F. oxysporum* and *F. solani* under greenhouse condition for 6 weeks.

MATERIAL AND METHODS:

Phytopathogens and Biocontrol Agents:

Two *Fusarium* isolates, namely: *F. oxysporum* f.sp. *radicis cucumerinum* and *F. solani* f.sp *cucurbitae* that have been isolated from roots of diseased cucumber, and two antagonistic organisms, namely: *Trichoderma viride* (isolate no. TM15) and *Bacillus subtilis* (isolate BM10) previously isolated from the rhizosphere of healthy cucumber plants (Al-Tuwaijiri, 2008) were employed. The latter two organisms have been found to enhance seed germination (P = 0.05), reduce the pathogenicity of *F. oxysporum* and *F. solani* to cucumber plants and appeared to exhibit strong antifungal potential versus the experimental phytopathogenic fungi *in vitro*, and significantly decrease disease incidence *in vivo* (Al-Tuwaijiri, 2008).

Inoculums Preparation, Seed Treatment, Soil Infestation and Cultivation:

The methods adopted by Pizzinatto and Freitas (1996) and Ehteshamul *et al*. (1990) were used. The procedures have been described in detail elsewhere (Al-Tuwaijiri, 2008).

Estimation of Photosynthetic Pigments:

Chlorophyll was extracted using the method of Harborne (1984). The absorbance of the extract was measured at 663, 646 and 470 nm using spectrophotometer (Spectronic DU 640). The concentrations of chlorophylls and carotenoides were calculated according to the formulae given by Wellbum and Lichtenthaler (1984), as follows:

Chlorophyll a = 12.21 E663 – 2.81E 646 ug/ml

Chlorophyll b = 20.13 E646 – 5.03E 664 ug/ml

C_{x+c} = 1000E470 – 3.27 chl.a – 104 chl.b / 229

c_{x+c} is the concentration of carotenoides (xanthophylls + carotenes)

Determination of Phenolic Compounds:

A known weight of fresh plant material was cut into small pieces and homogenized in 95% ethyl alcohol (20 ml/g). The homogenate was centrifuged at 3000 rpm for 20 min, and the precipitate was reextracted in 95% ethyl alcohol three times, the supernatants were combined and evaporated under vacuum to reduce the volume of ethanolic extract to 10 ml. The phenolic compounds notably: free phenols, total phenols and conjugated phenols were determined according to the method described by Swain and Hillis (1955).

Determination of Protein Content:

The protein content was estimated in air dry plant materials following the methods of Lowry *et al*. (1951).

Determination of Total Free Amino Acids Content:

Total free amino acids content was estimated in the ethanolic extract according to the method described by Rosein (1957).

Determination of Enzyme Activity:

a) Soluble enzymes:

The method adopted by Malik and Singh (1980) was used for extraction of soluble enzymes. The fresh plant material was ground in 0.1M sodium phosphate buffer, pH 7 (2 ml/g fresh weight) with acid washed fine sand at 4°C. The plant homogenate was maintained overnight at zero°C and rehomogenized again, then centrifuged at 6000 rpm for 30 min at 4°C. The clear extract was collected, completed to a known volume, then filtered through Millipore filter (0.450 μm) and used as enzymes preparation.

Determination of Phenol Oxidase Activity:

Phenol oxidase activity was determined by the method of Matta and Dimond (1963). The reaction mixture contained 0.2 ml enzyme source; 1 ml phosphate buffer (pH 7); 1 ml of 1.0 mM catechol and distilled water up to 6 ml, was incubated for 30 min at 30°C. The absorbance of the puspurogallin formed was measured at 420 nm. One unit of phenol oxidase was expressed as the change in absorbance at 420 nm/min/g fresh weight.

Determination of Catalase Activity:

Catalase activity was determined following the procedure of Beers and Sizer (1952).

Peroxidase Activity:

Peroxidase activity was determined using the method described by Allam and Hollis (1972).

b. Determination of Hydrolytic Enzymes:

Invertase activity:

The method adopted by Pressey and Avants (1980) was used. One unit of invertase activity was expressed as mg reducing sugar liberated per 30 min per 0.1 g fresh weight.

Amylase activity:

The method adopted by Mansour *et al*. (1994) was used. One unit of amylase activity was expressed as the amount of enzyme which liberates 1 mg of reducing sugar/ 30 min/ 0.1 g fresh weight.

