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Role of Biological Control on Some Physiological Aspects of *Zea mays* Infected by *Rhizoctonia solani*

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Abstract: The results revealed that treatment with either *Trichoderma harzianum* or *Bacillus subtilis* by soil inoculation or grain coating significantly increased the percentage of healthy seedlings as well as the length, fresh and dry weight of seedlings. Photosynthetic pigments content of the leaves significantly increased in absence of *Rhizoctonia solani* alone. The same almost applied to soluble sugar content, amino acid content or total nitrogen of the seedlings, though less apparent or insignificant when the grains were treated with *B. subtilis* before growing in soil treated with 3% *R. solani*. *R. solani* lowered the test elemental content of *Zea mays* seedlings, while the reverse was most prominent by sowing the grains in soil amended with *R. solani* and *T. harzianum*. The results also revealed that infestation by *Rhizoctonia solani* significantly lowered the length of the ears and weight of 100 grains. In the mean time the weight of 100 grains significantly dropped; a response that was hardly, if at all affected by implying *R. solani* with *Bacillus subtilis* or *T. harzianum*. The presence of the three microorganisms increased the fresh weight of the ears but the total count or weight of the grains was lowered. The presence of *R. solani* in soil lowered the lipid, total carbohydrates and protein content of corn flour. Meanwhile using the biological control agents *T. harzianum* or *B. subtilis* or both initiated the increase of these components.

Key words: Physiological activities, *Zea mays*, metabolic activities, *R. solani*, biological control, corn yield

INTRODUCTION

The *Zea mays* is considered a very important plant since it is used to feed humans as well as animals and in industrial production of starch, proteins and oil. The grains contain vitamin A, C, E and a number of amino acids and salts as well as rare elements (Bausch *et al*., 1982). The main problem for *Z. mays* sowing in Saudi Arabia is the root-rot of the seedlings caused mainly by *Rhizoctonia solani* (Öller *et al*., 1999; Priyatmojo *et al*., 2002). The chemical control, most commonly applied for the plant disease control, resulted in the appearance of pathogenic microbe strains, resistant to the fungicides used in such treatments. Now scientists try to apply the biological control to replace the chemical control in order to avoid the undesirable effects on the plant. The biological control has been used to minimize the density of the pathogenic organism or arrest the action of pathogenic microbes at its different stages of growth. This can be carried out by injecting one or more of the microbes in the natural atmosphere in the soil or by coating the seeds with such antagonists to protect the seedlings from the pathogenic microbes (Baker, 1986).

About 25% of the world production of *Zea mays* grains has been used as human food either as flour or as oil. The zea starch, dextrin have been used in the industry of acetone and alcohol. The draw back *Z. mays* cultivation in Saudi Arabia is the high infestation by microorganisms that leading to decreased yield and low quality of the product, more prominently by the roo-rot disease caused by *Rhizoctonia solani* (McGee, 1988). In spite of the importance of chemical control to counteract many of the plant disease yet their repeat use causes the appearance of pathogenic microbe strains resistant to them. Nowadays the biological control is one of the methods that has been used to replace the chemical control.

The aim of this study is to find out the response of some physiological activities of *Zea mays* seedlings and to detect the response of some metabolic activities of corn yield to the biological control of *R. solani* using *T. harzianum* and *B. subtilis*.

MATERIALS AND METHODS

Microorganisms: *Trichoderma harzianum* and *Rhizoctonia solani* were isolated from the rhizosphere of *Zea mays* plant and identified by R. Gashgary, Associate Professor in Girls College of Education, Jedda, Saudi Arabia.

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Bacillus subtilis was supplemented by Department of Plant Pathology, Faculty of Agriculture, Zagazeg University.

