# Efficacy of *Trichoderma* species as bio-control agents to control root rot disease of Cantaloupe plants under the impact of climate change

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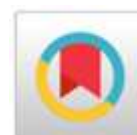
Abstract

In this study, several bio-agents, including *Trichoderma album*, *T. hamatum*, *T. harzianum* and *T. viride*; prepared as suspensions at a concentration of 30×10^6 conidia/ ml, in addition to bio-fungicides, such as Plant Guard (*T. harzianum*, 30×10^6) and Bio-zeid (*T. album*, 10×10^6), demonstrated significant *in vitro* and *in vivo* antagonistic efficacies against certain soil-borne fungal pathogens, which cause root rot diseases of cantaloupe plants during the growing seasons of 2019/ 2020 and 2020/ 2021. In the *in vitro* assays, *T. harzianum* caused a significant reduction of the pathogens' radial mycelial growth, including *Rhizoctona solani*, *Phytophthora* spp., *Sclerotium rolfsii* and *Fusarium oxysporum* by 79.69 %, followed by *T. viride* (76.26 %), and *T. album* (70.82 %), respectively. On the other hand, *T. hamatum* expressed the least antagonistic potential and decreased the pathogens' growth by 66.01 % only. *In vivo* assay results showed that applying inocula of 1 l/100 l water/ Feddan of the bio-control agents against the cantaloupe root rot pathogens significantly reduced the disease incidence, which recorded the highest reduction percentages of 79.66 % and 77.07 % by *T. harzianum*, during the two growing seasons of 2019/ 2020 and 2020/ 2021, respectively. Moreover, these applied bio-agents promoted the growth parameters and chemical components of the cantaloupe plants significantly, including percentage of the total soluble solids, total phenol content, protein, nitrogen, ascorbic acid, and total sugars, thus increasing the crop yield. The current work aimed to reduce the use of toxic chemical fungicides during the agriculture process, to produce safe food of high quality and quantity, and to find the most suitable bio-agent that has the ability to protect and control the cantaloupe plants against the soil-borne fungal diseases under the impact of climate change.

Keywords: Cantaloupe, *Trichoderma* spp., Root rot, Bio-control agents, Climate change

1. Introduction

Cantaloupe plant (*Cucumis melo* var. *cantaloupensis*) is one of the most important types of melon and popular fruity vegetables that are grown in Egypt. Melo *et al*., (2000) revealed that cantaloupe is an excellent source of carbohydrates, vitamins, and minerals (*i.e*., potassium). In addition, this plant is rich in several antioxidant compounds, which have the ability to protect the human body cells against cancer, and is low in fat content and calories (about 17 kcal/ 100 g) (Shafeek *et al*. 2015). In Egypt, the cultivated area of melons (cantaloupe) during 2020 was 18,678.24 Feddan (7848 ha.), which yielded approximately 275189 tons of fruits (FAO. 2021). According to the previous studies conducted by Uematsu *et al*., (1992); Chen *et al*., (2004); Cheraghali *et al*., (2012), cantaloupe is susceptible to several diseases that attack the roots, foliage, and fruits, which lead to serious losses in crop quality and quantity. The most commonly recorded diseases of cantaloupe, include root rot/ vine decline, damping off, and root-knot nematode (Abad *et al*., 2000; Aegerter *et al*., 2000; El-Desouky and El-Wakil, 2003; Cheraghali *et al*., 2012; El-Mougy *et al*., 2014).

In the fields of cantaloupe, several soil-borne fungi, including *Verticillium dahlia*, *Fusarium oxysporum* f.sp. *melonis*, and *Rhizoctonia solani* have been reported to cause wilt and root rot diseases (Buzi *et al*., 2002; Infantino *et al*., 2004; Boughalleb *et al*., 2018; Aiello *et al*., 2012; El-Kolaly and Abdel-Sattar, 2013). In the developing countries, climate change has a negative impact and consequences on agriculture. Furthermore, ecology of the fungal and pests diseases is affected by climate change, which have an impact on the cantaloupe crop production and protection (Pardossi *et al*., 2000; Baker and Reddy, 2001; Sarkar *et al*., 2013).

Santamarina and Rosello, (2006) studied the impact of climate change mainly, temperature and water availability on the antagonism of *T. harzianum* against the phytopathogenic *Verticillium* spp. and *Rhizoctonia* spp., where the recorded results revealed the ability of *T. harzianum* to control these pathogens upon the increase in water availability in the growth medium. On the contrary, growth of these pathogenic fungi decreased as the incubation temperature dropped from 25 °C to 15 °C.

A previous study of Abdel-Kader *et al*., (2017) highlighted that a preventive program that combines the use of good agricultural practices and biological control, has provided the best results of the control of root rot, and caused the increase in production of the cantaloupe plants. The current pathogens management strategy depends mainly on the intensive use of the synthetic fungicides, which do not provide satisfactory control of the root rot disease, in addition of being toxic to human. Recent trends in researches during the past 10 years have focused on finding alternatives to the chemical pesticides application; through the induction of plant defenses, which may provide protection against broad spectrum of disease-causing pathogenic microorganisms (El-Mougy *et al*., 2014).

