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Research Article
Green Chemicals and Bio-agents for Controlling Damping-off Diseases of Sugar Beet and Scaling up the Yield and Quality

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Abstract

Background and Objective: Sugar beet is one of the cash crops grown in temperate and cold zones of the world. In Egypt, it thrives in the northern part of the country. It comprises about 30% of total sugar production. Both foliage and pulps are feed of high value for the domestic animals. Since several soil-borne fungi attack this crop causing a significant reduction in the production, the present investigation aimed to evaluate the effectiveness of selected antioxidants including GAWDA formulation along with *Trichoderma* fungus and organic fertilizers (compost) to induce resistance against the root rot disease along with their possible role on increasing the yield and quality. **Materials and Methods:** *In vitro* studies were carried out to select the efficient concentration of the tested antioxidants on reducing the growth of the target pathogens. *In vivo* studies were applied in the field plots at Mansoura University Campus during the two successful seasons of 2015 and 2016. The growth parameters as well as the root weight and their quality characters were recorded. Data were statistically analyzed by CoStat 6.3 software of analysis of variance at $p \leq 0.05$ as outlined by Duncan. **Results:** Soaking seeds in water solution of GAWDA® formulation at a concentration of 4 g L^{-1} for 12 hours before planting and air dried for 1 h followed by coating them with *Trichoderma harazianum* (*T. harazianum*) before planting in soil supplemented with the composted agricultural residues significantly decreased the damping-off disease and scaled up the yield and quality. **Conclusion:** Results addresses a ramping up in the yield and quality of sugar beet as well as control damping-off disease when treated with friendly environmental materials.

Key words: Sugar beet, *Trichoderma* spp., organic fertilizer (compost), soil-borne fungi, disease control, GAWDA formulation and green chemicals

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sugar beet is classified as the second important sugar crop in Egypt and in many other countries after sugar cane[1-3]. The present world production of sugar beet reached nearly 269.714 million ton comes from about 4471580 ha with an average of 60.32 t ha^{-1}. In Egypt, the total cultivated area of this crop reached about 211806 ha with total production of 11.046 million tons of the fresh roots and average of 52.15 t ha^{-1} [4].

Several soil-borne fungi attack this crop causing a significant reduction in the production viz., *Rhizoctonia solani* (*R. solani*), *R. crocorum*, *Aphanomyces cochlioides* (*A. cochlioides*), *Phoma betae* (*P. betae*), *Macrophomina phaseolina* (*M. phaseolina*), *Fusarium oxysporum* f. sp., *radicis-betae, Pythium aphanidermatum* (*P. aphanidermatum*), *Phytophthora drechsleri* (*P. drechsleri*), *Rhizopus stolonifer* (*R. stolonifer*), *R. arrhizus* and *Sclerotium rolfsii* (*S. rolfsii*). Several fungicides have been used to control these diseases including, chlorothalonil, pencycuron, tebuconazole, azoxystrobin, trifloxystrobin and pyraclastrobin[5].

Some other fungi cause post-harvest losses, in storage piles. *Rhizoctonia* crown and root rot caused by *R. solani* is one of the most damaging sugar beet pathogen. While *R. solani* can also cause damping-off and crown and root rot of sugar beet and other crops including beans and soybean[6,7].

One of the potential tactics for management these diseases are the use of compounds of antimicrobial activities to increase the plant resistance[8]. Some chemical compounds i.e., salicylic acid (SA), mono and di-basic potassium phosphate (KH_2PO_4 and K_2HPO_4), hydrogen peroxide (H_2O_2) and Bion (BTH) have been shown to induce resistance in plants[9,10].

Benhamou and Belanger[11] illustrated antioxidants as a resistance inducer (SAR) of plants against pathogens and indicated that resistance more or less is associated with metabolic and structural changes inside plants. Vallad and Goodman[12] suggested that SAR may be induced as a result of the stress of both biotic or abiotic elicitors, resulting in the accumulation of salicylate such as salicylic acid leading to the expression of pathogen related (PR) genes. Van Loon and Bakker[13] revealed that SAR refers to a distinct signal transduction pathway that plays an important role in the ability of plants defense against plant pathogens. Recognition of plant pathogen immediately initiates a cascade of molecular signals and transcription of many genes, which eventually results in the production of defense molecules by the plant.