Biological control experiment: This study was carried out in the Girls College of Education, Jeddah, Saudi Arabia. Pots of 20 cm diameter were sterilized by soaking in 5% formaline solution for 20 min and then dried before filling with soil at the rate of 3% (w/w) of (growing *Rhizoctonia solani* in vermiculite). Fifteen surface sterilized *Z. mays* grains were sown in each pot, 3 replicates for each treatment. Treatments were 3% (w/w) of pathogenic fungus *R. solani* inoculated in the soil before sowing surface sterilized *Zea* grains. 3% (w/w) *R. solani* was inoculated in the soil with 3% (w/w) of *T. harzianum* followed by sterilized *Zea mays* sown (Sivan *et al*., 1984).

Soil inoculated with 3% (w/w) *R. solani* was used to implant the coated *Z. mays* grains with *B. subtilis*.

Soil inoculated with 3% (w/w) of the pathogenic fungus combined with both antagonistic microorganisms. There was a control for each treatment (free of pathogenic fungus *R. solani*). The 2-week-old seedlings were transplanted to the field till cropping.

After 2 weeks, samples from the seedling leaves of the different treatments were taken at random to record their morphological data as percentage of healthy seedling, length, fresh and dry weight of seedlings and estimate their pigments content, some carbohydrate, nitrogen and element components. Pigments were assayed using Lichtenthaler (1987) method. Total amino acids was determined by Mütting and Kaiser (1963), whereas total nitrogen was assayed applying the method by Naguib (1968). Total soluble sugars were estimated by Cooper and McDaniell (1970) method whereas the flame emission spectrophotometry (Stewart, 1972) was used to detect the test element.

RESULTS AND DISCUSSION

Table 1 shows that the various treatments with *B. subtilis* and/or *T. harzianum* restored the percentage of healthy corn seedlings infected with *R. solani* to the control level. This indicates that the metabolites of either organism suppressed (or even arrested) the production of the hydrolyzing enzymes cellulase, polygalacturonase and pectin methyl esterase responsible for the lysis of the host cell wall as well as minimized the toxins produced by this pathogenic fungus (Vazquez *et al*., 1993; Madhosingh, 1995). *B. subtilis* was least effective when provided alone. *R. solani* significantly lowered the fresh and dry weight of the seedlings as well as their length; a response that was encountered by the presence of *B. subtilis*. *B. subtilis* alone insignificantly affected these criteria. *T. harzianum* alone stimulated length, fresh and dry weight of the seedlings, a response that was almost unaffected by *R. solani*. Coupling both organisms hardly affected the suppressive effects of *R. solani*. This result is in agreement with Bae *et al*. (1995) using a mixture of the antagonistic fungus *Gliocladium virens* and the bacterium *Pseudomonas putida* lead to increased percentage of germination of seeds and increased the fresh weight of the shoot. Ran *et al*. (2005) used different types of *Pseudomonas* spp. to protect the *Eucalyptus* seedling from the wilt disease. Roberts *et al*. (2005) used the antagonistic microbes *Trichoderma virens* and *Serratia marceseem* to control the cucumber diseases caused by *R. solani*. They found that the coupling the antagonistic microbes inhibited the pathogenic fungus more than the single treatment. Also, the coupling inhibited several pathogenic microbes, thus changing the microbial equilibrium in fevour to the plant growth. These antagonistic microbes secrete antibiotic and plant hormones and growth-promoting which lead to stimulate the growth. Some of this metabolic secondary secretion dissolve some solid substance in the soil that can be absorbed by the plant and also increase the root branches so increase its area so help to absorb more nutrients (Xiong *et al*., 2005; Wen *et al*., 2005).

Table 2 shows soil amendment with *R. solani* favoured lower accumulation of chlorophyll a and b and carotenoid content; an indication that the fungus interfered with the biotransformation of chl a to chl b. i.e., suppressed the photosynthetic activity of corn leaves, as well as treating the grains with *B. subtilis* or *T. harzianum* significantly increased the chlorophylls and carotenoids

Table 1: Effect of biological control treatments of root-rot disease of *Zea mays* seedlings on the % of healthy seedlings, length, fresh and dry weights of 2 weeks-old *Zea mays* seedlings±SE

