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Enhancing Systemic Acquired Resistance in Cucumber to Control Root Rot and Wilt Diseases with Reference to Yield and Quality

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ABSTRACT
The current methods for controlling root rot and wilt disease caused by *Fusarium oxysporum* f. sp. radicis-cucumerinum, *F. solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*, the most destructive fungi on cucumber are not yet rely. Salicylic Acid (SA), tartaric acid, GAWDA® formulation, compost, *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichoderma viride* and *Gliocladium virens* were used in this investigation as promising methods for controlling such diseases. Results illustrated that soaking seeds in the antioxidants for 12 h before sowing followed by coating them with a combination of *Trichoderma* and compost, significantly reduced the disease incidence. It was also found that the triple combination of salicylic acid at 4 mM, *T. harzianum* and compost (2 t/fed) showed to be an intelligent tactic to enhance the resistance of cucumber plants against the root rot and wilt diseases. Moreover, this approach significantly promoted both yield and quality of the fruits.

Key words: Systemic acquired resistance, cucumber, antioxidants, wilt and root rot diseases, *Trichoderma,* Seed coating

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the oldest cultivated vegetables dating back to 5,000 years (Wehner and Guner, 2004) and classified in the Asian continent as the fourth most important vegetable crop following tomato, cabbage and onion (Tatlioglu, 1997). It ranks as the second vegetable crop in Western Europe behind tomato (Phu, 1997). The major disease contributing to yield losses is wilt disease caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* Schlecht (Ye *et al*., 2004; Morsy *et al*., 2009; Farrag and Fatouh, 2010; Hu *et al*., 2010). *Fusarium oxysporum*, *F. solani*, *S. rolfsii*, *R. solani* and *M. phaseolina* are the commonly associated fungi with cucumber plants (Morsy *et al*., 2009). Since the control of such disease depends mainly on intensive application of the fungicides, risks to human health and the environment are expected. Therefore, alternative eco-friendly approaches for the control of the disease were emphasized (Mandal *et al*., 2009). Scaling up the Systemic Acquired Resistance (SAR) is a newly strategy to control a number of diseases in different crops (Hoitink and Boehm, 1999; Vallad *et al*., 2000; Nelson and Boehm, 2002). The varieties of microbes present in composts successfully induce systemic resistance in plants (Wei *et al*., 1991; Liu *et al*., 1995a, b, c). At the time being Induced Systemic Resistance (ISR) is accepted as one of the most promising methods for controling plant diseases. Some bacteria and fungi have been reported as potential candidates to induce systemic resistance in plants (Van Loon *et al*., 1998). *Trichoderma* spp. have beneficial impact on plant protection if colonized on roots, as they trigger the plant defense mechanism including the production of phytoalexins, Pathogenesis Related (PR) proteins, jasmonic acid, peroxidases and chitinases (Djonovic *et al*., 2006). The antioxidants increase plant growth

parameters, resulting in enhancement of the yield and seed or fruit quality in a number of vegetables and field crops (Elwakil and El-Metwally, 2000; Elwakil, 2003; El-Mougy *et al*., 2004; Karlidag *et al*., 2009; Abd El-Hai *et al*., 2009; Zahra *et al*., 2010).

Therefore, the present research aimed to study the role of a number of selected antioxidants and their formulations (including Salicylic Acid (SA), tartaric acid, GAWDA® formulation, compost as well as *T. harzianum*, *T. hamatum*, *T. viride*, *Gliocladium virens*) for possible enhancement of (1) The systemic acquired resistance, (2) The control of root rot and wilt disease caused by *F. oxysporum* f. sp. radicis-cucumerinum, *F. solani*, *R. solani* and *M. phaseolina* the most aggressive fungi attacking cucumber roots, (3) The yield and quality of fruits. In this context, the increases in contents of the photosynthetic pigments and total phenols in the vegetative parts of the plants will be taken as indicators for the expected acceleration of plant defense mechanism against the attack of the damping off and wilt pathogens.

MATERIALS AND METHODS

Trials were conducted in laboratories and farm of Plant Pathology Department, Faculty of Agriculture, Mansoura University in the period from 2012-2015.

Sampling: Samples of cucumber roots presenting wilted plants grown in open fields at different areas of Dakahlia and Damietta Governorates of Egypt were collected to be used in the research.

Isolation and identification of the fungal pathogens: Roots from wilted plants washed thoroughly in tap water. Small pieces of their vascular tissues were then surface sterilized for 1 min in 0.5% sodium hypochlorite solution (NaOCl), washed again three times in sterilized distilled water and distributed on moist blotter papers containing Petri-dishes. Dishes were incubated at 22°C for 7 days in dark. The isolated fungi were identification based on the keys of morphological and cultural characteristics of the mycelium, conidiophores, conidia and colony morphology (Parmeter, 1970; Dhingra and Sinclair, 1978; Nelson *et al*., 1983; Booth, 1985; Burrges *et al*., 1988).

Pathogenicity test: Pots (20 cm in diameter), filled with clay sandy soil at a rate of 2.5 kg pot^{-1} were inoculated with the tested fungi prepared by growing each fungal isolate in glass bottles containing sterilized sorghum grain medium (100 g of sorghum seeds mixed with sand at a ratio of 2:1 and moisted with water, transferred to conical flasks and autoclaving at 121°C for 20 min), then incubated at 25±2°C for 15 days. Soil infestation was achieved by mixing the inoculum of each fungus with the upper 5 cm layer of the soil surface and rate of 2% (w/w) for *R. solani*, *M. phaseolina* and 4% (w/w) for *F. oxysporum*, *F. solani*. To make the soils ready for seeding, they were irrigated every 2 days for a period of a week and to insure the fungi adaptation. The 6 healthy looking cucumber seeds from previously tested lot were sown in each pot. The 3 pots were used as replicates for each fungus, while 3 uninfested pots were used as a check. The treatments were irrigated as needed. All pots were kept in a net house under natural conditions while the temperature was fluctuated between 25±5°C and the humidity between 55-75%.