Bigirimana *et al*.[14] presented a remarkable effects of *Trichoderma* spp. on plants due to their direct effects on the pathogenic fungi and localized resistance in plants. Bailey and Lumsden[15] revealed that the effective protection of plants against pathogen (biocontrol) exerted by *Trichoderma* strains is often unpredictable. The ability of *Trichoderma* strains as biocontrol agents is due to their high reproductive capacity, capability to survive under unfavorable conditions, effectiveness in the utilization of nutrients, ability to modify the rhizosphere, powerful aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms. Li *et al*.[16] indicated that many profitable properties of using the biocontrol agents are based on *Trichoderma* strains because they are living organisms and also because of their potential to survive in different environmental conditions. Until recently, the principal mechanisms for plant diseases control have been assumed to be those primarily acting upon the pathogens and included mycoparasitism, antibiosis and competition for resources and space[17].

Wasternack *et al*.[18] reported that this biocontrol fungus has two important modes of actions-direct suppression of the pathogen with production of antibiotics substances and enzymes and strong stimulation of the plants natural defence mechanism. Liu and Huany[19] found that the population density of *Fusarium* spp. was highly decreased, when the population density of *Trichoderma* spp. increased in rhizosphere zone of soil treated with bio-compost.

El-Mohamdy[20] showed that the bio-compost application as soil amendment were able to suppress diseases caused by *R. solani* and *Fusarium* spp. on a number of economic crops. Javaheri *et al*.[21] studied the effects of farm yard manure and other nutrients on quality and quantity of sugar beet and resulted that 20 t ha^{-1} (9 t ha^{-1}) of manure increased sugar yield by 10% with no significant effect on sugar loss in molasses. Research results show that manure could be a valuable source of nutrients for sugar beet, however, composted manure has different properties than the non-composted manure.

Cayuela *et al*.[22] found that application of composts to soil has been proposed to control different diseases. However, not all types of compost have been shown to exert beneficial effects on plant growth and health. Loffredo *et al*.[23] showed that a significant effect of humic fractions (HS) from soil and composts has been demonstrated on the mycelial growth and conidial germination of two formae speciales of *Fusarium oxysporum*) and on the growth and sclerotial formation of *Sclerotinia sclerotiorum* and two antagonistic *Trichoderma* species.

Loffredo and Senesi[24] showed that the five commercial types of compost were evaluated on suppressing the root-rot pathogens (*Fusarium solani*, *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotium rolfsii*) attacking sugar beet plants. El-Mohamedy *et al.*[25] found that amendment of compost to *T. harzianum* accelerate its suppressive effect on controlling the plant diseases.

Al-Mughrabi[26] showed that organic amendments play an important role as friendly environment and sustainable alternative approach to protect plants from the attack of the soil-borne pathogens. Soil amendments using composted agricultural wastes fortified with bio control agents is acceptable approaches in controlling a number of diseases. The use of organic agricultural wastes in this respect could be an advantageous in soil fertility, recycling of agricultural residues and provide a powerful tool for management of plant diseases. It has been reported that several composts and/or composts fortified with bio control agent used as soil amendments reduced the density of pathogen propagules and protected plants from the invasion of soil borne plant pathogens. Sabet *et al.*[27] illustrated a significant effect of isolated bacteria and fungi from composts on a number of fungal pathogens attacking sugar beet roots.

Based on the available data, the present investigation aimed to evaluate the effectiveness of some antioxidants including GAWDA formulation along with *Trichoderma* fungus and organic fertilizers (compost) for inducing resistance against root rot disease of sugar beet and their possible role on increasing the production and the quality.

MATERIALS AND METHODS

This study was carried out at Laboratory of Seed Pathology, Department of Plant Pathology, Mansoura University, Egypt during three successive seasons (2015-2016).

Samples of sugar beet roots suspected to be attacked by root rot and wilt fungi were collected from different growing sugar beet areas in Dakahlia Governorates, Egypt to be used in the present study.

Isolation: Diseased roots washed thoroughly in tap water followed by cutting small pieces, surface sterilized for 1 min in NaOCl (0.5%), re-washed three-times in distilled sterilized water and distributed on petri-dishes containing 3 layers of moist blotter papers. Plates were incubated for 7 days at 22°C in the dark. The grown fungi were identified depending on their habit characters, colony pigment, size and shape of conidia and other morphological structures described by Gilman[28], Parmeter[29], Dhingra *et al.*[30], Nelson *et al.*[31], Booth[32] and Burgess *et al.*[33].