Cellulase activity:

The method described by Mansour *et al*. (1985) was used. The reaction mixture contained 0.1 g cellulose powder; 1 ml 0.05M citrate buffer (pH 4.8) and 0.5 ml enzyme source was incubated for 1 h at 50°C. One unit of cellulose is defined as the amount of

enzyme which liberates 1 mg reducing sugar per 1 h/0.1 g fresh weight.

Carboxy methyl cellulose (CMCase):

The method described by Mansour *et al*. (1985) was used. The reaction mixture contained 5 ml of 1% carboxy methyl cellulose in phosphate buffer (pH 3.8) and 0.5 ml of crude enzyme preparation was incubated at 50°C for 30 min. One unit of CMCase activity was expressed as mg reducing sugar liberated/1 h/0.1 g fresh weight.

Statistical Analysis:

Data were analyzed with the statistical analysis system (SAS Institute, 1988). All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using least significance difference (LSD) at P = 0.05 (Daniel, 1978).

RESULTA AND DISSCUSSION:

i. Plant Growth:

The results depicted in Table 1 demonstrate that infection of cucumber plants with either *F. oxysporum* f. sp. *radicis cucumerinum* or *F. solani* f. sp. *cucurbitae* resulted in significant decrease in plant length, fresh, and dry weight of infected plants as compared to uninfected control. The magnitudes of decrease were accounted by 63, 82, and 83%, respectively, in plants infected with *F. oxysporum*, and by 60, 82 and 81%, respectively in plants infected with *F. solani*, indicating a basic inhibitory effect on one or several processes upon which plant growth are dependent.

Table 1.Effect of biocontorl agents, *Trichoderma viride* TM15 and *Bacillus subtilis* BM10 on growth of cucumber plants grown in soil infested with *Fusarium oxysporum* f. sp. radicis cucumerinum or *F. solani* f.sp. *cucurbitae* under greenhouse condition for 6 weeks at 27 ± 2°C

Treatment	Growth vigor of cucumber plants		
	Plant length (cm)	Fresh weight (grams)	Dry weight (grams)
Control healthy	67.62*	34.97	5.32[b]
F. oxysporum	24.95[c]	6.15[c]	0.93[c]
F. oxysporum + T. viride	68.12[a]	40.05[a]	6.40[a]
F. oxysporum + B. subtilis	51.86[b]	28.44[b]	4.33[b]
F. solani	27.05[c]	6.38[c]	0.97[c]
F. solani + T. viride	67.95[a]	39.88[a]	6.15[a]
F. solani + B. subtilis	51.60[b]	29.7[b]	4.61[b]

* Mean within a column followed by the same letter are not significantly different according to LSD test P = 0.05.

Incorporation, into the potting soil, of the biocontrol agents, *T. viride* TM15 or *B. subtilis* BM10 resulted in significant increases in all of these growth parameters. This may be attributed to the fact that the experimental biocontorl agents may secrete certain metabolites which lead to inhibition of pathogens conidia germination; suppressing their growth and/or decrease their infective potency (Roberts *et al*., 2005). Chang *et al*. (1986), Windham *et al*. (1986), Lynch *et al*. (1991), and Kleifeld and Chet (1992) reported that several *Trichoderma* strains increased some aspects of growth, such as fresh and dry weight of cucumber plants in potting compost or natural soil. Also Inbar *et al*. (1994) have demonstrated that cucumber plants treated with *T. harzianum* were much more developed and vigorous than untreated plants.

ii. Protein and Free Amino acids Contents:

As could be deduced from the results presented in table 2 protein and total free amino acid contents of cucumber plants were significantly as a consequence of infection with the experimental phytopathogens. This is in conformity with the results of Oke and Banjoko (1991), Cordero *et al*. (1992), and Rey *et al*. (1998). The increase in protein content following infection with fungal pathogen has been suggested to play an important role in host resistance (Andebrhan *et al*., 1980; Abou-Tabel *et al*., 1988).

Table 2. Effect of *Trichoderma viride* TM15 and *Bacillus subtilis* BM10 on protein and free amino acids contents of cucumber plants grown in soil infested with *Fusarium oxysporum* f. sp. radicis cucumerinum or *F. solani* f.sp. *cucurbitae* under greenhouse condition for 6 weeks at 27 ± 2°C

Treatment	Protein content (mg/g dry wt)	Free amino acids content (mg/g fresh weight)
Control healthy	31.55[b]*	6.51[b]
F. oxysporum	34.64[a]	8.82[a]
F.*oxysporum + T. viride*	31.89[b]	8.45[a]
F. oxysporum + B. subtilis	31.95[b]	8.57[a]
F. solani	35.63[a]	8.14[a]
F. solani + T. viride	31.76[b]	7.95[a]
F. solani + B. subtilis	31.65[b]	7.92[a]

* Mean within a column followed by the same letter are not significantly different according to LSD test P = 0.05.