Effect of the tested antioxidants on the fungal growth: The following antioxidants were selected to test their possible effects on retarding the growth of the pathogenic fungi under investigation i.e., salicylic acid, tartaric acid and GAWDA® formulation (Tri-Sodium Orthophosphate 1 mM+Tartaric acid 2 mM+Hydroxyquinoline 1 mM+Calcium Chloride 6 mM+Magnesium Chloride 5 mM+Calcium Borate 5 mM). The antioxidants salicylic acid, tartaric acid and GAWDA® formulation were dissolved in distilled water to obtain the desired concentrations of (2, 4, 6 and 8 mM), (5, 10, 15 and 20 mM), (1, 2, 3 and 4 g L^{-1}), respectively. Disks of each fungal growth (0.6 cm diameter) from 7 days old cultures were transferred onto centers of PDA plates supplemented with different concentrations of the selected antioxidants. The possible inhibitory effects of these antioxidants on the tested fungi were recorded. The 3 replicates were used to present one treatment. All cultures were incubated at 25±2°C for 7 days in dark. Linear growth of each fungus was measured and recorded.

***Trichoderma* spp.:** *Trichoderma harzianum*, *T. hamatum*, *T. viride* and *G. virens* obtained from the Department of Plant Pathology, Faculty of Agriculture, Mansoura University Egypt were used in this investigation. Seeds were coated with a formulation consists of one sp of *Trichoderma* spp. (at a rate of 1 g/20 g seeds) and Acacia gum at a concentration of (2 g/10 mL water), air dried for 2 h. At this stage, seeds were ready for sawing.

Antagonistic effects of *Trichoderma* spp. on the selected pathogens: The possible antagonistic competency of *T. harzianum*, *T. hamatum*, *T. viride* and *G. virens* to inhibit the growth of the tested fungi were investigated using dual culture technique (Baker and Cook, 1974). Discs of 0.6 cm taken from the growing edge of 7 days old cultures of *F. oxysporum*, *F. solani*, *R. solani* and *M. phaseolina* were placed at a distances of 1 cm from the edge of Sterilized Petri dishes containing sterilized PDA. At the inverse side of the medium, 0.6 cm diameter disc of 5 days old culture of *T. harzianum*, *T. hamatum*, *T. viride* and *G. virens* singly

placed at a distances of 1 cm from the edges. The PDA containing petri dishes were inoculated at their centers with 0.6 cm diameter discs of *F. oxysporum*, *F. solani*, *R. solani* and *M. Phaseolina* to be used as checks. All plates were incubated at 25±2°C under 12 h in dark for 8 days. The inward linear growth (distance between the center of the disc and the edge of the colony) was measured after 2, 4, 6 and 8 interval days and the interaction between the 2 antagonized fungi was recorded either in the form of inhibition zone or the over growth of *T. harzianum*, *T. hamatum*, *T. viride* and *G. virens* on *F. oxysporum*, *F. solani*, *R. solani* and *M. phaseolina* as described by Desai *et al*. (2002).

Source of seeds: Seeds of cucumber cv. Beta Alpha Hybrid (produced by Seminis Co, USA) were used in the research.

Impact of the antioxidants on seed germination: Groups of 10 g of cucumber seeds were separately soaked in one of the following antioxidants for 12 h. Salicylic acid at (2, 4, 6 and 8 mM), tartaric acid at concentration (5, 10, 15 and 20 mM) and GAWDA® at (1, 2, 3 and 4 g L^{-1}). After soaking seeds of each treatment were seeded onto moist blotter paper in 9 cm diameter Petri dishes while 3 replicates presented one treatment. The check was prepared by soaking healthy seeds in a tap water. All plates were incubated at 25±2°C for 7 days in dark, then the number of germinated seeds were rated to assess the impact of the antioxidants on seed germination. The best concentration of the tested antioxidants was determined and recorded.

Possible impact of the used antioxidants on *Trichoderma* spp.: Disks of 0.6 cm diameter from 5 days old cultures of each candidate (*T. harzianum*, *T. hamatum*, *T. viride*) were set onto centers of PDA plates each supplemented with 4 mM salicylic acid or 10 mM Tartaric acid or 1 g L^{-1} GAWDA® and incubated in dark at 25±2°C up to 7 days. Three replicates were used per each treatment. The check was prepared by placing each fungus on PDA free from the antioxidants. At the point in which *Trichoderma* cover the whole surface of the check treatments, colony diameters were measured and the possible percentage of growth retardation was calculated.

Field trails: Field trails were carried out at the experimental farm of Plant Pathology Department, Faculty of Agriculture, Mansoura University, to compare the *in vitro* results versus the *in vivo* studies. Split-Split Plot design of 6 replicates was applied. The compost were allocated in the main plots, antioxidant sub plots and Trichoderma sub plots. Cucumber seeds cv. Beta alpha hybrid were sown in ridges of 100 cm apart in hills spaced 35 cm apart on one side of the ridge.

Table 1: Chemical analysis of the used compost

Characters	Compost
Weight (m^3 kg)	650-700
Moisture content (%)	25-30
pH	7.5-8
EC (ds m^{-1})	2-4
Total nitrogen	1.2-1.4
Organic substance (%)	32-34
Organic carbon (%)	18.5-19.7
C/N ratio	1-14.1/1-1.18
Total phosphorus (%)	0.5 -0.75
Total Potassium (%)	0.8-1
Sodium Chloride (%)	1.1-1.75
Micro elements	
Iron ppm	1500-1800
Manganese (ppm)	25-50
Copper (ppm)	75-150
Zinc (ppm)	150-225

Compost: Commercial compost produced by the Egyptian Company for Solid Waste Recycling (ECARU) at the rate of 2 t/fed was used in this research. The composition of the compost is shown in Table 1. A daily observation for damping-off and wilt disease were recorded.

