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Control of Wilt, Stalk and Tuber Rots Diseases Using Arbuscular mycorrhizal fungi, *Trichoderma* Species and Hydroquinone Enhances Yield Quality and Storability of Jerusalem Artichoke (*Helianthus tuberosus* L.)

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ABSTRACT

Under field production phase, wilt, stalk and tuber rots diseases lead to considerable decline in Jerusalem artichoke (JA) yield and quality, and subsequently reduce storability of tubers. This study aimed to test and prepare suitable formula for increasing yield, quality and storability of JA tubers, through controlling the causative pathogens; *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. Among Arbuscular mycorrhizal fungi (AM), *Trichoderma* species (T) and hydroquinone (HQ), the combined application of AM+HQ, followed by AM+T reduced the disease incidence under greenhouse and field conditions, improved yield and enhanced the tuber quality (dry matter, total carbohydrates and protein). This in turns reasonably increased the storability parameters (decay, weight loss, dry matter and inulin content) during storage of JA tubers. So, such applications may introduce integrated solutions for field and storage problems of JA tubers.

Key words: Jerusalem artichoke, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, Abuscular mycorrhizal fungi, *Trichoderma* spp., Hydroquinone, Control.

INTRODUCTION

The perennial vegetable plant Jerusalem artichoke (JA) (*Helianthus tuberosus* L.) is an interesting plant as regards to its functional food constituents. Tuber flesh of the plant is one of the primary sources for fructooligosaccharides (inulin) in higher plants (Saengthongpinit and Sajjaanantakul, 2005), which act as sweeteners without affecting blood sugar level after ingestion (Seljåsen and Slimestad, 2007). This fructan and its fructose units can be used in human diet or in medical and industrial applications (Monti *et al*., 2005). Its protein has high food value due to the presence of almost all essential amino acids in good balance (Rakhimov *et al*., 2003). Another potential use of this species is as a forage crop (Seiler and Campbell, 2004) and as a biomass crop for ethanol and biogas production in the last decades (Denoroy, 1996).

JA is native to North America (Cosgrove *et al*., 1991) but it is also being grown commercially in the tropics (Puangbut *et al*., 2011). In such regions, soil moisture and temperature (20 to 25°C) conditions that are conducive to plant growth are favorable for germination and infection with tuber and stalk rots caused by *Sclerotinia sclerotiorum* and/or *Rhizoctonia solani* which are also occasionally the cause of the brown rot of tubers and wilt diseases (Kays and Nottingham, 2008). Yield losses of 22% of JA have been reported, especially when grown in land previously planted with JA or other hosts of *S. sclerotiorum* (Cassells and Walsh, 1995). These climatic conditions are being exist during April and May in Egypt, which limiting the production of the plant. Both soil borne diseases are prevalently noticed in the clay loam soils and in wet soils or under excessive irrigation. Under storage conditions, approximately 20 organisms have been shown to cause JA tuber rots, although losses can be circumvented through proper storage conditions. Among them, watery soft rot caused by *S. sclerotiorum* often develops on JA tubers that appear sound at harvest but subsequently succumb in storage. Rhizoctonia rot results in a brown discoloration of the tubers, caused by *R. solani* that occasionally isolated from diseased tubers (Kays and Nottingham, 2008).

Control of pathogens attacking JA in both field and storage, includes several methods, e.g. avoidance of JA production, for 3 to 5 years, near *S. sclerotiorum* hosts such as beans, sunflower and soybean (Cosgrove *et al*., 1991) and breeding for resistance (Kays and Nottingham, 2008). Fungicides application can be an integrated management system but their field application may not always be desirable (McCarterand Kays, 1984), however, none offers complete control. Alternatively, biological control of plant pathogens has increased considerably in the last few decades. *Trichoderma* spp. are effective biocontrol agents for several fungal plant pathogens including sclerotia producing pathogens (Sennoi *et al*., 2013 and Al-Askar *et al*., 2014). The biocontrol exercised by *Trichoderma* can occur by means of several antagonistic mechanisms such as nutrient competition, antibiotic production, and mycoparasitism, some species are also known for their abilities to enhance systemic resistance to plant diseases as well as overall plant growth (Saba *et al*.,

2012 and Al-Askar *et al*., 2014). Arbuscular mycorrhizal fungi (AM) has special significance being an eco-friendly and cost effective strategy for disease management, in addition to its positive effects on plant growth and nutrition. It was found that *Glomus mosseae*, *G. intraradices*, *G. clarum*, *Gigaspora gigantea*, and *G. margarita* have an important role in the enhancement of plant growth, nutrition, water relations and resistance to plant diseases caused by several pathogens on different host species (Abdel-Fattah *et al*., 2011 and Al-Askar *et al*., 2014). Hydroquinone (HQ) is an aromatic organic compound that is a type of phenol, having the chemical formula $C_6H_4(OH)_2$, this phenol can act as antioxidant. It was reported to inhibit some pathogenic fungi as well as improving the growth and yield of the plants (Al-Askar *et al*. 2013 and 2014). One possible approach for enhancing the efficacy and improving biological control ability of the bioagent against pathogens may be the use of mixtures or combinations of biocontrol agents (Sennoi *et al*., 2013 and Al-Askar*et al*., 2014), since a single strain of a bioagent is less effective and its impact may not consistence (Spadaro and Gullino, 2005).

Considering the difficulties of controlling *S. sclerotiorum* and *R. solani* that responsible for JA stalk rots, wilt and tuber rots in Egypt, the present work aimed to: 1) evaluate the efficacy of AM, *Trichoderma* spp. and/or hydroquinone for controlling stalk and tuber rots diseases as well as, improving yield and tuber quality under greenhouse and field conditions, and 2) study their role in enhancing the storability of the obtained tubers.

MATERIALS AND METHODS

Plant and chemicals

JA tuber cultivar (Fuseau) was obtained from Baramoon Horticulture Research Station, Dakahlia Governorate, Egypt. Hydroqinone was obtained from Sigma Chemicals Co., USA. Benzothiadiazole (Benzo-(1, 2, 3) thiadiazole-7-carbothioic acid S-methyl ester wettable granule 50% WG, Bion^R) (BTH), a new product of Novartis Company was used in this experiment.