Treatment with the biocontrol agents seemed to rebalance the accumulation of protein in cucumber plants grown in soil infested with either phytopathogen. The present results (Table 2) further showed that the total free amino acids contents of infected cucumber were significantly increased as compared to the control (healthy) plants. This is in agreement with the finding of Tyuterev *et al*. (1994) who reported that the free amino acids contents of cucumber plants were significantly increased following infection with

fungal pathogen; a phenomenon that has been suggested to play an important role in the physiological resistance of the host. However, the excessive increase of free amino acid in the tissues of infected plants may be due to the inhibitory action of the toxic metabolites (mycotoxins) produced by the pathogen (Bhandi and Kang, 1990) on incorporation of amino acids into protein; treatment with *T. viride* or *B. subtilis* appeared to nullify such action.

iii. Chlorophyll and Carotenoids Contents:

The results presented in table 3 revealed that infection of cucumber plants with either *F. oxysporum* or *F. solani* resulted in significant decreases in chlorophyll a, b and carotenoids contents Chlorosis of plant tissue is a common visible symptoms following infection with phytopathogenic fungi. It may result as a consequence of either a) photo oxidative destruction of existing pigments, or b) inhibition of pigment synthesis. It is possible that the effect of the phytopathogenic fungi on chlorophyll and carotenoids is an attenuation of the biosynthetic rate rather than a break down of pigments already formed. Kern (1972) reported that the adverse effect of fungal pathogen on chlorophyll pigments may be due to the fact that the fungal toxins form iron-chelate, transforming iron to become unavailable to participate in chlorophyll synthesis. Treatment with the biocontrol agents (*T. viride* or *B. subtilis*) appeared to stimulate chlorophyll synthesis or at least eliminate the adverse effect of the phytopathogens on pigment formation.

Table 3. Effect of *Trichoderma viride* TM15 and *Bacillus subtilis* BM10 on pigments contents of cucumber plants grown in soil infested with *Fusarium oxysporum* f.sp. radicis cucumerinum or *F. solani* f.sp. *cucurbitae* under greenhouse condition for 6 weeks at 27°C

Treatment	Pigments content (mg/g fresh weight)		
	Chlorophyll a	Chlorophyll b	Carotenoides
Control healthy	0.7425[b]*	0.3765[b]	0.2494[a]
F. oxysporum	0.2637[c]	0.1386[c]	0.0875[c]
F. *oxysporum* + *T. viride*	0.8823[a]	0.4631[a]	0.2376[a]
F. oxysporum + *B. subtilis*	0.8399[a]	0.4525[a]	0.2015[b]
F. solani	0.2877[c]	0.1386[c]	0.0955[c]
F. solani + *T. viride*	0.9625[a]	0.4616[a]	0.2592[a]
F. solani + *B. subtilis*	0.9432[a]	0.4382[a]	0.1693[b]

* Mean within a column followed by the same letter are not significantly different according to LSD test P = 0.05.

iv. Phenolic Compounds:

As can be seen from the results presented in table 4, infection of cucumber plants with *F. oxysporum* or with *F. solani* induced significant increases in free, conjugated and total phenol contents as compared to the uninfected (control) plants. Phenolic compounds have been authenticated to play an important role in the mechanism of disease resistance in higher plants; they accumulated rapidly during host-pathogen interactions and mediated disease suppression through inactivation of fungal enzymes or strengthening of plant structural components (Fuchs *et al*., 1997). Application the biocontrol agents resulted in significant decreases in phenolic compounds. In case of *F. oxysporum*-infected plants, the percentages of decrease in the contents of free phenol, conjugated phenol and total phenol were 41.8, 7.3, and 23.5%, respectively following treatment with *t. viride*, and 33.7, 2.4, and 22%, respectively following treatment with *B. subtilis*. In case of plants infected with *F. solani*, the percentage decreases due to T. viride were 33.2, 6.6, and 23.2%, respectively; and the corresponding percentage decreases due to treatment with *B. subtilis* were 33.7, 2.4, and 45%, respectively. In this connection it may be mentioned that Ferraris *et al*. (1987) have shown that *F. oxysporum* f. sp. *lycopersici* caused significant increase in phenolic contents of tomato seedlings as compared to the healthy plants. The present results (Table 4) revealed that application of the bioagents (*T. viride* and *B. subtilis*) to soil infested with *Fusarium* isolates caused significant decreases in phenolic compounds contents of treated plants as compared those of untreated plants infected with the phytopathogens, indicating that the bioagents-treated plants recovered from the stress status of the pathogens.