Forty days beyond seed sowing, a number of physiological characters were determined. In blades of third leaves of the growing plants, total phenols were measured following the method of Maliak and Singh (1980). Chlorophyll a, b and carotenoid were detected according to the method of Mackinney (1941). Ascorbic acid content (vitamin C) was detected following the method of (AOAC., 1970). Total soluble solids in fruits were detected by using a refractometer (ATAGO-MANUAL, Japan) (AOAC., 1970), fruit firmness was measured in 6 fruits from each sample employing a hand penetrometer and the measurements were taken on the 2 cheeks at the center of each fruit using 0.654 cm diameter head of penetrometer (Gullimex FT327, Italy), acidity in cucumber juice was measured following the method developed by Rangana (1979). Fruit moisture was calculated by taking a fixed weight, cut it to few slices, distribute them in opened Petri dishes for a partial dryness followed by incubation for 3 days at 70°C to insure a complete dryness. The difference between the 2 weights was recorded. Total carbohydrates in the fruit was measured following the method described by Sadasivam and Manickam (1996). At maturity stage, the following characters were recorded: yield (g)/plant, number of fruits/plant, fruit length and fruit diameter.

Statistical analysis: The obtained data were statistically analyzed using COSTAT (2005) software of analysis of variance (Gomez and Gomez, 1984). The means were compared using Least Significant Difference (LSD) at $p = 0.05$ as outlined by Duncan (1995).

RESULTS AND DISCUSSION

Results in Table 2 illustrate the degrees of variance in damping-off severity caused by *F. oxysporum*, *F. solani*, *R. solani* and *M. phaseolina* isolates. *F. oxysporum* diseased 78% of the total number of tested plants, *F. solani* 89%, *R. solani* 94% and *M. phaseolina* 78% when compare with the check.

Results in Table 3 show that salicylic acid at concentrations of 2, 4, 6, 8 mM, tartaric acid at (5, 10, 15, 20 mM) and GAWDA® formulation at (1, 2, 3 and 4 g L^{-1}) significantly reduced the linear growth of *F. oxysporum*, *F. solani*, *R. solani* and *M. phaseolina* isolated from cucumber roots. It was also noticed that the reduction in the linear growth was correlated to the increase in concentration of the tested antioxidants.

The effect of *T. viride*, *T. hamatum*, *T. harzianum* and *G. virens* on the mycelial growth of *F. oxysporum*, *F. solani*, *R. solani* and *M. phaseolina* is shown in Table 4. *Trichoderma harzianum* presented the highest antagonistic effect (100%) on the mycelial growth of *R. solani* and *M. phaseolina* while least inhibition percentage was shown from *T. viride* on the linear growth of *R. solani* (76.8%).

On the other hand, soaking cucumber seeds in salicylic acid at concentrations of (2, 4, 6, 8 mM), tartaric acid at concentrations of (5, 10, 15, 20 mM) as well as GAWDA® formulation at concentrations (1, 2, 3 and 4 g L^{-1}) for 12 h significantly increased the percentage of seed germination (Table 5).

The results also show that the combination of *T. viride*, *T. hamatum*, *T. harzianum* and *G. virens* and salicylic acid at 4 mM, tartaric acid 10 mM or GAWDA® formulation 1 g L^{-1} did not show any adverse effect on the linear growth of *Trichoderma* spp. used in this investigation (Table 6 and Fig. 1).

The triple combination consists of compost, *Trichoderma* and antioxidants significantly reduced the percentage of damping-off syndrome. Precisely the combinations of (compost, salicylic acid and *T. viride* or *T. hamatum* or *T. harzianum* or *G. virens*) or (compost, tartaric acid and *T. harzianum* or *G. virens*) as well as (compost, GAWDA® formulation plus *T. harzianum* or *G. virens*) were highly effective on reducing the incidence of damping-off as shown in Table 7.

The impact of compost, *Trichoderma* and antioxidants solely or in their combinations on the content of

Table 2: Pathogenicity of the isolated root rot and wilt fungi in cucumber and percentage of their severity

Fungi	Severity of damping-off (%)
Check	0
Fusarium oxysporum	78
F. solani	89
Rhizoctonia solani	94
Macrophomina phaseolina	78

Table 3: Effect of different concentrations of antioxidants on the linear growth of the tested pathogenic fungi attacking cucumber plants

Antioxidant	*Fusarium oxysporum*	*F. solani*	*Rhizoctonia solani*	*Macrophomina phaseolina*
Check	9.00^{a}*	9.00^{a}	9.00^{a}	9.00^{a}
Salicylic acid				
2 mM	8.50^{b}	8.63^{b}	9.00^{a}	9.00^{a}
4 mM	5.42^{hi}	7.42^{d}	8.03^{c}	9.00^{a}
6 mM	4.08^{l}	5.57^{gh}	4.43^{k}	7.23d^{e}
8 mM	2.93^{qr}	3.28^{op}	1.28^{w}	5.67^{gh}
Tartaric acid				
5 mM	4.75^{j}	3.98^{lm}	5.40^{hi}	8.70^{ab}
10 mM	3.42^{no}	2.75^{r}	4.07^{l}	6.42^{f}
15 mM	1.90^{s-u}	1.45^{vw}	1.85^{s-u}	5.12^{i}
20 mM	1.62^{uv}	1.32^{vw}	1.42^{vw}	3.65^{n}
GAWDA®				
1 g L^{-1}	7.53^{d}	6.97^{e}	3.20^{o-q}	5.78^{g}
2 g L^{-1}	5.15^{i}	5.52^{gh}	2.08st	3.97^{lm}
3 g L^{-1}	3.70^{mn}	4.17^{kl}	1.20^{w}	2.17^{s}
4 g L^{-1}	1.82^{tu}	3.03^{p-r}	0.00^{y}	0.90^{x}