Fungal isolation and identification

Rhizosphere soil and plant samples were collected from JA cultivated fields located at Dakahlia, Damietta, Behera and Giza Governorates, Egypt. At each site, an area of 500 m^2 was chosen for sampling. For the isolation of the pathogen from plants showing typical symptoms of stalk rots, wilt and tuber rots, the infected parts were separately washed, surface-disinfected for 3 min. in 0.5% (v/v) sodium hypocholorite, rinsed in sterilized water, and sections (1 cm) were placed on PDA plates (Difco, USA), supplemented with 5mg/L L-chloramphenicol and 5mg/L streptomycin sulphate, then incubated at 25°C for 5-7 days. Hyphal tip and/or the single spore technique were used to obtain pure cultures. Recovered isolates were then transferred into slant of potato carrot agar and kept at 4°C for further studies. The isolates were identified as described by Domsch *et al*. (1980).

Trichoderma species were isolated from the rhizospheric soil of healthy JA plants, collected in triplicates from each previously mentioned location, using a selective medium of Elad *et al*. (1991). Developed colonies were identified on malt extract agar according to Kubicek and Harman (2002). The identification was confirmed at the Mycological Center, Assiut University (AUMC), Egypt.

Antifungal activity of *Trichoderma* spp.

Antagonistic activity of the isolated *Trichoderma* spp. was carried out against *S. sclerotiorum* and *R. solani* on PDA plates. Growth of the fungi and interaction between dual mycelia were scored for degree of antagonism reaction, using a scale of 1 to 5 after the 5th day of dual growth:

*Growth of the pathogens (%) = (Radius growth of pathogen in the direction *Trichoderma* /Radius of growth in absence of *Trichoderma*) x 100

Where: 1= *Trichoderma* overgrowing pathogen and 5= pathogen overgrowing *Trichoderma* (Bell *et al.,* 1982). *S. sclerotiorum* and *R. solani* developed from plates of dual cultures were then microscopically investigated and the changes in the mycelium of the pathogen were recorded.

Inoculum preparation of *Trichoderma* spp.

A mixture of three *Trichoderma* isolates consisted of spores of *T. atroviride* (Ta1 and Ta2) and *T. reesei* (Tr1) in equal proportions were selected, based on test of possible antagonism among the *Trichoderma* isolates as well as the dual culture test with the pathogens. Each of antagonistic *Trichoderma* isolate was grown in bottle of sterilized sorghum: coarse sand: water (2:1:2, v/v) medium and incubated at 25±2°C for 10 days, then the three inoclua were mixed in equal portions to obtain a mixture of *Trichoderma* inoculum (T).

Inoculum and mass production of Arbscular mycorrhiza fungi (AM)

Suspension mixture (10^6 units L^{-1}) of multi-Arbscular mycorrhizal fungi consists of equal proportions of spores of *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. intraradices* Schenck & Smith, *G. clarum* Nicol. & Schenck, *G. gigantean* (Nicol. & Gerd.) Gerd. & Trappe, and *G. margarita* (Becker & Hall), was kindly provided by Prof. Safwat El-Haddad, Plant Pathology Institute, ARC, Giza, Egypt (El-Haddad *et al*., 2004). Mass production of AM inoculum was carried out by spores of the previous

formula using the pot culture technique and Sudan grass as a host plant.

Greenhouse experiment

For artificial infection, *S. sclerotiorum* or *R. solani* was cultured on PDA plates and incubated at (25±2°C) for 3 days. After incubation, mycelium plugs were transferred to sterilized medium of sorghum: coarse sand: water (2:1:2, v/v) and incubated at room temperature (25±3°C) for 10 days to obtain the inoculum. Pots (40 cm in diameter) were filled with 8 kg/pot disinfested soil; clay: sand (2:1, v/v) and singly infested with the previously prepared pathogen inoculum at the rate of 0.4% (w/w). Pot soil was mixed thoroughly with the inoculum then regularly watered to near field capacity with tap water and left for one week to ensure even distribution of the pathogenic fungus. Tubers of JA with 3 to 4 buds were incubated for one week in a moist sterilized peat moss to facilitate germination under open-side greenhouse. Apparently healthy tubers, surface sterilized with 1% sodium hypochloride, followed by washing with sterilized distilled water and were used herein. Accordingly, 8 treatments were studied; (1) control, (2) T, (3) AM, (4) HQ, (5) BTH, (6) AM+HQ, (7) AM+T and (8) T+HQ. Pots that were inoculated with AM and/or T received 40 g of the inoculum/ pot, as seed-bed before planting. In case of HQ (20 mM) and BTH (1 g/1000 ml), tubers were soaked for two hours and 20 min., respectively (Al-Askar *et al*., 2010), then dried using sterilized filter paper. In case of infection with the pathogens, each of *S. sclerotiorum* or *R. solani* was used to infest another two groups containing the same previous treatments. Two tubers were planted in each pot, one week after, inoculation with each of them. Pots were regularly watered to near field capacity with tap water. All pots were arranged in 9 replicates and kept under greenhouse conditions with average temperature 25±2°C.

Disease assessment and biochemical testes

Disease incidence of the tubers was rated at 30 days after sowing under greenhouse conditions, while root rot (defined as wilting of all leaves on a plant) was followed up by observing the plants daily until the 90th day after sowing. Number of rotten tubers and plants with permanent wilting in each treatment was later converted to disease incidence (percentage symptomatic plants). After 40 days from sowing, total phenol content was determined using Folin Ciocalteau reagent based on the method described by Malik and Singh (1980). Extraction and assay of polyphenoloxidase (PPO) and peroxidase (POD) were carried out according to the methods described by Maxwell and Bateman (1967) and Maria *et al*. (1981), respectively. By the end of greenhouse experiment, fresh root samples were evaluated for mycorrhizal colonization after clearing with 10% KOH and coloration with trypan blue (Phillips and Hayman, 1970), by the light microscopy at 40× magnification (Giovannetti and Mosse, 1980).