Treatment of *R. solani* (%) in potting soil	(%) of healthy seedlings	(%) of unhealthy seedlings	Length (cm seedling)	Fresh weight (g seedling)	Dry weight (g seedling)
0.0	95.55±2.22	4.45±2.22	66.33±0.88	3.85±0.35	0.44±0.01
3.0	57.78±2.22	42.22±2.22	53.67±1.33	2.79±0.13	0.27±0.01
0.0+ grains coated by *B. subtilis*	93.33±2.22	6.67±2.22	68.17±4.95	4.50±0.32	0.46±0.06
3.0+ grains coated by *B. subtilis*	75.55±2.22	24.45±2.22	62.67±4.06	3.37±0.43	0.36±0.05
0.0+ soil treated by *T. harzianum*	95.55±2.22	4.45±2.22	83.67±4.84	4.90±0.48	0.52±0.04
3.0+ soil treated by *T. harzianum*	91.11±4.45	8.89±4.45	81.83±0.44	3.85±0.12	0.41±0.02
0.0+ grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	97.78±2.22	2.22±2.22	81.67±3.84	4.82±1.14	0.37±0.29
3.0+ grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	95.55±2.22	4.45±2.22	77.67±0.88	3.52±0.20	0.29±0.02

content almost to the same level; a response that was slightly counteracted when the soil was injected with *R. solani* and counteracted when both prevailed in the soil. These responses may be attributed to the hormones secreted by either *B. subtilis* or *T. harzianum* that was absorbed by the corn seedlings during their growth (Levenfors *et al*., 2004). Straub and Lichtenthaler (1973) reported that addition of cytokinins to the plant maintains the natural level of both chorophyllase and ribonuclease enzymes which lead to increase the photosynthetic product and addition of kinetin stimulate the biological building of chlorophyll. This is in agreement with Seyer *et al*. (1975) and Gasque (1982) study. Marino and Bertazza (1990); Jordi *et al*. (2000) and Dertinger *et al*. (2003) indicated that the presence of hormones leads to increased chlorophyll content in the leaves of intact plants which stimulate the enzymes required for chlorophyll regeneration and increase the protection of chlorophyll's pigments.

Table 3 shows that soil infection with *R. solani* drastically lowered the total soluble sugars, total amino acid and total nitrogen contents of *Z. mays* seedling leaves. The presence of either *B. subtilis* or *T. harzianum* almost equally affected a remarkable increase, total soluble sugars and total amino acids without affecting total nitrogen; an indication of enhanced metabolic activity of either carbon or nitrogen without affecting nitrate-N absorption from the soil, a response was almost unaffected when both organisms were coupled in the soil. Injection of *R. solani* invariably affected the stimulating affects of either *B. subtilis* or *T. harzianum*; an indication of the counteracting action of the metabolic products of these two microorganisms against the pathogenicity of *R. solani*. In this connection Troshina and Jamaleen (1991); and Wilderm *et al*. (1992) postulated that parts of the metabolic products were withdrawn by the pathogenic fungus leading to a drop in storage metabolites in the leaves.

Table 4 shows that *R. solani* favoured a noticeable drop in the test elements content except Ca, a response that was reversed when the grains were treated with *B. subtilis* or *T. harzianum* or even reversed. Injection with *R. solani* slightly or hardly affected the tested elements of corn grains when coated with *B. subtilis* or *T. harzianum*. The presence of these two organisms stimulated the accumulating effect on all test elements. In this connection Howell *et al*. (2000) and Yedidia *et al*. (2001) reported that some bacteria and some fungi, used as antagonists especially *T. harzianum*, stimulate the root branches and also secrete hormones to the outside which increase the root surface leading to large area in the soil, thus absorbing large amounts of elements from the soil.