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

Table 4: *In vitro* effect of *Trichoderma* spp. on the linear growth of the tested fungi

	Check	*Trichoderma viride*		*Trichoderma hamatum*		*Gliocladium virens*		*Trichoderma harzianum*	
Fungus	*Trichoderma* growth	*Trichoderma* growth	Inhibition	*Trichoderma* growth	Inhibition	*Trichoderma* growth	Inhibition	*Trichoderma* growth	Inhibition
Fusarium oxysporum	9.00^{a}*	7.67^{cd}	85.2^{cd}	7.67^{cd}	85.2^{cd}	7.77^{bc}	86.2^{bc}	7.85^{b}	87.2^{b}
F. solani	9.00^{a}	7.73^{bc}	85.9^{bc}	7.75^{bc}	86.1^{bc}	7.53^{de}	83.6^{de}	7.67^{cd}	85.2^{cd}
Rhizoctonia solani	9.00^{a}	6.92^{g}	76.8^{g}	7.03^{fg}	78.1^{fg}	7.47^{e}	82.9^{e}	9.00^{a}	100.0^{a}
Macrophomina phaseolina	9.00^{a}	7.18^{f}	79.8^{f}	7.10^{f}	78.8^{f}	7.18^{f}	79.8^{f}	9.00^{a}	100.0^{a}

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

photosynthetic pigments and the total phenols in leaves of cucumber plants revealed that the triple combination of compost, salicylic acid at 4 mM and *T. harzianum* presented a significant increase in the content of chlorophyll A (1.905 mg g^{-1}), chlorophyll B (0.964 mg g^{-1}), total chlorophyll (2.869 mg g^{-1}) and carotenoid (0.296 mg g^{-1}) as well as the total phenols to present a rate of (153.3 mg g^{-1} fresh weight) (Table 8).

It was also found that the combination of compost and salicylic acid at 4 mM in the presence of *T. harzianum* present a significant increase in the content of total carbohydrate (51.2%), ascorbic acid 45.4% (16.00 mg/100 g fresh weight). Firmness 60% (16 lb/in^2). Moisture was reduced by 1.56% and the total soluble solids by 32% as shown in Table 9. The acidity reduced by 42.8% to record 1.12% when compare with the check (Table 9).

This triple combination also showed a significant increase in the yield 66.6%, as the number of fruits/plant increased by 220%, fruit length by 61% and the fruit diameter was reduced by 40% as shown in Table 10.

In this study 4 fungal isolates i.e.,: *F. oxysporum* f. sp. radicis-cucumerinum, *F. solani*, *R. solani* and *M. phaseolina* obtained from wilted roots of cucumber plants grown in fields at different areas in Dakahlia and Damietta Governorates.

Table 5: Effect of different concentrations of salicylic acid, tartaric acid and the formulation of GAWDA® on germination of cucumber seeds *in vitro*

Antioxidant	Concentration	No of seed germination
Check	80[a]*	
Salicylic acid	2 mM	80[a]
	4 mM	70[ab]
	6 mM	50[c]
	8 mM	20[d]
Tartaric acid	5 mM	80[a]
	10 mM	80[a]
	15 mM	70[ab]
	20 mM	60[bc]
GAWDA®	1 g L^{-1}	80[a]
	2 g L^{-1}	70[ab]
	3 g L^{-1}	60[bc]
	4 g L^{-1}	50[c]

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

Table 6: Effect of the tested antioxidants on the linear growth of *Trichoderma* spp.

Antioxidant	*Trichoderma viride*	*Trichoderma hamatum*	*Gliocladium virens*	*Trichoderma harzianum*
Check	9.0[a]*	9.0[a]	9.0[a]	9.0[a]
Salicylic acid				
4 mM	8.8[ab]	8.6[ab]	8.6[b]	8.7[ab]
Tartaric acid				
10 mM	8.6[ab]	8.7[ab]	8.6[b]	8.6[ab]
GAWDA®				
1 g L^{-1}	8.7[ab]	8.7[ab]	8.7[ab]	8.6[b]