Field experiment

The experiments were conducted at Baramoon Research Station, Dakahlia, Egypt (+ 7m altitude, 30° 11^{-} latitude and 28° 26^{-} longitude), during the summer seasons of 2012/13 and 2013/14. The soil is a clay loam textured, with 1.2% organic matter and pH 7.9. Extractable soil P and K levels in the plots used in this 2-yr trial were in the range of 11.3 to 11.8 mg kg^{-1} for P and 292 to 308 mg kg^{-1} for K. Local climate is Mediterranean type, warm and dry during the summer season. Temperature range was between 12.5 and 37.3 °C. Whole tubers were within a range of 20 to 25 g, sown was on April 10th in both seasons. To study the effect of the previous treatments, under natural infection in the open only 5 treatments were selected based on the results of the greenhouse experiments as follows; (1) control without application, (2) BTH, (3) AM+HQ, (4) AM+T and (5) T+HQ. Experimental plot area of both experiments was 18.0 m^2. It contained three rows with 6 m in long and 1 m distance among the rows. Calcium superphosphate was thoroughly mixed within the upper soil layer (0-25 cm) before planting. All treatments were planted in hills 50 cm apart on one side of row ridge. After planting, the soil was ridged up around the plants, either along rows or around individual plants as hills. This also was done when the plants were around 30 cm tall, as part manual wedding operations. Earthen up the soil around the base of young stalks favors tuber formation. Nitrogen (ammonium nitrate 33.5% N), phosphorus (mono-superphosphate 15.5% P_2O_5) and potassium (potassium sulphate 48% K_2O) were applied in the rate of 90, 45 and 75 kg fed^{-1}, respectively. Nitrogen fertilizer was added at three equal doses, *i.e*. the first after emergence, and then the second and third doses were applied with the second and third irrigation, respectively. Potassium was added at two times, one-half was added with the second addition of N-fertilizer, and the second half was added with the third doses of N-fertilizer. Other agricultural practices were carried out as recommended. At flower initiation stage (at 120 days after planting), a random sample of three plants from each experimental plot was picked up to determine the fresh and dry weight of shoot, at harvest time, 185 days after planting, total tuber yield, marketable and unmarketable yield were recorded.

Monitoring of disease incidence

Incidence of JA tuber and stalk rots as well as wilt diseases was assessed at 40 and up to 160 days from sowing for pre-and post-emergence damping-off, respectively.

Tuber quality

Quality of the harvested tubers for each treatment was determined. Percentage of tuber dry matter was calculated by drying 100 grams of fresh tubers in oven at 70 °C till a constant weight. Total carbohydrate percentage was determined calorimetrically in fine grained dry tubers, following the method described by Dubois *et al*. (1956). Protein percentage was calculated in tubers dry matter using multiplying N percentage by the conversion factor 6.25 (Robinson, 1973).

Storability of JA tubers

Tubers of each treatment were sorted, washed and dried to terminate soil. All the defected tubers were discarded and then ~500 g of each treatment was packed in perforated polypropylene bags (15 µm) and kept at zero °C and 95% RH for four months. Tubers weight loss, decay and tuber dry matter percentages were recorded at 30 days intervals. Inulin content was determined in tubers according to the method of Winton and Winton (1958).

Statistical analysis

Data obtained were subjected to statistical analysis by analysis of variance based on completely randomized blocks design. Means were compared using Duncan multiple range test at probability level (P)≤ 0.05 or 0.01, using the statistical analysis software CoStat (Version 6.4).

RESULTS AND DISCUSSION

The pathogenic fungus

Concerning the isolation of the causal fungal agent of wilt, stalk and tuber rots from the infected JA plants, the results showed that *S. sclerotiorum* and *R. solani* were the most prevalent pathogens (data not shown). So, these fungi were selected for further investigation. Pathogenicity test was carried out using different isolates to select the most aggressive one causing ideal symptoms of rots. Infected tubers by *S. sclerotiorum* developed watery soft rot symptoms that appear sound at harvest, but subsequently succumb in storage. The tubers became covered with a dense white mycelium and irregular sclerotia, which progress from white to dark brown or black (Fig. 1). On the other hand, a dry brown discoloration of the tubers was associated by *R. solani* infection. Later the pathogen developed on progeny tubers as reported by Kays and Nottingham (2008). As a consequence of this complex disease, malformed tubers and changes in tuber size and number were reported, which may result in considerable reduction in marketable yield, next in the present study. Kays and Nottingham (2008) reported similar observations.

Infected JA plants with *S. sclerotiorum* appeared initial symptoms consisted of tubers rot, wilting of new shoots and leaves, followed by browning and collapse of all foliage. Crown and lower stem tissues were colonized internally and externally by white, cottony mycelium. Dark brown to black, irregular sclerotia that measured approximately 1 cm or more in diameter were formed on surfaces of the affected crowns and stems (Fig. 2). This was also previously reported by Cassells and Walsh (1995). In respect to *Rhizoctonia* wilt symptoms, the disease was initially manifested as lesions on JA sprouts and sometimes death of sprouts, and as dark brown stem canker lesions on underground stems of the JA plants and on the developing stolons.