Bio-control of the soil-borne pathogens using antagonistic microorganisms has been considered as a good ecofriendly alternative to the chemical treatment methods (Eziashi *et al*., 2007). Many antagonistic microorganisms have proven to be effective *in vitro* and *in vivo* control the various phytopathogens. Previous studies conducted by Suárez-Estrella *et al*., (2007); De Cal *et al*., (2009); Shabir-U-Rehman *et al*., (2013) highlighted that *Trichoderma* spp. Pers. are significantly effective in protecting the root system of several crops against the phytopathogenic fungi, including *Fusarium solani* (Mart.) Sacc. and *Macrophomina phaseolina* (Malik and Dawar, 2003; Khalili *et al*., 2016).

On the other hand, the biological control agents (BCAs) inhibit the plant pathogens through one or more mechanisms, such as competition for nutrients

and colonization sites, mycoparasitism, stimulation of plant defence, and the production of antibiotics (Oerke *et al*., 1994; Ahmed, 2005; Ahmed, 2013; Ahmed and Shaheen, 2016; Ahmed and El-Fiki, 2017). *Trichoderma* spp. have significantly increased the antioxidant activities and phenolic contents of the methanolic extracts prepared from different parts of the cantaloupe plant (Mariod and Matthaus, 2008). Several BCAs have been reported as effective antagonists of the plant pathogens; however, only a small number of them have been applied on a commercial scale (Fravel, 2005).

The objectives of the current study were to evaluate the *in vitro* and *in vivo* potential of *Trichoderma* spp. as BCAs against Cantaloupe root rot pathogens, and their positive impacts toward promoting the chemical components and yield of the cantaloupe plant.

.2. Materials and methods

2.1. Isolation of the fungal root rot pathogens

Nine samples of infected cantaloupe plant roots showing typical symptoms of root rot disease were collected from Private Sadat farm, Kilo 93 Cairo-Alexandria Desert Road, Sadat City, Menoufia Governorate, Egypt, placed in paper bags, and then transported immediately to the Microbiology laboratory. The infected roots were washed under tap water to remove soil particles, air dried, surface sterilized by dipping in 1% NaOCl solution for 3 min., washed 3 times with sterilized dist. water, and then dried between 2 sterilized Whatman filter papers. The sterilized root fragments were cut into small pieces using a sterilized scalpel, and then 5-6 pieces were aseptically transferred to the surface of Petri plates, each containing 15 ml of Potato dextrose agar (PDA) medium (Ahmed, 2018). The plates were incubated at 25± 2°C, and were examined daily for development of hyphal fungal growth. After incubation, the developing mycelial hypha of each emerged fungus was picked up aseptically, and then transferred onto PDA plates.

Purification of each isolated fungus was carried out using the hyphal tip technique of Brown, (1924); Hawker, (1960). Identification of the isolated fungi was carried out according to their cultural and morphological characteristics, as described by Gilman, (1957); Barnett and Hunter, (1998); Singh, (1982). The stock cultures were maintained on PDA slants, and kept at 5°C for further studies.

2.2. Preparation of inocula of the fungal bio-control agents

Four strains of *Trichoderma* spp., including *T. album*, *T. hamatum*, *T. harzianum* and *T. viride*, isolated from the rhizosphere of healthy roots, were kindly provided by the Central Lab. of Organic Agriculture (CLOA), ARC, Giza, Egypt, and were used at the rate of 1 l/ 100 l water/ Feddan.

In this assay, suspensions containing propagules of the tested fungal BCAs were grown for 10 d at 25± 2 ºC on Gliotoxin Fermentation (GF) broth medium in darkness (Ahmed, 2005; Ahmed, 2013). After incubation, all the fungal cultures were individually blended using an electrical blender for 2 min., and then suspensions were prepared at a concentration of 30×10^4 conidia/ ml, and were applied at a dilution of 1:100.

2.3. Preparation of the bio-fungicides

The bio-fungicides were used in this study to compare their results to those of the fungal BCAs.

2.3.1. Plant Guard

The recommended bio-fungicide (Plant Guard) was provided by the Central Lab. of Organic Agriculture (CLOA). This bio-fungicide consisted of *T. harzianum* (30×10^6 conidia/ ml), and was used at the rate of 1 l/ 100 l water/ Feddan.

2.3.2. Bio-zeid

The commercial Bio-zeid bio-fungicide preparation consisted of *T. album* (10×10^6 conidia/ ml), and was obtained from the Organic

Biotechnology Co, is branch of Kafr el Zayat branch pesticides & chemicals Co. (S.A.E), Gharbeya, Egypt. The biocide conidial suspension was used at the rate of 2.5 g/ l, and applied at a dilution of 1:100.

2.4. Cantaloupe seeds cv. *Primal hybrid*

Seeds of Cantaloupe were obtained from the Syngenta, Agricultural Research Station in Qaha, Qalyubia Governorate, Egypt. The seeds of cantaloupe cv. Primal hybrid were sown in nursery beds prepared from well-manure soil. The growing healthy seedlings were transplanted in the field, approximately 21 d after sowing.