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

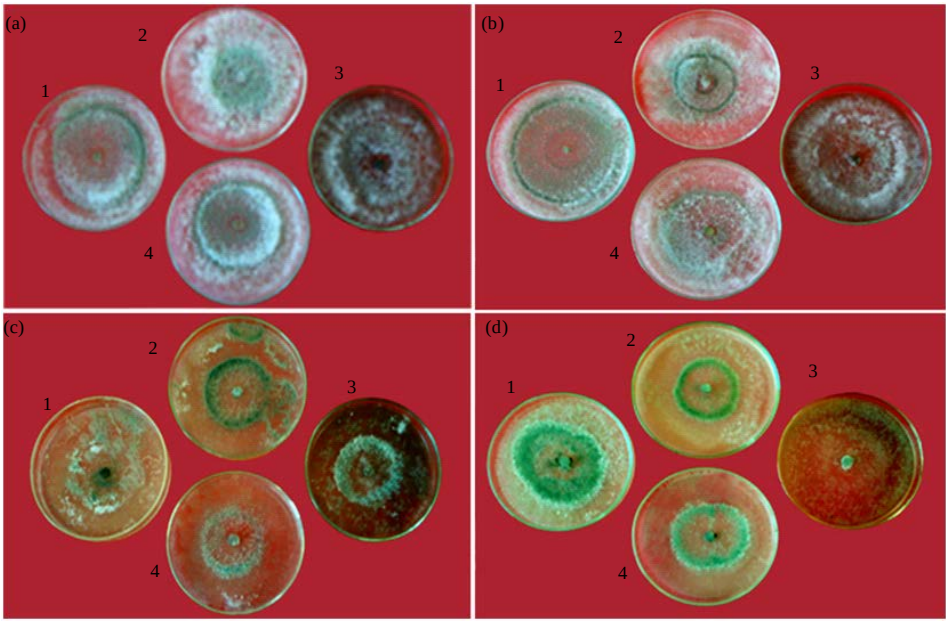

Fig. 1(a-d): Effect of the tested antioxidants on the linear growth of *Trichoderma* spp (a) *Trichoderma viride*, (b) *Trichoderma hamatum*, (c) *Gliocladium virens* and (d) *Trichoderma harzianum*, 1: Check, 2: Salicylic acid (4 mM), 3: GAWDA® (1 g L^{-1}) and 4: Tartaric acid (10 mM)

Table 7: Effect of the triple combinations of the antioxidants, *Trichoderma* and compost on the percentage of damping-off of cucumber seedlings under field conditions

Treatments		Damping-off	
Antioxidants	Antagonistic Fungi	No compost	Compost
Check	*Trichoderma viride*	33.3[b]*	16.7[c]
	Gliocladium virens	33.3[b]	16.7[c]
	Trichoderma hamatum	33.3[b]	16.7[c]
	Trichoderma harzianum	16.7[c]	16.7[c]
	Non *Trichoderma*	50.0[a]	33.3[b]
Salicylic acid	*Trichoderma viride*	16.7[c]	0.0[d]
	Gliocladium virens	16.7[c]	0.0[d]
	Trichoderma hamatum	16.7[c]	0.0[d]
	Trichoderma harzianum	16.7[c]	0.0[d]
	Non *Trichoderma*	33.3[b]	16.7[c]
Tartaric acid	*Trichoderma viride*	16.7[c]	16.7[c]
	Gliocladium virens	16.7[c]	0.0[d]
	Trichoderma hamatum	33.3[b]	16.7[c]
	Trichoderma harzianum	16.7[c]	0.0[d]
	Non *Trichoderma*	33.3[b]	33.3[b]
GAWDA®	*Trichoderma viride*	33.3[b]	16.7[c]
	Gliocladium virens	16.7[c]	0.0[d]
	Trichoderma hamatum	33.3[b]	16.7[c]
	Trichoderma harzianum	16.7[c]	0.0[d]
	Non *Trichoderma*	33.3[b]	33.3[b]

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

These fungi were purified and identified based on the identification keys while the pathogenicity tests revealed that all tested isolates have potential to attack cucumber roots cv. Beta alpha hybrid and cause a syndrome of damping-off, root rot and wilt, revealing a reduction in the number of healthy plants. These results are similar to those obtained by Morsy *et al*. (2009) and El-Mougy *et al*. (2012) who reported that *F. oxysporum*, *F. solani*, *S. rolfsii*, *R. solani* and *M. phaseolina* were associated with cucumber damping-off and Root Rot diseases.

The results also perform the role of the tested antioxidants i.e.,: Salicylic Acid (SA), tartaric acid, GAWDA® formulation as well as the antagonistic fungi *T. viride*, *T. hamatum*, *T. harzianum*, *G. virens* as potential triple combination for suppressing root rot and wilt pathogens of cucumber. The results are also in agreement with the finding of Abdel-Monaim (2013) who reported that salicylic acid and *T. viride* either individually or in combination inhibited the growth of pathogenic fungi attacking roots of faba bean. Ismail (2006) found that tartaric acid at 10 mM reduced linear growth of fungi causing damping-off, root rot, wilt in sesame (*Sesamum indicum* L). El-Metwally (2005) reported that GAWDA® formulation reduced the linear growth of the common seed borne fungi on Peanut. Hassan *et al*. (2014) reported that *T. harzianum* significantly reduced the linear growth of pathogenic fungi causing root rot and wilt diseases in Roselle. Mosaad (2008) reported that *T. harzianum*, *G. virens* reduced the radial growth of pathogenic of cucumber. Verma *et al*. (2007) reported that *Trichoderma* spp. is widely used as antagonistic fungal against several pests and enhance the plant growth. All these results support the finding of the authors.

However, the results presented here revealed that the suggested and tested triple combination of *T. viride*, *T. hamatum*, *T. harzianum* and *G. virens* and salicylic acid (4 mM), tartaric acid (10 mM) and GAWDA® formulation 1g L^{-1} did not present significant differences in their effect on linear growth of the root rot and wilt fungi of cucumber when compared with the solely uses of each one. Accelerate seed germination was also found when soaking seed in one of the tested antioxidants (4 mM of salicylic acid, 10 mM of tartaric acid or 1 g L^{-1} of GAWDA formulation). These results are also in a harmony with those obtained by El-Mougy *et al*. (2004) who reported that no significant reduction in seed germination was observed when lupine seeds were treated with SA and ASA up to 2 and 3 g kg^{-1}, respectively.