Occurrence of antagonistic fungi in JA rhizosphere

The fungal community in the rhizosphere of healthy JA plants was isolated and numerated. The aim was to find out strong antagonistic fungi to be used in biological control of tuber and root rots. Accordingly, twenty-five fungal isolates belonging to thirteen genera were identified. As presented in Fig. (3), *Penicillium* spp. were the highest counted genus (5.485 log. cycle). Meanwhile, members of the genus *Trichoderma* occupied from the second to the fifth order, being 5.379, (*T. atroviride*), 5.291 (*T. reesei*), 5.222 (*T. harzianum*) and 5.070 log. cycle (*T. koninige*) without significant differences among each others. On the opposite, members of the *Aspergilli* recorded wide variation in their counts; *Aspergillus ochraceus* (4.325 log. cycle) was the superior counted fungus. Members of the *Fusarium* spp. tended to distribute like *Aspergilli*, but *F. oxysporum* (3.251 log. cycle) was the highest among *Fusarium* spp. Other less frequent fungi were recorded, *e.g. Drechslera halodes, Gliocladium roseum* and *Mucor circinelloides* with equal counts (0.739 log. cycle). However, the isolation trial did not reveal important pathogenic fungi in the rhizosphere of the candidate plants. Because of their diversity, adaptability and ability to antagonize the pathogenic fungi, all *Trichoderma* isolates were tested against the previous two pathogens (*S. sclerotiorum* and *R. solani*).

***Trichoderma*-pathogen antagonism interaction**

Trichoderma isolates were tested against the two aggressive pathogens. Three isolates strongly inhibited the growth of *S. sclerotiorum* and *R. solani*. *T. atroviride* (Ta1 and Ta1) and *T. reesei* Tr1 were the most active isolates of the antagonistic fungi against the two pathogens and showed high degree of antagonism reaction after five days of dual culturing (Table 1). The lowest growth of *S. sclerotiorum* (34.6, 26.2 and 44.1) and *R. solani* (54.8, 52.7 and 55.7) were recorded by *T. atroviride* Ta1, *T. atroviride* Ta2 and *T. reesei*Tr1, respectively. The best antagonism reaction, being 1 was recorded by *T. atroviride* Ta1 on *R. solani* and *T. reesei*Tr1 on *S. sclerotiorum* and *R. solani*, which means the occurrence of strong

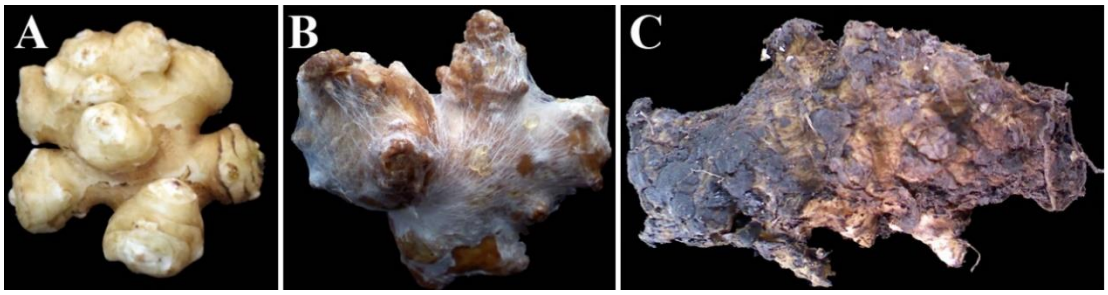


Fig. (1): Jerusalem artichoke tubers. A) Healthy tuber, B) watery soft rot caused by *Sclerotinia sclerotiorum* and C) *Rhizoctonia* rot results in dry and a brown discoloration caused by *Rhizoctonia solani*.

Fig. (2): Jerusalem artichoke plants affected by the tested treatments for controlling of *R. solani* (A) and *S. sclerotiorum* (B) under greenhouse conditions.

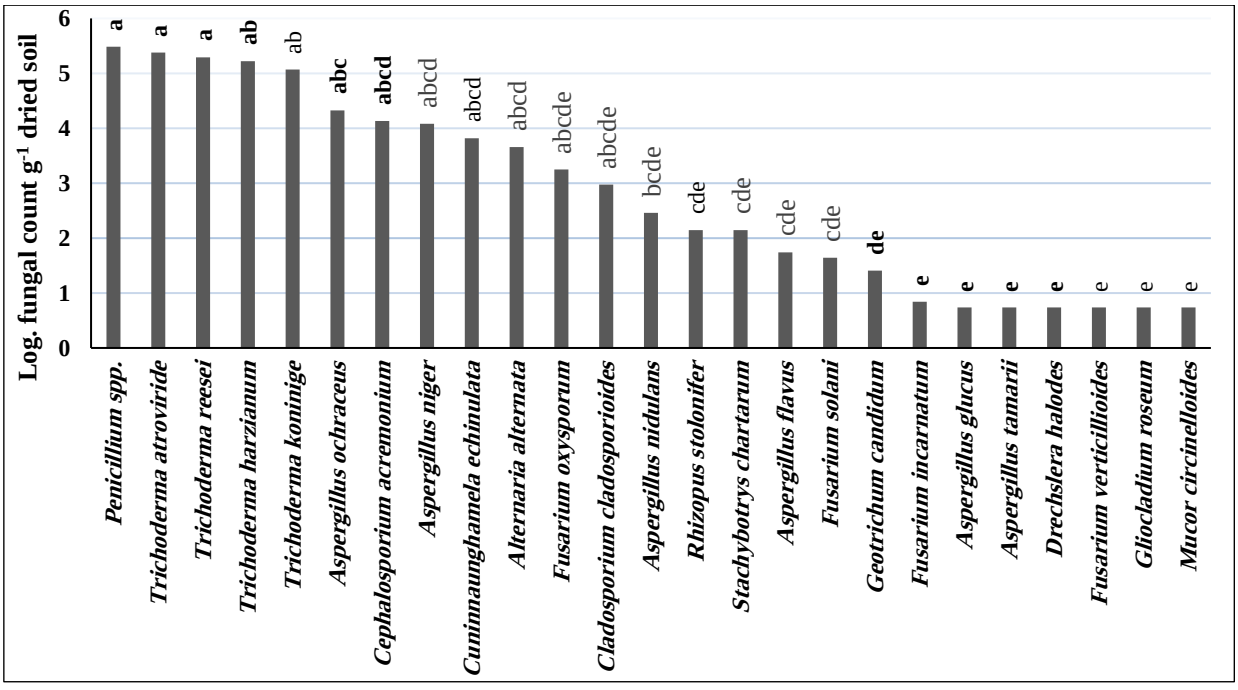
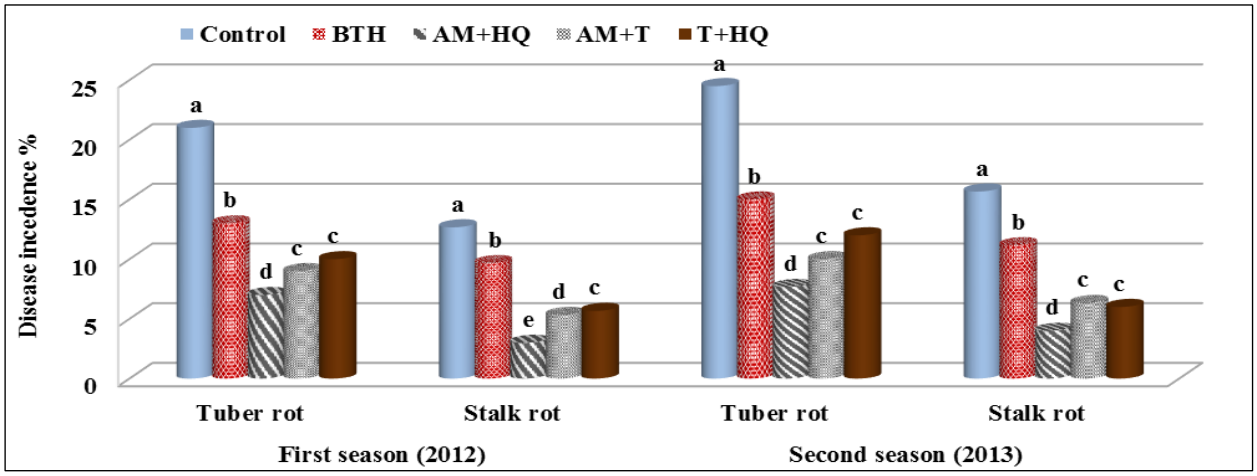