2.5. Pathogenicity assay

The pathogenicity tests were carried out in potted soils under greenhouse conditions at the CLOA. Plastic pots 20 cm diameter were sterilized by dipping in 5 % formalin solution for 5 min., then left in open air till dryness. Disinfected clay loam soil (with 5 % formalin) was distributed in plastic pots (3.0 kg sterilized soil/ pot), and then the soils were infested individually with different isolates of the recovered cantaloupe fungal pathogens. For preparing an inoculum of each pathogenic fungus, glass bottles (500 ml) were used; each containing 200 g of corn sand meal medium supplemented with 0.2 % peptone solution, and then autoclaved (Ahmed, 2005; Ahmed, 2013). The autoclaved bottles were inoculated individually with 6 agar discs (5 mm each) cut using a sterile cork borer from the periphery of *F. oxysporum* f.sp. *melonis*, *F. solani*, *M. phaseolina*, *Phytophthora* spp., *Pythium* sp., and *R. solani* cultures, and incubated for 15 d at 25± 1°C. After incubation, the seeded medium was added individually to the potted soil at the rate of 10 g/ kg soil, and mixed thoroughly. On the other hand, for the preparation of inoculum of *S. rolfsii*, glass bottles (500 ml) each containing 100 ml of solid PDA-medium were inoculated with one agar disc (5 mm) cut from the periphery of 5-d old culture of *S. rolfsii*, and then incubated for 15 d at 30± 1°C (Ahmed, 2005). After incubation, *S. rolfsii* sclerotia were harvested using a smooth brush, and

then added to the potted soil at the rate of 50 sclerotia/ kg. In all treatments, soil infestation was carried out 10 d before transplanting of the tested cantaloupe seedlings cv. Primal hybrid, which were used in this greenhouse assay. The cantaloupe seedlings were transplanted in the infested and non-infested (control) pots at the rate of 3 seedlings (21 d old)/ pot, and 3 replicate pots were used for each treatment. The percentages (%) of dead seedlings or plants showing root rot symptoms were recorded weekly, to determine the virulence of each tested fungal pathogen. The pathogenic fungi were re-isolated individually from the infected plants to verify Koch's postulates. Data were recorded as percentage of disease incidence (DI) in each treatment.

The percentages of root rot, as well as the % of healthy survived plants in each treatment were determined 45 d after transplanting of the seedlings, using the following formula of Ahmed, (2013):

$$\% \text{ of disease incidence (DI)} = \frac{\text{Number of rotted plants}}{\text{Total sowing seedlings}} \times 100$$

$$\% \text{ of survived plants} = \frac{\text{Number of survival plants}}{\text{Total sowing seedlings}} \times 100$$

$$\% \text{ Efficacy of each treatment} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

2.6. *In vitro* antagonistic potential of the fungal BCAs

The effect of the different fungal antagonists on the linear growth of the pathogenic fungi was conducted under *in vitro* conditions. As antagonistic fungi, isolates of *T. album*, *T. hamatum*, *T. harzianum* and *T. viride*, were evaluated during this study. Unless otherwise stated, the Gliotoxin fermentation agar (GFA) medium was used for growing the fungi. About 2 ml of each antagonistic fungal suspension was inoculated individually into 250 ml of GFA broth medium, and then incubated for 5-7 d at 25±1°C. After incubation, each growing broth culture was filtered using a sterile Whatman No. 1 filter paper, and then sterilized using a Millipore filter (0.2 µm), to obtain a sterile fungal filtrate. Approximately, 15 ml of each fungal filtrate was inoculated individually into 250 ml

of GFA medium. After solidification, each of the seeded plates was inoculated individually at the center with a fungal disc obtained from the periphery of 5 d old cultures of *F. solani*; *F. oxysporum*, *Phytophthora* spp., *Pythium* sp., *R. solani*, and *S. rolfsii*. Plates inoculated with the pathogens only served as control treatments. Three replicate plates were used for each treatment. The inoculated plates were incubated at 25±1℃. The incubation was terminated when the mycelial mats covered the GFA medium surface in the corresponding control treatments. After incubation, the diameter of each fungal pathogen colony was measured using a calibrated ruler, the percentage (%) reduction of radial mycelial growth of each pathogenic fungus was calculated, and then compared to the control treatments using the formula of Ahmed, (2005); Ahmed (2013) as follow:

% Reduction in liner growth of the pathogenic fungus $= \frac{G1-G2}{G1} \times 100$

Where; G1: diameter of growth of the pathogenic fungus in the control plate, G2: diameter of growth of the pathogen in the antagonist treated plate

2.7. Climatic data

The Sadat city follows Menoufia Governorate, Egypt (30.6 °N latitude, 31.1 °E longitude, 17 m altitude). The daily historical data of minimum and maximum air temperatures were provided from the automatic meteorological station of the CLAC, ARC. The climatic data from 2010 to 2019 years were collected from the Sadat City meteorological station, and represented as the monthly average of air temperature with Celsius degree (^{o}C), during the period of cantaloupe plant growing season.

The MAGICC/SCENGEN program (Wigley, 2003) was used to extract the projected changes in air temperature under the different four scenarios (A1, A2, B1 and B2) of Intergovernmental Panel on Climate Change (IPCC) as presented in Table (1). The model used for the 4 scenarios was the HadCM3 climate model. For each one of the four scenarios, a

simulation extracted the monthly air temperatures for the years 2050s and 2100s.

2.8. Field assays

The field experiments were carried out during the growing seasons of 2019/ 2020 and 2020/ 2021, in light loamy textured soil naturally infested with several root rot fungal pathogens, including *F. oxysporum* f.sp. *melonis*, *F. solani*, *M. phaseolina*, *Phytophthora* spp., *Pythium* sp., *R. solani*, and *S. rolfsii*, at a private farm, Kilo 93 Cairo-Alexandria Desert Road, Sadat City, Menoufia Governorate, Egypt, on the 1st April, 2019 and 2020 growing seasons. All the experiments were conducted in a complete randomized block design with three replicated plots. The area of the experimental plot was 50 m^2 and comprised of 3 rows (25 m long ×1.5 m width), with about 50 cm apart. Each row was planted with 50 seedlings of cantaloupe in a naturally infested soil. Regarding to fertilization of this farm, mature compost was applied at the rate of 30 m^3/ Feddan before the seedling date throughout the soil preparation (Abada *et al*., 2013). Nile water was available in this area with a drip irrigation system using well sources.