Pathogenicity: About 20 cm diameter pots, filled with sandy-clay soil (1:1) at a rate of 3 kg/pot, were inculcated with the tested fungi. Each fungus was grown in glass bottles containing sterilized moisted sorghum grain and incubated at 25±2°C for 15 days. Infestation was accomplished by mixing the inoculum with the upper 5 cm layer of the soil at a rate of 2% (w/w) for *Rhizoctonia solani*, *Sclerotium bataticola* and 4% (w/w) for *Fusarium monliforme*, *Fusarium solani*. Soil was irrigated 2 days intervally up to 8 days. Five healthy seeds from previously tested were sown in each pot. Three pots were used as lot replicate for each fungus, while three un-infested pots were used as a check. Pots were maintained under natural conditions. Observation for damping-off was recorded during the life span of the plants.

Antioxidant: The following antioxidants i.e., Salicylic acid, Tartaric acid and GAWDA® formulation Patent No. 23798, the Academy of Science and Technology of Egypt (Tri-sodium orthophosphate 1 mM+tartaric acid 2 mM+hydroxyquinoline 1 mM+calcium chloride 6 mM+magnesium chloride 5 mM+calcium borate 5 mM) were tested for their effect on the growth of the tested pathogenic fungi.

Compost: The Egyptian company for Solid Waste Recycling, Talkha, Dakahlia, Egypt kindly provided samples of its production used at the rate of 2 tons/fed was used in this study.

***Tricohderma* spp.:** *Tricohderma harzianum* and *Tricohderma viride* were obtained from Plant Pathology Department, Faculty of Agriculture, Mansoura University, Egypt.

Effect of antioxidants on the fungal growth: The following antioxidants; Salicylic acid, tartaric acid and GAWDA® formulation each was dissolved in distilled water while concentrations of (2, 4, 6 and 8 mM), (5, 10, 15 and 20 mM), (1, 2, 3 and 4 g L^{-1}) were used respectively. Disks presented 7 days old cultures were transferred onto the centers of PDA plates supplemented with the above antioxidants. The inhibitory effects were measured and recorded. Three replicates were used to present one treatment. All cultures were incubated for 7 days at 25±2°C in the dark. Linear growth of each fungus was recorded.

Effect of *Trichoderma* spp. on the fungal pathogens: The inhibition rate of *T. harzianum* and *T. viride* on the growth of *F. solani*, *F. monliforme*, *R. solani* and *S. bataticola* were investigated using the dual culture technique[34] while the interaction was recorded as described by Desai *et al.*[35].

Table 1: Designed combinations

Compost	Antioxidant	Fungus
Free compost	Free antioxidants	*T. viride*
		T. harzianum
		Non *Trichoderma*
	Salicylic acid	*T. viride*
		T. harzianum
		Non *Trichoderma*
	Tartaric acid	*T. viride*
		T. harzianum
		Non *Trichoderma*
	GAWDA® formulation	*T. viride*
		T. harzianum
		Non *Trichoderma*
Compost	Free antioxidants	*T. viride*
		T. harzianum
		Non *Trichoderma*
	Salicylic acid	*T. viride*
		T. harzianum
		Non *Trichoderma*
	Tartaric acid	*T. viride*
		T. harzianum
		Non *Trichoderma*
	GAWDA® formulation	*T. viride*
		T. harzianum
		Non *Trichoderma*

Effect of antioxidants on *Trichoderma* spp.: Disks from 5 days old cultures of *T. harzianum* and *T. viride* were transferred onto the center of PDA plates appended with 4 mM salicylic acid or 10 mM tartaric acid or 4 g L^{-1} GAWDA®. Cultures were incubated in dark for 7 days at 25±2°C. Three-replicates were used per each treatment. The inhibitory effect of the *Trichoderma* on pathogenic fungi were observed and recorded.

Formulation of *Trichoderma* spp.: After seeds soaking with selected antioxidants for 12 h, seeds were coated with one species of *Trichoderma* (1 g/20 g seeds) plus Acacia gum (1 g/100 mL water), then air dried.