Fig. (3): Counts and occurrence of fungi isolated from the rhizosphere of healthy JA plants. Columns designated with different letter(s) differ significantly ($P \leq 0.01$).



Columns of each group superscripted with different letter differ significantly ($P \leq 0.05$).

Fig. (4): Tuber and stalk rots of JA affected by the tested treatments under field conditions during two growing seasons 2012/13 and 2013/14.

Table (1): Growth of JA pathogens affected by *Trichoderma* spp. in dual culture test

Trichoderma isolate	Growth of pathogens (%)*					
	S. sclerotiorum			*R. solani*		
	3 day	5 day	Antagonism reaction**	3 day	5 day	Antagonism reaction**
T. atroviride Ta1	10.1 cd	34.6 bc	2	32.8 ab	54.8 ab	1
T. atroviride Ta2	5.4 d	26.2 c	2	33.8 ab	52.7 b	2
T. harizanum Th1	19.2 bc	54.8 a	2	35.1 ab	58.3 ab	3
T. harizanum Th2	18.4 bc	50.0 a	3	33.8 ab	57.3 ab	4
T. harizanum Th3	20.3 a-c	44.1 ab	2	39.2 a	60.0 ab	4
T. koninige Tk2	32.0 a	51.6 a	3	33.8 ab	57.9 ab	4
T. reesei Tr1	16.3 b-d	44.1 b	1	31.9 b	55.7 ab	1
T. viride Tv1	24.3 ab	54.8 a	2	36.5 ab	56.6 ab	3
T. viride Tv2	24.6 ab	47.1 a	2	35.1 ab	58.3 ab	3
T. viride Tv3	27.1 ab	51.2 a	2	36.5 ab	61.7 a	3

**The antagonism reactions. Different letters within a column indicate significant difference ($P \leq 0.01$).

Table (2): Disease incidence of JA as affected by the tested treatments under greenhouse conditions

Treatment		Tuber rot, %	Root rot, %	Survivals, %
Pathogen	Biotic and/or abiotic			
Control (uninfested soil)	Without	1.3 f	0.8 e	97.9 a
	T	0.7 f	0.5 e	98.8 a
	AM	0.8 f	0.7 e	98.5 a
	HQ	0.5 f	0.3 e	99.2 a
	BTH	0.3 f	0.3 e	99.4 a
	AM+HQ	0.3 f	0.2 e	99.5 a
	AM+T	0.8 f	0.3 e	98.9 a
	T+HQ	0.7 f	0.5 e	98.8 a
S. sclerotiorum	*S. sclerotiorum* only	29.2 ab	18.1 ab	52.7fg
	T	23.6 b-e	13.9 a-c	62.5 c-f
	AM	26.4 a-c	16.7 ab	57.0 d-g
	HQ	20.8 b-e	12.5 b-d	66.7 b-f
	BTH	26.4 a-c	13.9 a-c	56.9 d-g
	AM+HQ	15.3 e	5.6 de	79.1 b
	AM+T	19.4 c-e	11.1 b-d	69.5 b-e
	T+HQ	20.8 b-e	15.3 a-c	63.9 c-f
R. solani	*R. solani* only	33.3 a	20.8 a	45.9 g
	T	23.6 b-e	13.9 a-c	62.5 c-f
	AM	25.0 a-d	18.1 ab	56.9 d-g
	HQ	20.8 b-e	12.5 b-d	66.7 b-f
	BTH	27.8 a-c	16.7 ab	55.5 e-g
	AM+HQ	15.3 e	8.3 cd	76.4 bc
	AM+T	16.7 de	11.1 b-d	72.2 b-d
	T+HQ	19.4 c-e	12.5 b-d	68.1 b-f

Means followed by the same letter(s) within each column do not significantly differ ($P \leq 0.05$).