Seeds of sage were first sown in nursery beds with a clay soil on the 7th of March 2019 and 202 growing seasons, and then the growing seedlings of about 10-15 cm height were transplanted in a sandy loam soil on the 1st April 2019 and 2020 growing seasons. In all field experiments, the cantaloupe seedlings were used and dipped individually in diluted recommended dose (1:100) of the different bioagents, *i.e*., *T. album*, *T. hamatum*, *T. harzianum*, and *T. viride*, which were prepared as suspension at a concentration of 30×10^6 conidia/ ml. Moreover, cantaloupe seedlings were dipped individually in the tested bio-fungicides (mixed with 5 % Arabic gum and 0.3 % Tween 80) for 30 min. before transplanting. Transplants soaked in water only for the same period act as control treatments. The plots were irrigated regularly and received all the other normal agricultural practices.

Table 1. Description of the predicted IPCC scenarios of climatic changes

Scenario	Description
A1	Low population growth, regional convergence, quick adoption of new technology, enhanced social engagement, capacity building, and less regional variations in per capita income, which all contributed to the rapid economic growth. The temperature rose from 1.4 to 6.4 ^{o}C.
A2	Regionally-specific economic growth, high population increase, technological progress and dispersed economics, all are characteristics of a heterogeneous world. The temperature rose by 2.0 to 5.4 ^{o}C.
B1	The shift to a service and information economy, low population growth, the use of clean technologies and increased resource productivity, all are characteristics of the convergent world. The temperature rose by 1.1 to 2.9 ^{o}C.
B2	Moderate levels of economic growth, diverse planet with modest population increase, and slower technological change; with an emphasis on the local solutions to environmental, social and economic sustainability. The mercury rose by 1.4 to 3.8 ^{o}C.

2.9. Field assessments

2.9.1. Disease assessment

In all field experiments, the DI of root rot, percentage of healthy survived plants, and percentage of efficacy of each treatment were recorded 90 d after transplanting, according to the methods adopted by Ahmed, (2005); Ahmed, (2013).

2.9.2. Assessment of the enhanced plant growth parameters and yield components

The survived cantaloupe plants were counted, uprooted and then used for determining the vegetative growth and yield components. To evaluate the enhancement in vegetative growth, samples of 9 plants from each subplot were randomly taken at the flowering stage (60 d after transplanting date), and several characteristics were recorded, including plant height (cm), number of leaves and branches per plant, and fresh and dry weight of the whole plant and its organs. The total yield and quality of fruits were determined by weekly picking of the cantaloupe fruits throughout the harvesting period, counting the number of fruits, and determining the fruits weight (kg) per plot.

2.9.3. Determination of chemical components of the growing plants

All the following plants chemical determinations assays were carried out at the Central Lab. of CLOA, ARC, Giza, Egypt, and the Central Lab. of Environmental Studies & Research Institute, University of Sadat City, Egypt.

Nine samples of cantaloupe fruits at the harvesting stages were randomly collected from each plot, and the nitrogen percentage (N %) in the fruits tissue was determined using the Kjeldahl method described by Black, (1965). The crude protein content was estimated by multiplying the total nitrogen by 6.25 (A.O.A.C. 2005). Ascorbic acid (mg/ 100 g FW) was determined in reference to Offor *et al*., (2015). The total soluble solids (TSS %) were determined using a Carlzeiss hand Refractometer (A.O.A.C. 2005). The percentage of total sugars in the fruits tissue was determined in reference to Dubois *et al*., (1959). Total phenolic contents (TPC) of the cantaloupe extracts were determined using the Folin–Ciocalteu assay of Singleton *et al*., (1999); Quettier-Deleu *et al*., (2000); Meda *et al*., (2005); Saravanan and Parimelazhagan, (2014). Gallic acid (GA) was used as a standard; at concentrations that ranged from 1-200 µg/ ml, to prepare the standard curve required for estimating the TPC contents.

2.10. Statistical analysis

All the obtained data were subjected to statistical analysis and compared according to the least significant difference (L.S.D.), as described by Snedecor and Cochran, (1989).

3. Results and Discussion

3.1. Frequency of fungi isolated from the rotted roots of cantaloupe plants

The fungal pathogens recovered from the infected cantaloupe plants were purified, and then identified as *F. oxysporum*, *F. solani*, *M. phaseolina*, *Phytophtora* spp., *Pythium* sp., *R. solani*, and *S. rolfsii*. Data presented in Table (2) indicate that *R. solani*, *Phytophthora* spp., *S. rolfsii*, and *F. oxysporum* expressed the highest frequency among the isolated fungi.

3.2. Pathogenicity assays

The ability of the recovered fungi to induce root rot disease incidence was evaluated *in vivo* under greenhouse conditions. Data presented in Table (3) revealed that most of the isolated fungi have caused root rot disease of cantaloupe plants. *R. solani* was the most aggressive soil-borne fungus that caused the highest root rot incidence (90.30 %), and showed the lowest percentage (09.70 %) of plants survival, followed by *Phytophtora* spp. (86.60 %), respectively. On the contrary, *Pythium* spp. recorded the lowest incidence (45.50 %) of root rot disease, and showed the highest percentage (54.50 %) of survived plants. However, no significant variations in incidences of root rot disease were detected among *F. oxysporum*, *F. solani*, and *M. phaseolina* treatments.