Table 5 showed that the drop in the length of *Z. mays* ears of plants sown in soils infested with *R. solani*

Table 2: Effect of biological control treatments of root-rot disease of *Zea mays* seedlings on photosynthetic pigments (mg g fresh wt)±SE

Treatment of *R. solani* (%) in potting soil	Chlorophyll a	Chlorophyll b	Carotenoids
0.0	6.50±0.41	5.48±0.06	2.31±0.14
3.0	6.11±0.80	5.02±0.29	1.58±0.08
0.0+ grains coated by *B. subtilis*	11.31±0.60	8.80±0.44	3.58±0.19
3.0+ grains coated by *B. subtilis*	9.74±0.55	5.99±0.69	2.96±0.42
0.0+ soil treated by *T. harzianum*	11.24±1.92	8.48±0.06	3.74±0.61
3.0+ soil treated by *T. harzianum*	8.52±0.22	5.91±0.11	2.71±0.33
0.0+ grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	11.49±1.08	9.73±0.66	3.75±0.07
3.0+ grains coated by B. subtilis and soil treated by 3.0% *T. harzianum*	10.88±0.98	9.34±0.60	3.39±0.31

Table 3: Effect of biological control treatments of root-rot disease of *Zea mays* seedlings on total soluble sugars, amino acids and total nitrogen content in healthy seedlings leaves (mg/g dry wt.)±SE

Treatment of *R. solani* (%) in potting soil	Total soluble sugars (mg/g D.wt.)	Total amino acids (mg/g D.wt.)	Total nitrogen (mg g D.wt.)
0.0	32.35±2.68	11.89±0.30	194.50±27.11
3.0	27.59±0.95	10.45±0.14	131.18±6.68
0.0+ grains coated by *B. subtilis*	38.17±2.39	13.09±0.15	196.90±17.73
3.0+ grains coated by *B. subtilis*	32.92±2.22	12.47±0.13	178.75±9.64
0.0+ soil treated by *T. harzianum*	37.33±0.88	14.23±0.12	197.25±8.42
3.0+ soil treated by *T. harzianum*	32.11±1.33	13.40±0.23	188.123±14.63
0.0+ grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	38.20±0.87	14.99±0.30	198.00±4.81
3.0+ grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	33.20±0.87	14.15±0.85	191.25±16.42

Table 4: Estimation of biological control of treatment of some elements (mg/g dry wt.) In the leaves of healthy *Zea mays* seedlings after±SE

Treatment of *R. solani* (%) in potting soil	Fe	Mg	Mn	Na	K	Ca	P
0.0	1.58±0.11	73.50±2.61	1.32±0.05	17.57±0.67	59.19±21.02	18.68±1.11	16.34±0.72
3.0	1.04±0.25	58.38±2.26	1.21±0.06	14.51±1.45	49.44±2.33	16.13±2.41	14.87±0.81
0.0+grains coated by *B. subtilis*	1.63±0.52	78.15±3.68	1.40±0.01	18.62±0.57	68.54±8.83	20.42±0.79	17.56±0.29
3.0+grains coated by *B. subtilis*	1.56±0.99	75.50±5.06	1.33±0.04	17.04±0.16	60.58±3.01	19.60±1.42	16.28±0.37
0.0+soil treated by *T. harzianum*	1.34±0.26	84.58±2.46	1.37±0.04	18.76±0.14	59.93±3.73	19.40±1.11	16.40±0.45
3.0+soil treated by *T. harzianum*	1.68±0.17	81.83±1.83	1.29±0.04	17.19±0.75	57.08±3.74	18.16±1.69	16.17±0.58
0.0+grains coated by *B. subtilis* and soil	1.81±0.07	89.08±1.18	1.41±0.02	19.92±1.02	69.85±7.88	22.67±0.33	18.00±0.32
3.0+grains coated by *B. subtilis* and soil	1.67±0.05	77.33±0.68	1.35±0.16	18.21±0.59	63.71±2.51	21.42±3.47	16.65±0.37

Table 5: Effect of some biological control treatments on the length and weight of ears and on the weight of 100 grains (mean±SE)