Table (3): Physiological responses of JA affected by the tested treatments under greenhouse conditions

Treatment		Total phenol	Polyphenoloxidase	Peroxidase (Unit min^{-1}
Pathogen	Biotic and/or abiotic	(mg catechol g^{-1} fresh weight)	(Unit min^{-1} g^{-1} fresh wt.)	g^{-1} fresh wt.)
Control (uninfested soil)	Without	0.305 gh	0.87 e-i	1.37 b-e
	T	0.325 f-h	1.73 b-d	1.37 b-e
	AM	0.319 f-h	0.93 e-h	1.20 b-g
	HQ	0.312 f-h	1.23 d-g	0.93 c-h
	BTH	0.325 f-h	1.13 d-g	3.07 a
	AM+HQ	0.412 de	1.27 d-g	1.13 b-g
	AM+T	0.285 h	0.80 e-i	1.90 b
	T+HQ	0.325 f-h	0.70 f-i	1.20 b-g
S. sclerotiorum	*S. sclerotiorum* only	0.385 e-g	0.23 hi	1.47 b-e
	T	0.392 d-f	0.23 hi	1.60 b-d
	AM	0.572 ab	0.93 e-h	1.30 b-f
	HQ	0.559 ab	0.90 e-h	1.30 b-f
	BTH	0.298 h	1.53 c-e	0.83 d-h
	AM+HQ	0.285 h	2.30 ab	0.93 c-h
	AM+T	0.285 h	2.17 bc	0.53 f-h
	T+HQ	0.292 h	2.90 a	0.23 h
R. solani	*R. solani* only	0.385 e-g	0.50 g-i	1.67 bc
	T	0.305 gh	0.53 g-i	1.23 b-f
	AM	0.465 cd	1.33 d-f	1.43 b-e
	HQ	0.319 f-h	1.33 d-f	1.00 c-h
	BTH	0.606 a	0.60 f-i	0.97 c-h
	AM+HQ	0.532 a-c	1.10 d-g	0.87 d-h
	AM+T	0.539 a-c	0.10 i	0.70 e-h
	T+HQ	0.519 bc	0.27 hi	0.43 gh

Means followed by the same letter(s) within each column do not significantly differ ($P \leq 0.05$).

mycoparasitism. The light microscopy investigation showed the mycelium of the three pathogens to be fragmented hyphae, vacuolated and disrupted. Moreover, when plates were observed for more than 5 days, the three isolates of *Trichoderma* (Ta1, Ta2 and Tr1) were found to produce inhibition halos and sporulated over the colonies of the pathogens, with different degrees. However, mycoparasitism has been proposed as the major antagonistic mechanism displayed by *Trichoderma* spp. Saba *et al*. (2012) reported that *Trichoderma* spp. can attach to and coil around the pathogen, in some cases, form appressoria on the host surface, wherein *Trichoderma* spp. produce several cell wall degrading enzymes and probably also antibiotics, the combined activities of these compounds result in parasitism and dissolution of the cell walls forming holes, which acts as direct entry of *Trichoderma* hyphae into the target fungus. The antagonism among the three isolates of *T. atroviride* Ta1, *T. atroviride* Ta2 and *T. reesei* Tr1, was carried out to insure the growth compatibility of these isolates when used in combined inoculum. The test showed no visible antagonism, which encourages the use of a mixture of the three *Trichoderma* isolates.

Control of JA pathogens under greenhouse conditions

The most aggressive pathogens (*S. sclerotiorum* SS6 and *R. solani* RS9) were selected, based on a pathogenicity test, were used in this evaluation trial. *Trichoderma* (T), AM and HQ were applied individually or combined under greenhouse conditions. There were considerable differences among treatments in the disease incidence on JA seedlings infected with the tested pathogens (Table 2). Tuber treatment by AM+HQ was the most effective in increasing the survival of JA seedlings infected by *S. sclerotiorum* (79.2 %) and *R. solani* (76.4 %) as compared to the infected controls without treatment (52.5 and 45.8%, respectively). However, dual inoculation by AM+T came next in this respect. Generally, the combined treatments were found to protect JA plants more than applying the individual agent alone. It is of special importance to mention that mycorrhizal colonization in AM-treated JA plants, either single or in combinations, was examined to ensure the presence of effective mycorrhizal infection which was found to range from 80 to 100% colonization. The compatible synergism between T and AM stimulates the spore production of AM and subsequent formation of small vegetative spores (De Jaeger *et al*., 2011). Another mechanism suggested that the symbiosis between mycorrhizae and roots of many crops had a positive influence on the plant's nutrition and changes in root morphology that enhances resistance and/or tolerance of plants against the pathogen (Sennoi *et al*., 2013). In addition, hydroquinone recorded positive effect on disease parameters caused by *S. sclerotiorum* SS6 and *R. solani* RS9. That is why HQ was reported to be a potential inhibitor for some seed-borne pathogenic fungi (Elwakil, 2003 and Al-Askar *et al*., 2013).

Physiological aspects of JA

The physiological profile of JA plants as affected by the tested treatments under pathogen stress (Table 3) could not draw specific trend. However, total phenol and the two tested enzymes were induced in

Table (4): Vegetative growth characters, yield and yield components of JA as affected by the various applications in 2012/13 and 2013/14 seasons

Treatment	Shoot fresh weight plant^{-1}(g)		Shoot dry weight plant^{-1}(g)		Tuber yield (ton fed.$^{-1}$)					
					Marketable yield		Unmarketable yield		Total	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Control	1962 c	2717 a	495.23 c	476.66 c	17.240 c	18.036 d	2.190 a	2.187 a	19.430 b	20.223 c
BTH	2100 b	1996 c	514.03 c	467.50 c	18.883 c	19.001 cd	1.127 c	1.306 b	20.010 b	20.307 c
AM+HQ	2398 a	2717 a	606.93 a	590.33 a	23.107 a	23.843 a	1.023 c	0.967 b	24.130 a	24.810 a
AM+T	2371 a	2416 b	571.72 b	557.97 b	20.860 b	22.260 ab	1.300 b	1.186 b	22.160 a	23.446 ab
T+HQ	2390 ac	2682 a	581.84 b	589.61 a	22.233 ab	22.810 bc	1.393 b	1.133 b	23.627 a	21.943 bc

Means followed by the same letter(s) within each column do not significantly differed ($P \leq 0.05$).