In accordance with the current results, *Rhizoctonia* sp. was shown to cause root rot on watermelon in Italy, where it was isolated among other root rot pathogens from collapsed watermelon plants (Aiello *et al*., 2012). Moreover, a previous study conducted by Nischwitz *et al*., (2013) recorded a new root rot disease of watermelon caused by *Rhizoctonia* sp., which had been observed sporadically in commercial fields of central Arizona, USA. In agreement with the current results, *F. oxysporum* f.sp. *melonis* was reported to induce wilt disease of cantaloupe plants under field conditions (Zitter *et al*.,1996; Aegerter *et al*., 2000; Buzi *et al*., 2002, 2004; El-Kolaly and Abdel-Sattar, 2013). A previous study of El-Sheshtawi *et al*., (2014) reported that destruction of melon roots caused by *Fusarium* spp. as a soil-borne pathogen was attributed to the synergistic action between polygalactuornase and oxalic acid produced by this pathogenic fungus.

3.3. *In vitro* effect of the antagonists on linear growth of the pathogenic fungi

Results presented in Tables (2 and 3) showed that *R. solani*, *Phytophthora* spp., *S. rolfsii*, and *F. oxysporum* were the most 4 aggressive isolates causing cantaloupe root rot; as a result, their *in vitro* resistance response against the fungal antagonists was tested. Data in Table (4) show that *T. harzianum* caused the highest significant reduction of mycelial growth of all the tested pathogens by 79.69 %, followed by *T. viride* (76.26 %), and *T. album* (70.82 %). On the other hand, *T. hamatum* expressed the least antagonistic potential, and the average recorded decrease in diameter of the pathogen growth was 66.01 %. The average reduction in radial growth of *R. solani* by the 4 antagonists was 80.21 %, where such result represented the highest sensitivity to the fungal antagonists, followed by *Phytophtora* spp. (76.58 %), *S. rolfsii* (70.63 %), and *F. oxysporum* (65.36 %). According to the previous studies conducted by Harman *et al*., (2004); Ahmed, (2005); Harman, (2006); Ahmed, (2013); Ahmed and Shaheen, (2016); Ahmed and El-Fiki, (2017); Ahmed, (2018), this phenomenon might be attributed to the fact that different pathogens own different defense mechanisms against the enzymes and toxic substances produced by the various antagonists.

Furthermore, Hajieghrari *et al*., (2008); Ahmed, (2013) reported that *Trichoderma* spp. degrade the cell wall of the fungal pathogen through the production of several lytic enzymes, including peroxidase, chitinases, glucan 1-3 β-glucosidases, and polyphenoloxidase.

1688

Table 2. Frequency (%) of fungi recovered from the rotten roots of cantaloupe plants

Isolated fungi	Frequency of isolated fungi	
	No.	(%)
F. oxysporum (Schlecht)	6	12.00
F. solani (Marti "Sacc.")	5	10.00
M. phaseolina (Tassi) Goid, (1947)	3	06.00
Phytophthora **spp.**(Bary, 1876)	11	22.00
Pythium **sp.** (Pringsheim)	4	08.00
R. solani (Kuhn)	12	24.00
S. rolfsii (Sacc.)	9	18.00
Total	50	100.00

Table 3. Pathogenicity assay of the tested fungi expressing disease incidence on cantaloupe plants under greenhouse conditions

Tested fungi	DI (%)	Plant Survival (%)
F. oxysporum (Schlecht)	76.30	23.70
F. solani (Marti "Sacc.")	65.50	34.50
M. phaseolina (Tassi) Goid. (1947)	55.00	45.00
Phytophtora **spp.**(Bary 1876)	86.60	13.40
Pythium **sp.** (Pringsheim)	45.50	54.50
R. solani (Kuhn)	90.30	09.70
S. rolfsii (Sacc.)	80.50	19.50
Control (Untreated)	00.00	100.00
L.S.D. at 0.05 %	1.27	1.62

-Results are averages of 3 replicates for each treatment

Table 4. Effect of the antagonistic fungi on percentage (%) of *in vitro* reduction in linear growth of the pathogenic fungi

Antagonistic fungi	% Reduction in linear growth of the pathogenic fungi				
	R. solani	*Phytophthora* spp.	*S. rolfsii*	*F. oxysporum*	Mean
T. album	76.33	74.66	69.95	62.33	70.82
T. hamatum	73.82	68.83	61.67	59.71	66.01
T. harzianum	88.20	82.50	77.33	70.71	79.69
T. viride	82.50	80.33	73.55	68.67	76.26
Control	00.00	00.00	00.00	00.00	
Mean	80.21	76.58	70.63	65.36	73.19

L.S.D at 1% for:
Pathogenic fungi (P) = 0.88,
For antagonistic fungi (A) = 1.22
A × P= 1.33

-Results are averages of 3 replicates for each treatment

3.4. Field experiments

3.4.1. Effect of different fungal treatments on the incidence of cantaloupe root rot

Data presented in Table (5) indicate that all the tested fungal BCAs significantly reduced the incidence of root rot disease caused by the soil borne pathogens, and increased the percentage of the survived cantaloupe plants in both seasons of 2019/ 2020 and 2020/ 2021. In this regard, the different antagonistic isolates varied in their effects on reducing the root rot DI. Compared to the control treatment, *T. harzianum* bio-agent demonstrated the highest reduction of DI by 79.66 and 77.07 %, followed by *T. viride* (67.10 and 66.79 %), during the two growing seasons of 2019/ 2020 and 2020/ 2021, respectively. On the other hand, *T. hamatum* showed the lowest efficacy in controlling the root rot disease during 2019/ 2020 and 2020/ 2021, recording 37.05 % and 35.46 %; respectively. As bio-fungicides, Plant Gard recorded the highest efficiency of DI reduction recording 59.72 and 59.15 %, compared to the Bio-zeid biocide that recorded 49.22 and 47.12 %, during the two growing seasons of 2019/ 2020 and 2020/ 2021, respectively.