Table 4. Effect of *Trichoderma viride* TM15 and *Bacillus subtilis* BM10 on phenolic compounds contents of cucumber plants grown in soil infested with *Fusarium oxysporum* f.sp. radicis cucumerinum or *F. solani* f.sp. *cucurbitae* under greenhouse condition for 6 weeks at 27°C

Treatment	Phenolic compounds contents (mg/g fresh weight)		
	Free phenol	Conjugated phenol	Total phenol
Control healthy	0.7698[c]*	0.4209[c]	1.1907[c]
F. oxysporum	1.3333[a]	0.8040[a]	2.1373[a]
F.oxysporum + *T. viride*	0.8903[b]	0.7451[a]	1.6354[b]
F. oxysporum + *B. subtilis*	0.8856[b]	0.7582[a]	1.6735[b]
F. solani	1.3071[a]	0.7782[a]	2.0954[a]
F. solani + *T. viride*	0.8795[b]	0.7365[a]	1.6193[b]
F. solani + *B. subtilis*	0.8672[b]	0.7694[a]	1.6365[b]

* Mean within a column followed by the same letter are not significantly different according to LSD test P = 0.05.

v. Oxidative Enzymes:

The results given in table 5 indicated that there were remarkable increases in the relative activities of oxidative enzymes, notably catalase, peroxidase and phenol oxidase in cucumber plants in response to fungal infection; the percentages of increase were accounted by 14.8, 17.7, and 53%, respectively, as a consequence of infection with *F. oxysporum*, and by 14.3, 22.2, and 49%, respectively following infection with *F. solani*, over uninfected (healthy) control. This is in conformity with the finding of Storti *et al*. (1992) and Xue *et al*. (1998). Peroxidase and polyphenol oxidase are involved in the oxidation of specific host metabolites which in turn act as inhibitors of growth of the phytopathogen (Chen *et al*., 2000). Meanwhile, treatment with the biocontrol agents (*T. viride* and *B. subtilis*) resulted in a significant decrease in the activity of all of the oxidative enzymes tested in the tissues of infected plants as compared to infected untreated plants; the rates of activity became more or less comparable to that of the healthy plants.

Table 5. Effect of *Trichoderma viride* TM15 and *Bacillus subtilis* BM10 on catalase, peroxidase and phenol oxidase activities of cucumber plants grown in soil infested with *Fusarium oxysporum* f.sp. radicis cucumerinum or *F. solani* f.sp. *cucurbitae* under greenhouse condition for 6 weeks at 27°C

Treatment	Enzyme activity (unit/100 mg fresh weight)		
	Catalase	Peroxidase	Phenoloxidase
Control healthy	1.4264[b]	0.5566[b]	0.1375[b]
F. oxysporum	1.6753[a]	0.6767[a]	0.2940[a]
F.oxysporum + T. viride	1.6045[a]	0.5459[b]	0.1437[b]
F. oxysporum + B. subtilis	1.5960[a]	0.6578[a]	0.2545[a]
F. solani	1.6637[a]	0.7156[a]	0.2697[a]
F. solani + T. viride	1.4170[b]	0.5617[b]	0.1359[b]
F. solani + B. subtilis	1.4196[b]	0.5812[b]	0.1425[b]

* Mean within a column followed by the same letter are not significantly different according to LSD test P = 0.05.

vi. Hydrolytic Enzymes:

The results presented in table 6 show that the relative activities of hydrolytic enzymes namely, invertase, amylase, cellulose and carboxymethyl cellulase, of cucumber plants were significantly increased in response to infection with the phyto-pathogenic fungi as compared to healthy plants (control). Mitchell and Cook (1976) found that the invertase activity of *Brassica oleracea* significantly increased following infection with *Plasmodiophora brassica*. Also, amylase, cellulose and carboxymethyl cellulose activities of tomato plants displayed significant increases in response to infection with *F. oxysporum* f. sp. lycopersici (Ferraris *et al*., 1976; Bhaskaran and Kandaswamy, 1977). Treatment with the experimental biocontrol agents (*T. viride* and *B. subtilis*) appeared to counteract such drastic increase in activities of hydrolytic enzymes and brought about their magnitudes of activities to levels comparable to those of the healthy cucumber plants.