Treatment of *R. solani* (%) in potting soil	Length (cm ear)	Fresh weight (g ear)	Total count of grains	weight of (g 100 grains)
0.0	18.3±0.67	90.0±4.93	198.7±6.36	30.1±0.43
3.0	15.3±0.33	67.0±7.04	164.0±12.49	21.9±0.46
0.0+grains coated by *B. subtilis*	21.3±1.86	119.2±19.03	298.7±49.19	33.3±0.95
3.0+grains coated by *B. subtilis*	17.3±1.33	108.9±8.27	227.3±36.99	29.2±0.30
0.0+soil treated by *T. harzianum*	19.3±0.88	109.1±7.15	204.0±20.74	28.9±0.51
3.0+soil treated by *T. harzianum*	17.7±0.88	90.3±7.78	190.0±20.82	27.9±1.04
0.0+grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	20.7±1.30	110.2±8.00	233.3±3.33	31.6±0.41
3.0+grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	19.7±0.88	102.2±2.50	196.7±11.67	27.4±0.41

Table 6: Effect of some biological control treatments on the lipid, carbohydrates and protein content in corn flour (mg/g dry weight; mean±SE)

Treatment of *R. solani* (%) in potting soil	Lipids (mg g)	Carbohydrates (g g)	Protein (mg g)
0.0	8.3±0.13	0.5±0.00	76.1±2.04
3.0	6.5±0.73	0.5±0.0	73.0±2.24
0.0+ grains coated by *B. subtilis*	9.3±0.19	0.6±0.01	80.7±0.93
3.0+ grains coated by *B. subtilis*	8.47±0.49	0.6±0.0	76.2±1.53
0.0+ soil treated by *T. harzianum*	10.8±0.35	0.6±0.02	81.5±1.16
3.0+ soil treated by *T. harzianum*	10.5±0.52	0.5±0.01	80.2±1.26
0.0+ grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	12.1±0.97	0.6±0.02	85.8±0.52
3.0+ grains coated by *B. subtilis* and soil treated by 3.0% *T. harzianum*	10.7±0.32	0.6±0.01	82.7±1.60

was alleviated by the presence of *B. subtilis* and/or *T. harzianum*. In the meantime *T. harzianum* increased the fresh weight of the ears in presence or in absence of the other test microorganism. The number of grains per ear was hardly affected by either microbes except for a minor drop when all prevailed. On the other hand, the weight of 100 kernels slightly, but significantly dropped by all treatments. This in agreement with other results (Yedidia *et al*., 2001; Harman *et al*., 2004).

Table 6 showed that the suppressive effect of *R. solani* on the lipid or protein content of *Zea mays* flour was counteracts by the presence of *B. subtilis* and reversed by the presence of *T. harzianum* either of these microorganisms stimulated the accumulation of these components. *B. subtilis* or *T. harzianum*, alone, stimulated carbohydrate accumulation in corn flour, a response that was hardly, if at all affected by infection with *R. solani*. On the contrary, infection by *R. solani* decreased the carbohydrate accumulation in corn flour.

The results revealed that the presence of *B. subtilis* and/or *T. harzianum* initiated better accumulation of the test metabolic components and the morphology of the corn ear; a response that was counteracted by infestation with *R. solani*. This indicates that the secretion of either microorganism (hormones and/or other metabolites) counteracted the suppressive effects of *R. solani* metabolites. Generally the root-rot disease led to decreased number of plants especially in the 1st stages. Also the flowering stage and ears of the healthy plants was weak as a result the yield was reduced (Sidorov, 1990). The increase of protein level in corn flour has been explained as a result of good nitrogen metabolism in the seedling and the *R. solani* fungus can't use part of the metabolic process in the plant tissue for its growth or it can be explained as a result of presence of antagonistic microbes used in the biological control, since Hynes *et al.* (1994) found that the coated grains by bacteria inoculum caused sensitive reaction in the seedlings leading to appearance of metabolites suppressing the activity of the pathogenic microbes in the soil. Thus stimulating the growth of the plant and its metabolic activity. There is high relation between the hormones especially cytokinin and benzyl adenine and nitrogen metabolism since this substance increase the total nitrogen (De-Freites, 1991). This indicated that *T. harzianum* and *B. subtilis* secrete these hormones.

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