These currently recorded results may be attributed to the antagonistic potential of *Trichoderma* spp. (Suárez-Estrella *et al*., 2007; De Cal *et al*., 2009; Shabir-U-Rehman *et al*., 2013), which were significantly effective in protecting the root system of some crops against the action of several strains of pathogenic fungi, including *F. solani* (Mart.) Sacc. and *M. phaseolina* (Malik and Dawar, 2003; Khalili *et al*., 2016). Moreover, the BCAs inhibit the plant pathogens through several mechanisms, such as competition for key nutrients and colonization sites, mycoparasitism, stimulation of plant defence, and production of antibiotics (Oerke *et al*., 1994; Ahmed, 2005; Ahmed, 2013; Ahmed and Shaheen, 2016; Ahmed and El-Fiki, 2017). *Trichoderma* spp. are present in all soil types and represent the most cultural fungi (Hajieghrari *et al*., 2008). A previous study conducted by Gava and

Menezes, (2012) reported that *Trichoderma* spp. produce hydrolytic enzymes, which play important roles in the mycoparasitism against the phytopathogenic fungi, and in the field control of the soil-borne pathogens of melon.

3.4.2. Effects of the BCAs on the cantaloupe plant growth parameters

Concerning the influence of the BCAs on growth parameters of cantaloupe plant, results presented in Table (6) demonstrated that all the tested BCAs significantly increased all the plant growth parameters, including plant height (cm); number of leaves/ plant; number of shoots/ plant; fresh weigh of shoots and leaves/ plant (g) and dry weight of shoots and leaves/ plant (g), compared to the control treatments. *T. harzianum* recorded the highest significant promotion of plant height (167.67 and 168.33 cm); number of leaves/ plant (47.50 and 47.78), number of shoots/ plant (5.00 and 5.50), fresh weigh of shoots and leaves/ plant (167.67 and 169.18 g), and dry weight of shoots and leaves/ plant (27.80 and 28.31 g), during the two growing seasons of 2019/ 2020 and 2020/ 2021 , respectively.

On the other hand, *T. hamatum* exhibited the lowest promoting effects on plant height (144.50 and 146.44 cm); number of leaves/ plant (39.83 and 41.22), number of shoots/ plant (3.67 and 3.83), fresh weigh of shoots and leaves/ plant (147.18 and 149.56 g), and dry weight of shoots and leaves/ plant (23.83 and 24.17 g), during the two growing seasons of 2019/ 2020 and 2020/ 2021, respectively.

These results are consistent with those previously reported by Harman *et al*., (2004); Harman, (2006); Bernal-Vicente *et al*., (2009), who mentioned that *Trichoderma* spp. caused conspicuous improvement in the aforementioned crop parameters. In addition, these differences in results may be attributed to variations in the genetic pool of the cantaloupe cultivars, and/ or effects of the climatic change on the vegetative growth. Similar results were reported by Gava and

1690

Menezes, (2012); Ahmed and Shaheen, (2016), who revealed that *Trichoderma* spp. were considered as plant growth promoters, as they produce plant growth hormones, and enhance the transfer of minerals to the plant roots.

Table 5. Effects of bio-control agents on reducing the disease incidence of cantaloupe root rot diseases under field conditions, during the growing seasons of 2019/ 2020 and 2020/ 2021

Different antagonists	2019/2020 growing season			2020/2021 growing season		
	DI (%)	Efficacy of reduction DI (%)	Plant survival (%)	DI (%)	Efficacy of reduction DI (%)	Plant survival (%)
T. album	45.3	41.32	54.7	46.8	41.35	53.2
T. hamatum	48.6	37.05	51.4	51.5	35.46	48.5
T. harzianum	15.7	79.66	84.3	18.3	77.07	81.7
T. viride	25.4	67.10	74.6	26.5	66.79	73.5
Bio-zeid (*T. album*)	39.2	49.22	60.8	42.2	47.12	57.8
Plant Guard (*T. harzianum*)	31.1	59.72	68.9	32.6	59.15	67.4
Control	77.2	00.00	22.8	79.8	00.00	20.2
LSD at 5 %	1.22	--	--	1.12	--	--

-Results are averages of 3 replicates for each treatment

Table 6. Effect of the BCAs on the growth parameters of cantaloupe plants var. *Primal hybrid* under field conditions, during the 2019/ 2020 and 2020/ 2021 growing seasons