Table 6. Effect of *Trichoderma viride* TM15 and *Bacillus subtilis* BM10 on hydrolytic enzyme activity of cucumber plants grown in soil infested with *Fusarium oxysporum* f.sp. radicis cucumerinum or *F. solani* f.sp. *cucurbitae* under greenhouse condition for 6 weeks at 27°C

Treatment	Hydrolytic enzyme activity (unit/100 mg fresh weight)			
	Invertase	Amylase	Cellulase	CMCase
Control healthy	2.96[b]*	1.77[c]	3.35[b]	11.84[c]
F. oxysporum	5.75+a	4.35[a]	5.72	15.65[a]
F.oxysporum + T. viride	2.87[b]	2.05[b]	3.18[b]	13.88[b]
F. oxysporum + B. subtilis	3.25[b]	2.18[b]	3.37[b]	13.92[b]
F. solani	5.39[a]	3.95[a]	4.95[a]	14.25[a]
F. solani + T. viride	3.18[b]	1.98[b]	3.55[b]	12.80[b]
F. solani + B. subtilis	3.05[b]	2.26[b]	4.46[a]	13.73[b]

* Mean within a column followed by the same letter are not significantly different according to LSD test P = 0.05.

Perusal of the aforementioned pattern of results, it is emerged out that *T. viride* (isolate no. TM15) and *B. subtilis* (isolate BM10) proved to have good efficacy for controlling root-rot disease of cucumber caused by *F. oxysporum* f. sp. *radicis cucumberinum* or by *F. solani* f. sp. *cucurbitae*.

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دور عاملي المكافحة الحيوية- تريكوديرما فيريدي وبا سيللس ستلس- في إزالة التأثيرات الضارة على نمو نبات الخيار وأنشطته الأيضية والأنزيميةالناجمة عن الأصا بة بفطري تعفن الجذور فيوزاريوم أوكسيسبورام وفيوزاريوم سولاني

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يهدف هذا البحث نحو دراسة قدرة معزولتين من الكائنات الدقيقة ذات النشاط الضد إحيائي(القدرة على المكافحة الحيوية)- أحدهما تابعة لفطر ترايكود يرما فيريدي TM15، و الأخرى تابعة لبكتيرة با سيللس ستلس BM10– على إزالة التأ ثيرات ا لمرضية لفطري فيوزاريوم أوكسيسبورام و فيوزاريوم سولاني المسببين لمرض تعفن جذور نبات الخيار على نمو النبات و أ نشطته الأيضية و الإنزيمية. و قد اظهرت النتائج أ ن الإصابة بأي من الفطرين الممرضين فيوزاريوم أوكسيسبورام. أو فيوزاريوم سولاني تسبب نقصا معنويا في طول النبات و وزنه الرطب و الجاف ، في حين أدت المعاملة بكل من كائني المقاومة الحيوية (ترايكود يرما فيريدي و با سيللس ستلس) إلى زيادة معنوية في جميع ظواهر النمو المذكورة ، و في نفس الوقت أدت الإصابة بالفطرين الممرضين إلى زيادة ملحوظة في محتوى النبات من البروتين و الأحماض الأمينية بينما أدت المعاملة بالكائنين المضادين إلى إعادة قيم هذه النواتج الأيضية إلى قيم متقاربة أو معادله لها بنباتات المجموعة الضابطة غير المصابة ، كما تبين على ضوء نتائج هذه الدراسة أن الميكروبات المضادة (كائني المقاومة الحيوية) لا تعمل فقط على إزالة التأثير الضار للميكربات الممرضة على محتوى النبات من الأصباغ الكلوروفيلية والكاروتينويدات بل تؤدي فضلا عن ذلك إلى زيادة معنوية في معدلاتها. هذا وقد أظهرت النتائج أن الإصابة بأى من الفطرين الممرضين فيوزاريوم أوكسسيسبورام أو فيوزاريوم سولاني تؤدي بشكل عام إلى زيادة معنوية في محتوى النبات من المركبات الفينولية (الحرة و المرتبطة) و في النشاط الإنزيمي لكل من إنزيمات الفينول أوكسيد يز ، الكاتاليز، الإنفرتيز، الأميليز، السيليوليز، الكاربوكسي ميثيل سيليوليز مقارنة بمثيلاتها بنباتات المجموعة الضابطة ،وقد أدت المعاملة بكل من كائني المقاومة الحيوية (تريكوديرما فيريدي TM15 وباسيللس ستلس BM10) إلى إعادة التوازن لتلك النواتج الأيضية و الأنشطة الإنزيمية إلى معدلاتها الطبيعية حيث كانت قياساتها متقاربة أو معادلة لمثيلاتها لنباتات المجموعة الضابطة.

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