Efficacy of antioxidants and GAWDA formulation as well as *Trichoderma* and the organic fertilizer (compost) on the sugar beet growth: Field plots located at the Campus of Mansoura University were used for the *in vivo* studies. The experiment was carried out according to Split-Split Plot design of six replicates. Compost was presented the main plots while the antioxidants were presented in the sub plots, *Trichoderma* sub-sub plots. Sugar beet seeds cv. Tenor were grown in ridges of 20 cm apart in hills spaced 60 cm apart on one side of the ridge.

Experimental design: The design made for the use of each component and their combination to assess their effect on retarding the severity of the pathogens under field condition was shown in Table 1.

Assessment of the photosynthetic pigments in the leaves: The third upper parts of a number of sugar beet leaves collected from plants were used to determine their contents of the photosynthetic pigments according to the method described by Mackinney[36].

Total phenols: Fresh leaves of sugar beet plants were collected to determine their contents of the total phenols using Foline-ciocalteau reagent[37].

Plant growth characters: After 200 days of sowing date, the following characters were measured:

- Plant weight (g), root weight (g), root length (cm), root diameter (cm), shoot weight (g), shoot length (cm), number of leaves/plant

Total soluble solids (TSS) and sucrose content: The TSS was measured in juice of fresh roots by using hand refractometer (Hycle groupe lifasa bio 21320 Pouuilly by Auxxois-Fransa). Sucrose percent was determined by using polarimetric on lead acetate extract of fresh macerated roots according to the method of Carruthers and Oldfield[38] and Fatouh[39].

Statistical analysis: Obtained data were statistically analyzed according to CoStat 6.311[40] of analysis of variance[41] at $p \leq 0.05$ as outlined by Duncan[42].

RESULTS

Pathogenicity test: Data in Table 2 show that the degree of variance in damping-off percentage caused by the selected fungi was 42% in case of *F. monliforme*, 34% in case of *F. solani*, *R. solani* (60%) and *S. bataticola* (46%).

***In vitro* effect of the selected antioxidants on the growth of the isolated fungi:** Results in Table 3 presented salicylic acid at a concentration of 2, 4, 6, 8 mM, tartaric acid at (5, 10, 15, 20 mM) and GAWDA at (1, 2, 3 and 4 g L^{-1}) significantly reduces the linear growth of *F. monliforme*, *F. solani*, *R. solani* and *S. bataticola*.

Interaction between *Trichoderma* spp. and the isolated fungi: *Tricohderma viride* and *T. harzinum* retarded the mycelial growth of *F. monliforme*, *F. solani*, *R. solani* and *S. bataticola* as shown in Table 4. *T. harzinum* recorded the highest antagonistic effect (100%) on the mycelial growth of both *R. solani* and *S. bataticola* only.

Table 2: Pathogenicity of damping-off pathogens isolated from sugar beet roots

Fungi	Damping-off (%)
F. monliforme	42[c]*
F. solani	34[d]
R. solani	60[a]
S. bataticola	46[b]
Check	0[e]

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$

Table 3: Effect of selected antioxidants on the linear growth of the pathogenic fungi attacking sugar beet plants

Antioxidant	*F. monliforme*	*F. solani*	*R. solani*	*S. bataticola*
Salicylic acid				
2 mM	6.51[b]*	6.61[b]	5.89[b]	6.89[b]
4 mM	4.15[d]	5.68[c]	5.15[c]	5.79[d]
6 mM	3.12[g]	4.26[e]	3.39[e]	5.53[e]
8 mM	0.00[k]	0.00[i]	0.00[l]	0.00[l]
Tartaric acid				
5 mM	3.64[f]	3.05[f]	4.13[d]	6.66[c]
10 mM	2.62[i]	2.11[g]	3.12[f]	4.91[f]
15 mM	1.45[j]	1.11[h]	1.42[i]	3.92[h]
20 mM	1.24[j]	1.01[h]	1.09[j]	2.79[j]
GAWDA® formulation				
1 g L^{-1}	5.76[c]	5.34[d]	2.45[g]	4.42[g]
2 g L^{-1}	3.94[e]	4.23[e]	1.59[h]	3.04[i]
3 g L^{-1}	2.83[h]	3.19[f]	0.92[k]	1.66[k]
4 g L^{-1}	0.00[k]	0.00[i]	0.00[l]	0.00[l]
Check	9.00[a]	9.00[a]	9.00[a]	9.00[a]