Treatments	Plant height (cm)		Number of leaves/ plant		Number of shoots/ plant		Fresh weight of shoots and leaves (g/ plant)		Dry weight of shoots and leaves (g/ plant)	
	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21
T. album	149.33	149.67	42.44	42.67	3.89	4.00	153.11	155.44	25.28	25.98
T. hamatum	144.50	146.44	39.83	41.22	3.67	3.83	147.18	149.56	23.83	24.17
T. harzianum	167.67	168.33	47.50	47.78	5.00	5.50	167.67	169.18	27.80	28.31
T. viride	161.33	163.22	46.67	45.83	4.50	4.67	163.83	165.50	26.72	26.83
Bio-zeid	155.22	156.67	43.50	44.22	4.00	4.06	157.80	159.34	25.87	26.00
Plant Guard	157.44	158.00	44.83	45.33	4.15	4.46	161.00	162.75	26.08	26.14
Control	122.12	123.10	25.06	26.12	2.83	2.87	125.21	126.11	18.19	19.14
LSD at 5 %	2.66	2.62	1.03	1.04	2.74	2.73	1.76	1.77	1.02	1.03

-Results are averages of 3 replicates for each treatment

3.4.3. Effect of the BCAs on the crop yield and components of the cantaloupe plant

Results presented in Table (7) demonstrate that all of the tested BCAs significantly increased the crop yield of the cantaloupe plant, the % of total soluble solids (TSS), and the total phenol contents, compared to the non-treated control plants during the two growing seasons. *T. harzianum* expressed the highest significant increase in the number of fruits/ plant (312.67 and 313.15), the crop yield (93.80 and 93.94 Kg/ plot), TSS (16.20 and 16.92 %), and total phenol content (79.27 and 80.22 mg GAE/ 100 g FW), during the two growing seasons; respectively, followed by *T. viride*. On the other hand, *T. hamatum* was the least effective BCA during the two growing seasons. Plant Guard as a biocide recorded the highest efficiency in crop yield promotion, compared to the tested Bio-zeid bio-fungicide, during the two growing seasons.

These results are in agreement with those reported by El-Desuki *et al*., (2000); Harman *et al*., (2004); Adebayo *et al*., (2014); Martínez-Medina *et al*., (2014), where the BCAs effectively managed the different pathogens, and simultaneously promoted growth of the plants, which was expressed through a significant increase in the crop yield and components. According to the previous studies conducted by Vouldoukis *et al*., (2004); Mariod and Matthaus, (2008); Gava and Pinto, (2016); Ahmed and El-Fiki, (2017); Fernondo *et al*., (2018), the current increase of crop yield may be attributed either to the existence of healthy root system that absorbs and supplies adequate amounts of raw nutrients to the plant shoot, or due to the effective synthesis of raw nutrient materials by the BCAs, which was expressed in the present study through the recorded high amounts of TSS, phenol, and protein, which accordingly led to more fruit yield.

Table 7. Effect of the BCAs on yield component, TSS and total phenol of cantaloupe under field conditions, during the 2019/ 2020 and 2020/ 2021 growing seasons

Treatments	Number of fruits/ plot		Weight of fruits/ plot (Kg)		Total soluble solids (TSS %)		Total phenols (mg GAE/ 100 g FW)	
	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21
T. album	302.00	302.08	90.60	90.62	12.73	12.94	66.70	68.70
T. hamatum	299.07	299.79	89.72	89.94	11.65	11.90	64.50	65.65
T. harzianum	312.67	313.15	93.80	93.94	16.20	16.92	79.27	80.22
T. viride	309.87	310.91	92.96	93.27	15.06	15.10	78.67	78.83
Bio-zeid	304.80	305.04	91.44	91.51	13.30	13.77	69.67	69.83
Plant Guard	307.33	307.55	92.20	92.26	14.84	14.85	74.33	75.67
Control	180.40	181.25	54.12	54.38	9.10	9.22	38.67	41.20
LSD at 5%	3.3	3.4	2.1	2. 3	0.45	0.44	1.06	1.07

-Results are averages of 3 replicates for each treatment

3.4.4. Effect of the BCAs on chemical components of the cantaloupe plant

Data obtained from these chemical analyses are presented in Table (8), beside the results previously mentioned about effects of the BCAs on cantaloupe root rot; just to correlate and understand the role of these bio-agents on the changes that may occur in the plant chemical components, and reflection of these changes on the degree of resistance to pathogens, and the increase in crop yield. Compared to the control treatment, *T. harzianum* was the highest effective BCA, which recorded the highest increase in the amount of total nitrogen (1.24 and 1.26 %); protein (7.75 and 7.88 %), sugar (13.80 and 14.15 %), and Ascorbic acid (38.00 and 38.10 mg), during the 2019/ 2020 and 2020/ 2021 growing seasons; respectively, followed by *T. viride*, and then the Plant Guard bio-fungicide.

On the contrary, *T. hamatum* represented the least effective BCA, as this isolate did not cause significant increase in the total nitrogen recording 1.12 and 1.13 %, protein (7.00 and 7.06 %), sugar (11.47 and 11.77 %), and Ascorbic acid (25.84 and 26.17 mg) of the plant. The interactions among the other treatments had no significant differences on the percentages of nitrogen, protein, total sugars, and Ascorbic acid contents of the cantaloupe fruit tissues, as demonstrated in Table (8). These results are consistent with those obtained by Karaoglu *et al.*, (2018). Currently, treatments with the BCAs had high positive effects on the plant protection and % of disease reduction, combined with a significant increase in the amounts of total nitrogen, protein, sugar (%) and Ascorbic acid, in agreement with those results previously obtained by Gava and Pinto, (2016).