Table (5): Tuber quality of JA as affected by the various applications in 2012/13 and 2013/14 seasons

Treatment	Tuber dry matter (%)		Total carbohydrates (mg g^{-1} dry weight)		Protein (%, on dry matter base)	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Control	22.14 c	21.86 c	41.49 c	36.36 e	12.00 c	11.17 b
BTH	22.30 c	22.12 c	40. 98 c	37.67 d	12.22 c	12.34 ab
AM+HQ	24.60 a	24.52 a	46.30 a	44.48 a	13.46 a	13.49 a
AM+T	23.52 b	23.08 b	44.03 b	43.49 b	12.64 b	12.16 a
T+HQ	24.11 ab	23.53 b	44.45 b	42.10 c	12.75 b	12.81 a

Means followed by the same letter(s) within each column do not significantly differed ($P \leq 0.05$).

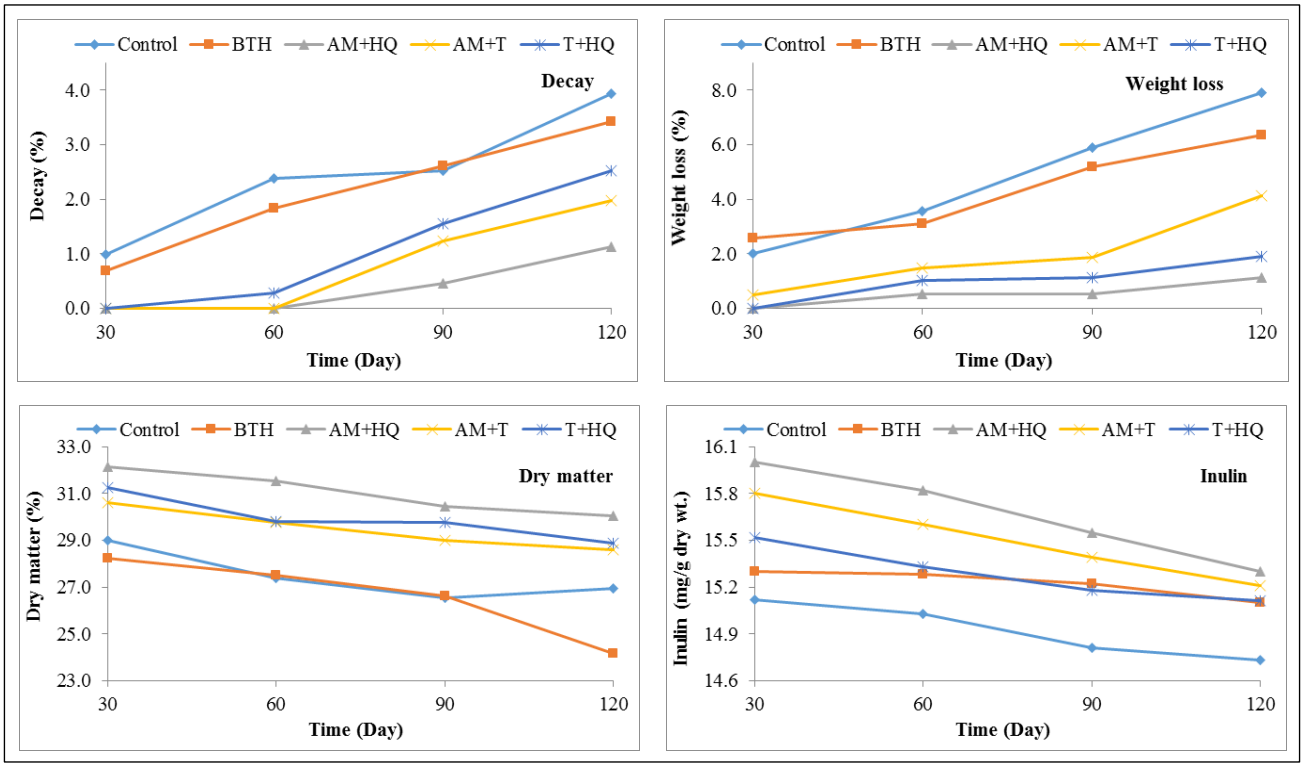


Fig. (5): Changes in decay, weight loss, dry matter and inulin content of JA tubers during storage as affected by the various treatments (average of the two seasons).

the treated plants even in the presence of the pathogen. In this respect, AM and HQ were the most inducers for total phenol in the plants infected with *S. sclerotiorum*. Plants infected with *R. solani* recorded high content in total phenol when treated with BTH, AM+T, AM+HQ or T+HQ. Polyphenoloxidase (PPO) of plants infected with *S. sclerotiorum* significantly induced by AM+HQ, T+HQ or AM+T compared with all other treatments, as well as plants infected with *R. solani* and the control treatments. Moreover, the present data revealed slight reduction of peroxidase activity in plants treated with the combined treatment, compared with the single. Because of the action of phenols as antioxidant, antimicrobial, and photoreceptor, their rapid accumulation at the infection site was the first step of the defense mechanism, which restricts the growth of the pathogen (Saber *et al*., 2009). There are possible mechanisms for defense role of peroxidase such as its toxicity to pathogens, accelerating the death of the infected cells, which reduces bioavailability of cellular proteins to the pathogen, forming a physical barrier to pathogens in the cell wall, and quinone redox cycling leading to H_2O_2 and other reactive

oxygen species, which are known as defense signaling (Raj *et al*., 2006). There was immediate 5-fold increase in PPO in the 1st day of plant infection, that activity is probably induced in plants to some degrees at all times in the field due to the environmental conditions, peroxidase can aid in the production of toxic secondary metabolites and its simultaneous oxidant and antioxidant activity can help defense response of plants under stress conditions (Thakker *et al*., 2013).

***In vivo* evaluation**

Based on the greenhouse results, only five treatments that excreted high performance on the tested pathogens were selected to be applied under field conditions.