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$

Table 4: Interaction between *Trichoderma* spp. and the isolated fungi

	Check		*T. viride*		*T. harzinum*	
Fungus	Growth (cm)	Inhibition (%)	Growth (cm)	Inhibition (%)	Growth (cm)	Inhibition (%)
F. monliforme	9.00[a]*	0.00[a]	1.50[c]	83.38[b]	1.29[b]	85.62[b]
F. solani	9.00[a]	0.00[a]	1.43[d]	84.13[a]	1.50[a]	83.37[c]
R. solani	9.00[a]	0.00[a]	2.34[a]	74.01[d]	0.00[c]	100.00[a]
S. bataticola	9.00[a]	0.00[a]	2.05[b]	77.26[c]	0.00[c]	100.00[a]

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$

Table 5: Effect of the selected antioxidants on the mycelial growth of *Trichoderma* spp.

Antioxidants	*T. viride*	*T. harzianum*
Salicylic acid		
4 Mm	8.7[ab]*	8.6[ab]
Tartaric acid		
10 mM	8.6[ab]	8.5[ab]
GAWDA formulation®		
4 g L^{-1}	8.4[ab]	8.0[b]
Check	9.0[a]	9.0[a]

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$

Effect of the tested antioxidants on *Trichoderma* spp.: The results presented in Table 5 revealed that salicylic acid at 4 mM, tartaric acid at 10 mM or GAWDA formulation at 4 g L^{-1} show a significant effect on the linear growth of *Trichoderma* spp.

Table 6: Effect of the selected formulations of the antioxidants, *Trichoderma* and compost on the damping-off percentage of sugar beet

Treatment	Pre	Post	Stunt	Survival
Non-compost				
Check				
Control	10.3[a]*	7.0[a]	11.3[a]	71.0[n]
T. viride	8.0[d]	5.0[d]	9.0[d]	78.0[k]
T. harziunum	5.3[g]	4.0[e]	6.3[g]	84.3[h]
Tartaric acid				
Control	9.6[b]	6.6[ab]	11.0[ab]	72.6[mn]
T. viride	7.0[e]	5.0[d]	8.0[e]	80.0[j]
T. harziunum	4.6[b]	3.3[f]	5.0[h]	87.0[g]
Salicylic acid				
Control	9.0[c]	6.0[c]	10.0[c]	75.0[l]
T. viride	6.3[f]	4.0[e]	7.0[fg]	82.3[i]
T. harziunum	3.0[i]	2.0[g]	3.6[ij]	91.6[de]
GAWDA formulation				
Control	9.3[bc]	6.3[bc]	10.3[bc]	74.3[lm]
T. viride	6.3[f]	4.3[e]	7.3[ef]	82.0[i]
T. harziunum	2.0[j]	1.0[h]	2.0[l]	95.0[b]
Compost				
Check				
Control	3.3[i]	2.3[g]	4.0[i]	89.6[f]
T. viride	3.0[i]	2.0[g]	3.0[jk]	92.0[de]
T. harziunum	2.0[j]	1.0[h]	2.0[l]	94.6[bc]
Tartaric acid				
Control	3.3[i]	2.3[g]	3.3[ij]	90.3[ef]
T. viride	2.0[j]	2.0[g]	3.0[jk]	93.0[cd]
T. harziunum	2.0[j]	1.0[h]	2.0[l]	94.6[bc]
Salicylic acid				
Control	3.0[i]	2.0[g]	3.0[jk]	91.0[ef]
T. viride	2.0[j]	1.3[h]	2.3[kl]	94.0[bc]
T. harziunum	0.0[k]	0.0[i]	0.0m	100.0[a]
GAWDA formulation				
Control	3.0[i]	2.0[g]	3.3[ij]	91.0[ef]
T. viride	0.0[k]	0.0[i]	0.0[m]	100.0[a]
T. harziunum	0.0[k]	0.0i	0.0[m]	100.0[a]

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$

Effect of the combination of antioxidants, *Trichoderma* and compost on the damping-off percentage: The combination consists of GAWDA formulation at 4 mM and *T. harzianum* before sowing in soil amended with organic fertilizer (compost) has a significantly effects on decreasing the incidence of damping-off as shown in Table 6.

Effect of the combination of antioxidants, *Trichoderma* and compost on the content of the photosynthetic pigments and total phenols in leaves: The combination consists of GAWDA formulation at 4 mM and *T. harzianum* before sowing in soil amended with organic fertilizer (compost) significantly increased the content of chlorophyll A to record (2.592 mg g^{-1} fresh weight), chlorophyll B (1.719 mg g^{-1} fresh weight), total chlorophyll (4.311 mg g^{-1} fresh weight) and carotenoid (0.116 mg g^{-1} fresh weight). The total phenols recorded (943 mg/100 g fresh weight) as shown in Table 7.