Table 8. Effect of BCAs on the chemical components of cantaloupe plant under field conditions, during the 2019/ 2020 and 2020/ 2021 growing seasons

Treatments	Nitrogen (%)		Protein (%)		Total sugar (%)		Ascorbic acid (mg/100 g FW)	
	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21
T. album	1.14	1.14	7.13	7.13	11.90	12.10	27.14	27.83
T. hamatum	1.12	1.13	7.00	7.06	11.47	11.77	25.87	26.17
T. harzianum	1.24	1.26	7.75	7.88	13.80	14.15	38.00	38.10
T. viride	1.22	1.23	7.63	7.69	12.77	13.14	37.20	37.80
Bio-zeid	1.16	1.15	7.25	7.19	12.22	12.34	29.56	30.75
Plant Guard	1.18	1.19	7.38	7.44	12.45	12.53	36.59	36.82
Control	0.85	0.86	5.31	5.38	9.04	9.07	14.20	15.10
LSD at 5%	0.10	0.11	0.22	0.23	0.26	0.27	0.34	0.35

Where; mg/ 100 g FW: refers to the amount of ascorbic acid in mg/ 100 g fresh weight of cantaloupe. Results are averages of 3 replicates for each treatment

3.4.5. Impact of climate change on air temperature during 2019 growing season of cantaloupe cultivation and its effect at the future

The data demonstrated in Table (1) describes the IPCC climate change scenarios and their impact on using the different BCAs to control the cantaloupe root rot disease. Meanwhile, the data in Table (9) displays the average monthly trend of air temperature in Sadat City meteorological station during the growing season of 2010-2019, and the projected (2050s–2100s) conditions. The greatest average temperature values in both of the actual and forecasted data were noted on August, while the lowest was recorded on April.

These findings demonstrated that the average temperature ranged from 23.4 to 36.2 o C. The scenario A1 consistently produced the highest average temperature, while the B2 scenario produced the lowest one. B2 and A1 scenarios showed the average air temperature differences that varied from 2.7 to 4.6 °C. In addition, under the A2 scenario, the 2050s average air temperature was shown to be the lowest. Furthermore, the A1 scenario had the highest average monthly air temperature values, compared to the other scenarios for the majority of the growing season periods.

Table 9. Average air temperature under present and future conditions of Sadat region during the periods of cantaloupe growing seasons

Month	Average air temperature (°C)								
	(2010-2019)	2050s A1	2100s A1	2050s B1	2100s B1	2050s A2	2100s A2	2050s B2	2100s B2
April	23.4	25.0	26.5	25.5	24.9	24.4	25.5	23.9	25.0
May	25.8	28.0	29.8	29.0	28.2	26.6	29.3	26.9	28.9
June	28.9	31.9	34.4	33.4	32.1	30.9	34.1	30.6	32.9
July	29.2	32.9	35.5	34.1	32.9	31.5	34.8	30.6	32.9
Aug.	30.1	33.5	36.2	35.6	33.3	32.5	35.9	31.4	33.8
Sep.	27.5	31.0	33.6	36.8	31.1	29.6	33.2	28.7	31.3
Average	27.5	30.4	32.7	32.4	30.4	29.2	32.1	28.7	30.8

According to IPCC scenarios, climate change led to raising the average air temperature. The optimum air temperature for cantaloupe growth ranged between 18- 28 °C. The rise in average air temperature will make a biological stress, which will lead to the decrease in the growth rate and development of the cantaloupe plants. On the other hand, the increase of air temperature will affect the irrigation requirements of cantaloupe plant, due to the increase in the evapotranspiration (Eto) rate. So,

farmers should increase the irrigation amounts to compensate for the lost water from the soil by evaporation. The increase in average air temperature and soil moisture under climate change conditions will encourage the infection of cantaloupe roots with the soil-borne pathogens.

The use of biological control and development of new varieties of cantaloupe plant will be more effective at the future, to control the root rot disease

of cantaloupe plants. These results are in agreement with those of Baker and Reddy, (2001); Santamarina and Rosello, (2006); Sarkar *et al*., (2013).

Conclusion

Recently, the growers realized that using chemical pesticides might have injury on the environment and human health, because they are highly toxic substances, which caused great disturbance in the biological balance. This disturbance led to the appearance of new pests; caused reduction in the number of natural enemies, and increased the accumulation of toxic chemicals in the human food chain. The present work was designed to reduce the use of toxic chemicals in the agriculture process, in order to produce sufficient food of high quality; enhance the biodiversity system, maintain and increase the long-term fertility of the soils. In addition to finding the most suitable biological control method, to protect the cantaloupe plants from the soil-borne fungal pathogens under the impact of climate change. Several BCAs, including *T. album*, *T. hamatum*, *T. harzianum*, and *T. viride*, and the tested bio-fungicides (i.e., Plant Guard and Bio-zeid), showed high *in vitro* and *in vivo* efficacy in their antagonistic actions against the tested soil-borne fungal pathogens, which cause root rot disease of cantaloupe. Results showed that, applying an inoculum of the BCAs on the cantaloupe roots at the rate of 1 l/100 l of water/ Feddan, significantly reduced the DI of root rot disease caused by the soil-borne fungal pathogens, and increased the percentage of the survived cantaloupe plants. In addition, the BCAs significantly increased all the tested plant growth parameters, compared to the control treatment. Furthermore, all the fungal bio-agents increased the chemical components of the cantaloupe plants. These changes have reflected on the degree of plant resistance, and on the increase in crop yield. This trend was true during 2019/ 2020 and 2020/ 2021 growing seasons.

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