Disease incidence

As described in Fig. (4), all treatments significantly reduced the incidence of diseases symptoms of JA plants during the two successive seasons. The combined application of AM+HQ was the most effective treatment, which reduced disease incidence of tuber rots and plant mortality in the first and the second seasons. The dual treatment by T+HQ or AM+T ranked second in this respect. On the other hand, the single treatment with BTH recorded lower efficiency on disease incidence in both seasons. Pre-treatment of JA tuber greatly decreased root and stalk rot incidence hence increased survivals under field conditions. Several reports indicated that treating seeds of JA with AM and/or HQ effectively controlled soil-borne diseases (Sennoi *et al*., 2013). Moreover, AM significantly decreased disease incidence and severity. This could be due to direct antagonistic effect of AM on the pathogen and/or improvement of the growth conditions of the host plant. Hydroquinone is thought to have relative toxicity to microorganisms, with evidence that increased hydroxylation associated with increased toxicity, so the more highly oxidized phenols are the more inhibitory effect to the pathogen (Scalbert, 1991). HQ as an antioxidant is a molecule capable of inhibiting the oxidation of other molecules, which delay or inhibit oxidative damage to target molecules such as lipids, proteins, nucleic acids and carbohydrates. Additionally, Elwakil (2003) found that HQ improved the growth of peanut and raised the yield by up to 50 %. Recently, Al-Askar *et al*. (2014) found that treatment by AM + hydroquinone or AM + *Trichoderma* spp. minimized disease incidence, improved plant growth and yield and enhanced the tuber quality of JA.

Vegetative growth, yield and yield components of JA

Application of AM+HQ had a significant effect on shoot fresh and dry weights of plant, marketable and total tuber yield in comparison with other treatments (Table 4). This is true in both seasons of the study. On the other hand, considerable yield losses of control plants were observed, which caused by tuber and stalk rots as well as *Rhizoctonia* wilt diseases. Most of these losses returned to tuber rot phase by soil-borne *S. sclerotiorum* and *R. solani* pathogens. The highest tuber yield (24.13 and 24.81in both seasons, respectively) was obtained from the application of AM+HQ. Total tuber yield increase was due to primarily the increase in marketable tuber yield in larger grade and decrease of the unmarketable yield. These pronounced positive effects on the vegetative growth parameters of JA plants may be attributed to the fact that plants under inoculation with AM increased utilization of water and nutrients, particularly phosphorus element, and that in turns, enhanced the vegetative growth. The acquisition of carbon is strongly modulated by the surface area of photosynthesizing leaves; hence, understanding leaf area development is germane to the efforts to increase yield (Kays and Nottingham, 2008). In this respect, Sennoi *et al.* (2013) found that vascular AM (*G. clarum*) alone gave better control of *S. rolfsii* on JA. The inoculation with AM could improve plant growth and biomass accumulation of bio-energy crops (*Galega orientalis* and *H. tuberosus*) even in non-sterile soil containing naturally occurring Mycorrhiza (Püschel *et al*., 2011). According to Artursson *et al*. (2006), AM can increase growth and phosphorus content of many crops. Generally, application of AM improved the plant growth and productivity by its direct effect on improving the internal status of the crop or indirectly by reducing the harmful effect of the pathogen (Al-Askar *et al*., 2014).

Tuber quality of JA

Tuber quality appeared to respond to the inoculation with AM+HQ in a similar manner to total yield. Increase of carbohydrates and protein were found as a result of AM+HQ. These changes corresponded with the increase in dry matter in both seasons (Table, 5). The content of inulin (main components of carbohydrates) depends upon many factors, such as the plant source from which it is extracted, the climate and growing conditions, the tuber maturity and the storage time after harvest (De Leenheer and Hoebregs, 1994). In this respect, Modler *et al.* (1993) found that higher stress conditions encouraged breakdown of inulin and utilization of monosaccharides formed from breakdown, presumably due to higher respiration and other metabolic activities. Antioxidant effects of HQ, against the toxic and degradable effects of reactive oxygen species (O_2^-, OH^- and H_2O_2), which probably generated were found (Cakmak and Marschner, 1992) in stressed tissues. Wu *et al.* (2013) mentioned that the inoculation of micro-propagated plant material with MA fungus improved both mini-tuber

production in the seedling plate and tuber production and quality in the field.

Storability of JA tubers

Storability of harvested JA tubers from different treatments was evaluated along 120 days at 0°C (Fig. 5). Average values of storability characteristics (decay %, weight loss %, dry matter % and inulin content) of the two seasons revealed that AM+HQ treatment gave the lowest decay and weight loss percentages. However, applying BTH or control recorded the maximum decay and weight loss. On the other side, during the storage period, tubers previously treated with AM+HQ before sowing recorded slight decrement in dry matter and inulin. Whereas, a gradual decrease in dry matter and inulin content was shown in control treatment, and reached the maximum values at the end of storage period. Tubers obtained from AM+HQ before sowing could help in preservation during storage, although weight loss and decay of tuber were influenced by storage period. Data collected from this study suggest that enhancement of plant growth and tuber quality, especially by AM+HQ application, resulted in reasonably increment in storability parameters of the tubers. Vigorous growth during the growing season may affect tuber storability. Reduction in dry matter during the last period of storage might be attributed to higher rate of sugar loss through respiration than the water loss through transpiration (Gathungu *et al*., 2013). Ben Chekroun *et al*. (1996) reported that the dry matter of JA tubers maintained for the first 7 weeks of cold storage beyond this period, a decrease trend takes place until the end of the storage. Saengthongpinit and Sajjaanantakul (2005) stated that long term storage of JA tubers would inevitably affect inulin composition, *i.e.* degradation to shorter chains. The pronounced worst storability of control tubers included progressive sprouting, weight loss and decay incidence, probably due to the same previously mentioned considerations, thus, it can be suggested that the control tubers had less or no capabilities to protect and preserve against such stressful effects (Ezzat, *et al*., 2011). So, the proposed application of (AM+HQ) during field phase may improve tuber quality, by reducing the respiration process during storage period, and leading to better storability of the tubers.

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