Table 7: Effect of the combination of antioxidants, *Trichoderma* and compost on the content of the photosynthetic pigments and the total phenols in the leaves of healthy sugar beet plants

Treatments	Total phenol mg catechol/100 g fresh weight	Chlorophyll A mg g^{-1} fresh weight	Chlorophyll B mg g^{-1} fresh weight	Total chlorophyll mg g^{-1} fresh weight	Carotenoid mg g^{-1} fresh weight
Non-compost					
Check					
Control	319^{l*}	0.876^{l}	0.581^{l}	1.457^{l}	0.039^{l}
T. viride	397^{k}	1.093^{k}	0.752^{k}	1.819^{k}	0.049^{k}
T. harziunum	399^{k}	1.096^{k}	0.727^{k}	1.824^{k}	0.049^{k}
Tartaric acid					
Control	334^{l}	0.92^{l}	0.610^{l}	1.530^{l}	0.041^{l}
T. viride	478^{h-j}	1.314^{h-j}	0.871^{h-j}	2.186^{h-j}	0.059^{h-j}
T. harziunum	558^{fg}	1.534^{fg}	1.018^{fg}	2.552^{fg}	0.069^{fg}
Salicylic acid					
Control	411^{k}	1.131^{k}	0.750^{k}	1.882^{k}	0.050^{k}
T. viride	557^{fg}	1.531^{fg}	1.016^{fg}	2.548^{fg}	0.068^{fg}
T. harziunum	637^{e}	1.752^{e}	1.162^{e}	2.914^{e}	0.078^{e}
GAWDA formulation					
Control	425^{jk}	1.168^{jk}	0.775^{jk}	1.943^{jk}	0.052^{jk}
T. viride	558^{fg}	1.533^{fg}	1.017^{fg}	2.550^{fg}	0.068^{fg}
T. harziunum	717^{d}	1.971^{d}	1.307^{d}	3.279^{d}	0.088^{d}
Compost					
Check					
Control	438^{i-k}	1.204^{i-k}	0.799^{i-k}	2.003^{i-k}	0.054^{i-k}
T. viride	571^{f}	1.569^{f}	1.041^{f}	2.611^{f}	0.07^{f}
T. harziunum	797^{c}	2.190^{c}	1.453^{c}	3.643^{c}	0.098^{c}
Tartaric acid					
Control	478^{h-j}	1.314^{h-j}	0.871^{h-j}	2.186^{h-j}	0.059^{h-j}
T. viride	584^{ef}	1.606^{ef}	1.065^{ef}	2.672^{ef}	0.071^{ef}
T. harziunum	823^{bc}	2.263^{bc}	1.501^{bc}	3.765^{bc}	0.101^{bc}
Salicylic acid					
Control	491^{hi}	1.350^{hi}	0.895^{hi}	2.246^{hi}	0.060^{hi}
T. viride	637^{e}	1.752^{e}	1.162^{e}	2.914^{e}	0.078^{e}
T. harziunum	876^{b}	2.109^{b}	1.598^{b}	4.008^{b}	0.107^{b}
GAWDA formulation					
Control	505^{gh}	1.387^{gh}	0.92^{gh}	2.307^{gh}	0.062^{gh}
T. viride	717^{d}	1.971^{d}	1.307^{d}	3.279^{d}	0.088^{d}
T. harziunum	943^{a}	2.592^{a}	1.719^{a}	4.311^{a}	0.116^{a}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$

Effect of the combination of antioxidants, *Trichoderma* and compost on the growth parameters of healthy sugar beet plants: The combination of the compost, GAWDA formulation at 4 mM and *T. harzianum* significantly increased the plant weight to record (2619 g), plant height (328 cm), root weight (1405 g), root length (124 cm), shoot weight (1213 g), shoot length (205 cm), root diameter (20.3 cm) and leave numbers (96 leaves) (Table 8).

Effect of the selected formulations on the content of the total sugar and TSS in healthy sugar beet plants: The combination of the compost, GAWDA formulation at 4 mM and *T. harzianum* significantly increased the content of total sugar to record (23.66%) and TSS (28.08%) (Table 9).