The *in vivo* results insured that the triple combination of compost+salicylic acid 4 mM+*T. harzianum* has a significant impact on damping-off of cucumber seedlings under the natural field condition, significant increase in the content of the photosynthic pigment and the total phenols in the cucumber plants. Also improved fruit quality including total carbohydrates, ascorbic acid, firmness, moisture, TSS and acidity %. A significant increase in the yield (g)/plant, number of fruits/ plant, fruit length and fruit diameter were significant. In conclusion it is recommended to use a triple designed formulation of Salicylic Acid (SA) at 4 mM and *T. harzianum* for treating seeds following by sawing in soil supplemented with compost at a rate of (2 t/fed). This tactic is an intelligent

Table 8: Effect of the triple combinations of the antioxidants, *Trichoderma* and compost on the content of the photosynthetic pigments and the total phenols in the upper leaves of cucumber plants

		No compost					Compost				
Treatments		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	Total phenols (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	Total phenols (mg catechol/100 g) fresh weight
Antioxidant	Antagonistic fungus										
Check	*Trichoderma* viride	1.375^{I*}	0.674^{L}	2.049^{L}	0.129^{K}	103.3^{K}	1.572^{r}	0.757^{r}	2.329^{r}	0.231^{r}	122.6^{r}
	Gliocladium virens	1.384^{H}	0.677^{K}	2.061^{K}	0.141^{J}	104.1^{J}	1.595^{q}	0.761^{q}	2.356^{q}	0.233^{q}	123.4^{q}
	Trichoderma hamatum	1.366^{J}	0.671^{M}	2.037^{M}	0.117^{L}	101.6^{L}	1.572^{r}	0.751^{s}	2.323^{s}	0.230^{s}	122.2^{s}
	Trichoderma harzianum	1.393^{G}	0.680^{J}	2.073^{J}	0.156^{I}	107.5^{I}	1.615^{p}	0.832^{p}	2.447^{p}	0.235^{p}	124.0^{p}
	Non Trichoderma	1.313^{K}	0.666^{N}	1.979^{N}	0.113^{M}	99.2^{L}	1.571^{s}	0.746^{t}	2.317^{t}	0.229^{s}	121.7^{t}
Salicylic acid	*Trichoderma* viride	1.536^{u}	0.736^{w}	2.272^{w}	0.222^{v}	120.3^{v}	1.865^{c}	0.952^{c}	2.818^{c}	0.290^{c}	138.8^{c}
	Gliocladium virens	1.536^{u}	0.738^{v}	2.274^{v}	0.226^{u}	120.8^{u}	1.880^{b}	0.955^{b}	2.835^{b}	0.294^{b}	139.9^{b}
	Trichoderma hamatum	1.531^{v}	0.732^{x}	2.263^{x}	0.217^{w}	119.5^{w}	1.818^{d}	0.934^{d}	2.752^{d}	0.287^{d}	137.1^{d}
	Trichoderma harzianum	1.539^{t}	0.739^{u}	2.278^{u}	0.228^{t}	121.3^{t}	1.905^{a}	0.964^{a}	2.869^{a}	0.296^{a}	153.3^{a}
	Non Trichoderma	1.526^{w}	0.727^{y}	2.253^{y}	0.215^{x}	118.9^{x}	1.792^{e}	0.930^{e}	2.722^{e}	0.278^{e}	135.6^{e}
Tartaric acid	*Trichoderma* viride	1.501^{z}	0.712^{B}	2.213^{B}	0.208^{A}	117.4^{A}	1.718^{h}	0.905^{h}	2.623^{h}	0.252^{h}	131.3^{h}
	Gliocladium virens	1.505^{y}	0.717^{A}	2.222^{A}	0.209^{z}	118.0^{z}	1.765^{g}	0.915^{g}	2.680^{g}	0.256^{g}	132.1^{g}
	Trichoderma hamatum	1.501^{z}	0.709^{C}	2.210^{C}	0.202^{B}	116.4^{B}	1.679^{i}	0.895^{i}	2.574^{i}	0.248^{i}	129.8^{i}
	Trichoderma harzianum	1.521^{x}	0.721^{z}	2.242^{z}	0.212^{y}	118.5^{y}	1.771^{f}	0.927^{f}	2.698^{f}	0.259^{f}	134.4^{f}
	Non Trichoderma	1.462^{A}	0.705^{D}	2.167^{D}	0.200^{C}	115.7^{C}	1.672^{j}	0.887^{j}	2.559^{j}	0.246^{j}	129.3^{j}
GAWDA®	*Trichoderma* viride	1.426^{D}	0.691^{G}	2.117^{G}	0.185^{F}	111.7^{F}	1.641^{m}	0.873^{m}	2.514^{m}	0.239^{m}	127.6^{m}
	Gliocladium virens	1.436^{C}	0.696^{F}	2.132^{F}	0.187^{E}	114.2^{E}	1.646^{l}	0.880^{l}	2.526^{l}	0.241^{l}	128.3^{l}
	Trichoderma hamatum	1.416^{E}	0.688^{H}	2.104^{H}	0.181^{G}	111.0^{G}	1.638^{n}	0.859^{n}	2.497^{n}	0.238^{n}	126.2^{n}
	Trichoderma harzianum	1.457^{B}	0.701^{E}	2.158^{E}	0.189^{D}	115.2^{D}	1.671^{k}	0.884^{k}	2.555^{k}	0.245^{k}	128.8^{k}
	Non Trichoderma	1.414^{F}	0.685^{I}	2.099^{I}	0.172^{H}	110.1^{H}	1.633^{o}	0.834^{o}	2.467^{o}	0.236^{o}	124.9^{o}

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

Table 9: Effect of the triple combinations of the antioxidants, Trichoderma and compost on the fruit contents of total carbohydrates, ascorbic acid, firmness, moisture, total soluble solids and acidity