DISCUSSION

The results illustrate obvious incidence of *F. moniliforme*, *F. solani*, *R. solani* and *S. bataticola* in wilted roots of sugar beet plants grown in different areas at Dakahlia Governorate of Egypt.

A modern method for controlling diseases attacking sugar beet roots caused by *F. moniliforme*, *F. solani*, *R. solani* and *S. bataticola* was applied as a friendly environmental method of fungal control. The application of compost, antioxidant i.e.: Salicylic acid, Tartaric acid, GAWDA® formulation and *T. harzianum* and *T. viridae* individually or combinations accelerate the resistance of sugar beet plants against *F. moniliforme*, *F. solani*, *R. solani* and *S. bataticola*.

Table 8: Effect of the selected formulations of the antioxidants, *Trichoderma* and compost on growth parameters in healthy sugar beet plants

Treatment	Plant weight	Plant height	Root weight	Root length	Shoot weight	Shoot length	Root diameter	Leave numbers
Non-compost								
Check								
Control	885 ^{l*}	37^{n}	475^{l}	11^{m}	410^{l}	26^{n}	7.0^{i}	32^{l}
T. viride	1105^{k}	41^{l}	593^{k}	14^{k}	512^{k}	27^{m}	9.0^{h}	40^{k}
T. harziunum	1108^{k}	46^{k}	595^{k}	14^{k}	514^{k}	32^{l}	9.0^{h}	40^{k}
Tartaric acid								
Control	929^{l}	39^{m}	499^{l}	12^{l}	431^{l}	27^{n}	7.3^{i}	34^{l}
T. viride	1328^{h-j}	48^{jk}	712^{h-j}	16^{i}	615^{h-j}	32^{l}	10.0^{fg}	48^{h-j}
T. harziunum	1550^{fg}	64^{g}	832^{fg}	19^{g}	719^{fg}	45^{gh}	12.0^{e}	57^{f}
Salicylic acid								
Control	1143^{k}	48^{jk}	613^{k}	14^{k}	530^{k}	34^{k}	9.0^{h}	41^{k}
T. viride	1548^{fg}	64^{g}	830^{fg}	19^{g}	717^{fg}	45^{gh}	12.0^{e}	56^{fg}
T. harziunum	1770^{e}	74^{e}	950^{e}	22^{e}	820^{e}	52^{f}	14.0^{d}	65^{e}
GAWDA formulation								
Control	1180^{jk}	49^{jk}	633^{jk}	14^{k}	547^{jk}	35^{jk}	9.3^{gh}	43^{jk}
T. viride	1549^{fg}	64^{g}	831^{fg}	19^{g}	718^{fg}	45^{gh}	12.0^{e}	56^{f}
T. harziunum	1992^{d}	83^{d}	1069^{d}	25^{d}	923^{d}	58^{e}	15.0^{d}	73^{d}
Compost								
Check								
Control	1217^{i-k}	51^{j}	653^{i-k}	15^{j}	564^{i-k}	36^{j}	9.6^{f-h}	44^{i-k}
T. viride	1586^{f}	66^{fg}	851^{f}	20^{f}	735^{f}	46^{gh}	12.3^{e}	58^{f}
T. harziunum	2213^{c}	92^{c}	1187^{c}	27^{c}	1026^{c}	65^{d}	17.0^{c}	81^{c}
Tartaric acid								
Control	1328^{h-j}	55^{i}	712^{h-j}	16^{i}	615^{h-j}	39^{i}	10.0^{f-h}	48^{h-j}
T. viride	1623^{ef}	67^{f}	871^{ef}	20^{f}	752^{ef}	47^{g}	12.6^{e}	59^{ef}
T. harziunum	2287^{bc}	95^{bc}	1227^{bc}	28^{bc}	1060^{bc}	67^{c}	17.6^{bc}	84^{bc}
Salicylic acid								
Control	1364^{hi}	57^{h}	732^{hi}	17^{h}	632^{hi}	40^{hi}	10.3^{fg}	49^{hi}
T. viride	1770^{e}	74^{e}	950^{e}	22^{e}	820^{e}	52^{f}	14.0^{d}	65^{e}
T. harziunum	2439^{b}	101^{b}	1306^{b}	30^{b}	1128^{b}	71^{b}	18.6^{b}	89^{b}
GAWDA formulation								
Control	1401^{gh}	58^{h}	752^{gh}	17^{gh}	649^{gh}	41^{h}	10.6^{f}	51^{gh}
T. viride	1992^{d}	83^{d}	1069^{d}	25^{d}	923^{d}	58^{e}	15.0^{d}	73^{d}
T. harziunum	2619^{a}	109^{a}	1405a	35^{a}	1213^{a}	74^{a}	20.3^{a}	96^{a}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$

The *in vitro* studies showed that salicylic acid, tartaric acid, GAWDA® formulation and antagonistic fungi; *T. harzianum* and *T. viridae* in the presence of the selected compost inhibit damping-off pathogens. These results were in support with the finding of Abdel-Monaim[43], Ismail[44] and Elwakil and El-Metwally[45].

Salicylic acid at 2, 4, 6 and 8 mM, tartaric acid at 5, 10, 15 and 20 mM and GAWDA® formulation at 1, 2, 3 and 4 g L^{-1} significantly decreased the linear growth of *F. monliforme*, *F. solani*, *R. solani* and *S. bataticola*. Moreover, the presented results are also in support with the finding of Galal *et al.*[46], Shahda[47], Shabana *et al.*[48] and Abd El-Hai *et al.*[49].

Giridhar and Reddy[50] presented the antioxidants as a friendly green chemicals which inhibit of functions of several enzymes by oxidized compounds, dissolved membrane lipids and interfere with membrane functions including transport of nutrients and interferes with proteins, RNA and DNA synthesis.

It was also, obvious that the best concentration for soaking seeds in salicylic acid was 4 mM, tartaric acid 10 mM and GAWDA® one g L^{-1} and this was in a harmony with those obtained by El-Mougy *et al.*[51].

The application of selected antioxidants increases the sugar beet growth parameters. This increase may attribute to the role of antioxidants in stimulating of the physiological processes and reflecting an improvement in the vegetative growth followed by active translocation of the photo assimilation. In this respect, antioxidants might also increase enzyme activates such as α-amylase and nitrate reductase, which accelerates the sugar translocation from the leaves to developing fruit[52].

Table 9: Effect of selected formulations of antioxidants, *Trichoderma* and compost on the content of the total sugar and TSS in healthy sugar beet plants

Treatment	Sucrose (%)	TSS (%)
Non-compost		
Check		
Control	15.52^{l*}	18.28^{l}
T. viride	16.55^{k}	19.50^{k}
T. harziunum	16.57^{k}	19.52^{k}
Tartaric acid		
Control	15.73^{l}	18.65^{l}
T. viride	17.60^{hij}	20.73^{ghi}
T. harziunum	18.64^{fg}	21.97^{ef}
Salicylic acid		
Control	16.73^{k}	19.81^{jk}
T. viride	18.62^{fg}	21.95^{ef}
T. harziunum	19.67^{e}	23.18^{d}
GAWDA formulation		
Control	16.91^{jk}	20.12^{ijk}
T. viride	18.63^{fg}	21.95^{ef}
T. harziunum	20.71^{d}	24.41^{c}
Compost		
Check		
Control	17.08^{ijk}	20.42^{hij}
T. viride	18.81^{f}	22.26^{e}
T. harziunum	21.75^{c}	25.63^{b}
Tartaric acid		
Control	17.60^{hij}	20.73^{ghi}
T. viride	18.98^{ef}	22.57d^{e}
T. harziunum	22.10^{bc}	26.24^{b}
Salicylic acid		
Control	17.77^{hi}	21.04^{gh}
T. viride	19.67^{e}	23.18^{d}
T. harziunum	22.79^{b}	27.47^{a}
GAWDA formulation		
Control	17.94^{gh}	21.34^{fg}
T. viride	20.71^{d}	24.41^{c}
T. harziunum	23.66^{a}	28.08^{a}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at $p \leq 0.05$

CONCLUSION

This research highlights that the designed formulation of GAWDA at 4 mM and *T. harzianum* used for treating sugar beet seeds before sawing in soil supplemented with compost at a rate of (2 ton/fed) is an innovated method to overcome the incidence of damping off disease of sugar beet plants and significantly improve the yield and quality of the harvested roots.

SIGNIFICANCE STATEMENT

This data may help the researchers to direct their attention to use green chemicals as safe alternative to the toxic chemicals used in the agriculture regime, subsequently produce healthy food and keep the environment and soil clean.

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