		No compost						Compost					
Treatments		Total carbohydrates	Ascorbic acid (mg/100 g)	Firmness	Moisture		Acidity	Total carbohydrates	Ascorbic acid (mg/100 g)	Firmness	Moisture		
Antioxidant	Antagonistic fungus	(%)	fresh weight	(lb/in^2)	(%)	TSS (%)	(%)	(%)	fresh weight	(lb/in^2)	(%)	TSS (%)	Acidity (%)
Check	*Trichoderma viride*	8.63^{A}*	11.37^{x-z}	10.1^{L}	96.77^{a-c}	3.22^{DE}	1.95^{ab}	10.55^{mn}	13.80^{m}	13.0^{r}	96.07^{k-m}	3.63^{lm}	0.150^{i-m}
	Gliocladium virens	8.67^{zA}	11.20^{z}	10.2^{K}	96.66^{b-d}	3.25^{CD}	1.89^{a-c}	10.57^{m}	13.80^{m}	13.3^{q}	96.05^{lm}	3.65^{kl}	0.146^{j-n}
	Trichoderma hamatum	8.51^{B}	11.37^{x-z}	10.1^{L}	96.79^{ab}	3.20^{EF}	1.95^{ab}	10.44^{no}	13.70^{m}	12.9^{s}	96.09^{kl}	3.61^{mn}	0.150^{i-m}
	Trichoderma harzianum	8.75^{z}	11.30^{yz}	10.3^{J}	96.67^{b-d}	3.27^{BC}	1.86^{a-d}	10.64^{lm}	14.00^{l}	13.5^{p}	96.03^{lm}	3.67^{jk}	0.144^{j-o}
	Non *Trichoderma*	8.13^{C}	11.00^{A}	10.0^{M}	96.82^{a}	3.18^{FG}	1.96^{a}	10.41^{o}	13.70^{m}	12.8^{t}	96.12^{j-l}	3.58^{n}	0.154^{h-l}
Salicylic acid	*Trichoderma viride*	10.18^{pq}	13.00^{p}	12.3^{w}	96.25^{h-j}	3.53^{tu}	1.60^{f-k}	11.91^{c}	15.69^{bc}	15.6^{c}	95.42^{vw}	3.97^{gh}	0.118^{rs}
	Gliocladium virens	10.23^{pq}	13.20^{o}	12.5^{v}	96.24^{h-j}	3.55st	1.57^{g-l}	12.10^{b}	15.80^{b}	15.8^{b}	95.33^{w}	4.10^{fg}	0.112^{s}
	Trichoderma hamatum	10.17^{q}	13.00^{p}	12.0^{x}	96.25^{h-j}	3.51^{uv}	1.60^{f-k}	11.80^{cd}	15.60^{cd}	15.5^{d}	95.47^{uv}	3.95^{hi}	0.115^{rs}
	Trichoderma harzianum	10.29^{p}	13.40^{n}	12.6^{u}	96.20^{i-k}	3.57^{s}	1.57$_{g-l}$	12.30^{a}	16.00^{a}	16.0^{a}	95.30^{w}	4.20^{ef}	0.112^{s}
	Non *Trichoderma*	10.12^{qr}	12.80^{q}	11.9^{y}	96.25^{h-j}	3.49^{vw}	1.63^{e-j}	11.73^{d}	15.43^{de}	15.3^{e}	95.56^{tu}	3.92^{ij}	0.121^{q-s}
Tartaric acid	*Trichoderma viride*	9.74^{t}	12.20^{t}	11.5^{B}	96.30^{hi}	3.43^{yz}	1.70^{c-h}	11.28^{g}	15.00^{g}	14.8^{h}	95.65^{r-t}	3.85^{HI}	0.128^{n-s}
	Gliocladium virens	9.92^{s}	12.40^{s}	11.6^{A}	96.29^{hi}	3.45^{xy}	1.66^{e-i}	11.41^{f}	15.20^{f}	14.9^{g}	95.61st	3.88^{GH}	0.125^{o-s}
	Trichoderma hamatum	9.59^{u}	12.20^{t}	11.4^{C}	96.34^{f-h}	3.41^{zA}	1.70^{c-h}	11.11^{h}	14.80^{h}	14.6^{i}	95.73^{q-s}	3.82^{IJ}	0.128^{n-s}
	Trichoderma harzianum	10.05^{r}	12.60^{r}	11.8^{z}	96.26^{hi}	3.47^{wx}	1.66^{e-i}	11.54^{e}	15.40^{e}	15.0^{f}	95.58^{tu}	3.91^{FG}	0.122^{p-s}
	Non *Trichoderma*	9.45^{v}	12.00^{u}	11.3^{D}	96.31^{g-i}	3.39^{AB}	1.73^{c-h}	11.01^{hi}	14.80^{h}	14.5^{j}	95.75^{p-r}	3.79^{J}	0.134^{m-r}
GAWDA®	*Trichoderma viride*	9.15^{xy}	11.50^{wx}	10.8^{G}	96.48^{e}	3.32^{yz}	1.79^{a-f}	10.82^{ijk}	14.20^{k}	13.9^{m}	95.60^{s-u}	3.73^{c}	0.141^{k-p}
	Gliocladium virens	9.21^{wx}	11.60^{vw}	10.9^{F}	96.46^{ef}	3.34^{xy}	1.70^{d-h}	10.88^{j}	14.40^{j}	14.0^{l}	95.88^{n-p}	3.75^{b}	0.138^{l-q}
	Trichoderma hamatum	9.09^{y}	11.50^{wx}	10.6^{H}	96.54^{de}	3.30^{zA}	1.61^{f-j}	10.76^{k}	14.20^{k}	13.8^{n}	95.95^{m-o}	3.70^{cd}	0.141^{k-p}
	Trichoderma harzianum	9.31^{w}	11.70^{v}	11.0^{E}	96.44^{e-g}	3.36^{wx}	1.76^{b-g}	10.93^{ij}	14.60^{i}	14.3^{k}	95.86^{o-q}	3.77^{a}	0.134^{m-r}
	Non *Trichoderma*	8.60^{AB}	11.40^{xy}	10.5^{I}	96.64^{cd}	3.28^{AB}	1.82^{a-e}	10.71^{kl}	14.00^{l}	13.6^{o}	96.00^{l-n}	3.69^{de}	0.144^{j-o}

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p = 0.05, TSS: Total soluble solid

Table 10: Effect of different combinations of the antioxidants, Trichoderma and compost on Yield (g)/plant, number of fruits/plant, fruit length and fruit diameter

Treatments		No compost				Compost			
Antioxidant	Antagonistic fungus	Yield (g)/plant	Fruits/plant	Fruit length (cm)	Fruit diameter (cm)	Yield (g)/plant	Fruits/plant	Fruit length (cm)	Fruit diameter (cm)
Check	*Trichoderma viride*	820^{K}*	8.6^{H}	12.10^{y}	4.4^{b}	1015^{r}	16.6^{q}	15.03^{m}	3.0^{op}
	Gliocladium virens	830^{J}	9.3^{G}	12.30^{x}	4.3^{c}	1025^{q}	17.2^{p}	15.40^{l}	3.0^{o-q}
	Trichoderma hamatum	800^{L}	8.3^{I}	12.03^{y}	4.4^{ab}	990^{s}	16.6^{q}	14.96^{m}	3.0^{o}
	Trichoderma harzianum	850^{I}	9.6^{F}	12.46^{x}	4.2^{d}	1035^{p}	18.0^{o}	15.43^{kl}	2.9^{p-r}
	Non *Trichoderma*	750^{M}	8.0^{J}	11.80^{z}	4.5^{a}	985^{t}	16.3^{r}	14.86^{m}	3.0^{o}
Salicylic acid	*Trichoderma viride*	970^{w}	15.3^{u}	14.53^{no}	3.2^{mn}	1220^{c}	24.6^{c}	18.50^{b}	2.8^{s-v}
	Gliocladium virens	975^{v}	15.6^{t}	14.60^{no}	3.1^{mn}	1230^{b}	25.0^{b}	18.60^{b}	2.8^{u-w}
	Trichoderma hamatum	965^{x}	15.3^{u}	14.43^{op}	3.2^{mn}	1215^{d}	24.3^{d}	18.00^{c}	2.9^{r-u}
	Trichoderma harzianum	980^{u}	16.0^{s}	14.64^{n}	3.2^{mn}	1250^{a}	25.6^{a}	19.00^{a}	2.7^{w}
	Non *Trichoderma*	945^{y}	15.0^{v}	14.26^{pq}	3.2^{lm}	1210^{e}	24.0^{e}	17.23^{d}	2.9^{q-s}
Tartaric acid	*Trichoderma viride*	935^{A}	13.3^{y}	13.83^{s}	3.5^{i}	1170^{h}	21.6^{h}	16.63^{f}	2.8^{t-w}
	Gliocladium virens	935^{A}	13.6^{x}	14.06^{r}	3.4^{j}	1181^{g}	22.0^{g}	16.90^{e}	2.8^{vw}
	Trichoderma hamatum	930^{B}	13.0^{z}	13.70st	3.6^{h}	1160^{i}	21.0^{i}	16.53^{f}	2.9^{r-u}
	Trichoderma harzianum	940^{z}	14.6^{w}	14.18^{qr}	3.3^{kl}	1200^{f}	23.6^{f}	16.93^{e}	2.8^{vw}
	Non *Trichoderma*	920^{C}	12.6^{A}	13.66st	3.7^{h}	1140^{j}	19.6^{j}	16.26^{g}	2.9^{r-t}
GAWDA®	*Trichoderma viride*	880^{F}	11.6^{C}	13.33^{v}	4.0^{e}	1100^{m}	18.6^{m}	15.66^{ij}	3.3^{jk}
	Gliocladium virens	890^{E}	12.3^{B}	13.37^{uv}	3.8^{f}	1111^{l}	19.2^{l}	15.80^{i}	3.3^{k}
	Trichoderma hamatum	870^{G}	11.3^{D}	13.20^{v}	4.0^{e}	1080^{n}	18.2^{n}	15.60^{jk}	3.5^{i}
	Trichoderma harzianum	910^{D}	12.6^{A}	13.53^{tu}	3.8^{g}	1121^{k}	19.4^{k}	16.03^{h}	3.1^{n}
	Non *Trichoderma*	860^{H}	10.3^{E}	12.93^{w}	4.1^{d}	1050^{o}	18.2^{n}	15.60^{jk}	3.6^{h}

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

method to overcome the incidence of root rot and wilt disease of cucumber and improve its yield and quality of the fruits. It is also recommended for the growers to follow it for increasing their income and market competition. These results are also in agreement with the finding of Abdel-Kader *et al*. (2013) who reported that combined treatments (compost+*T. harzianum*+ thyme or compost+*T. harzianum*+lemongrass) were effective in controlling peanut crown rot disease under field conditions and reduced the disease incidence in both pre and post-emergence growth, respectively. Abo-Elyousr *et al*. (2014) reported that under greenhouse and field conditions, *T. harzianum* (isolates No. 1 and 2) and compost individually or in combination were effective in controlling *Rhizoctonia* root rot disease and showed a suppressive effect on severity of the disease and increased the yield of soybean. On the other hand, Elwan and El-Hamahmy (2009) found that spraying salicylic acid at a rate of (10^{-6} and 10^{-4} M) was a promising compound to control pepper diseases. This application at a low concentration (10^{-6} M) positively increased the foliage fresh and dry weight, fruit number, average fruit weight, fruit yield, vitamin C, carotenoids content, cuticle thickness of fruit pericarp and translocation of sugars from leaves to fruits.

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