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Improving Growth and Productivity as well as Controlling *Sclerotium rolfsii* in Jerusalem Artichoke using Biotic and Abiotic Agents

Abdul Aziz A. Al-Askar[1], Khalid M. Ghoneem[2], Abd El-Badea S. Ezzat[3] and Wesam I.A. Saber[4]

[1]Department of Botany and Microbiology, Faculty of Science, King Saud University, Saudi Arabia.
[2]Department of Seed Pathology Research, Plant Pathology Res. Institute,Agric. Res. Center, Giza, Egypt.
[3]Department of Vegetable Research, Hort. Res. Institute, Agric. Res. Center, Giza, Egypt.
[4]Department of Microbiology, Soils, Water and Environment Research Institute, Agric. Res. Center, Giza, Egypt.

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Sclerotium stem and tuber rot diseases caused by *Sclerotium rolfsii* is a significant problem facing the Jerusalem artichoke cultivation. The objective of this study was to evaluate the efficacy of biotic and a biotic agents in control of stem and tuber rots of Jerusalem artichoke. For this purpose, *Trichoderma atroviride* and *T. reesei* were isolated from rhizosphere of healthy Jerusalem artichoke plants. These isolates positively antagonized *S. rolfsii*. Arbuscular mycorrhiza, mixture of the previous *Trichoderma* species and/or hydroquinone were evaluated under greenhouse and field conditions against stem and tuber rots. The treatments of arbuscular mycorrhiza+ hydroquinone and arbuscular mycorrhiza + *Trichoderma* minimized the disease incidence, improved the plant growth and yield and enhanced the tuber quality of Jerusalem artichoke. These data encourage the incorporation of such agents in the rots control and the production strategies of Jerusalem artichoke.

Key words: *Sclerotium rolfsii*, Mycorrhiza, *Trichoderma* spp., Hydroquinone, *Helianthus tuberosus*.

Helianthus tuberosus L. (Asteraceae), known as Jerusalem artichoke is an important non-traditional tuberous crop, which recently introduced to Egypt for its high nutritional and medicinal values. Because of its high content of inulin, the plant tubers are currently used for production of healthy food, since, inulin can prevents obesity, enhances immunity, reduces blood cholesterol and the risk of insulin-dependent diabetes mellitus (type 2) and heart disease[1]. Jerusalem artichoke is also used to produce a variety of products such as animal feed[2], and bioethanol[3]. Jerusalem artichoke originated in North America[4] but can also be grown commercially in the tropics[5]. In such regions, the high temperature and humidity conditions raise the severity of stem rot disease caused by *S. rolfsii* causing losses up to 60 % [6,7]. These climatic conditions are being exist during August and September in Egypt, which limiting the production of the plant.

Various methods for management of *S. rolfsii* have been investigated on Jerusalem artichoke plants; however, these methods are not completely effective. Although some chemical fungicides are commonly used successfully for controlling stem rot disease of Jerusalem

* To whom all correspondence should be addressed.
E-mail: aalaskara@ksu.edu.sa

artichoke[8], their field application may not always be desirable. In recent years, several antagonistic organisms have been successfully used as biocontrol agents for controlling soil-borne pathogens and post-harvest diseases of fruits[9]. Many of these antagonists are soil inhabitants. Several strains of *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants. The mode of action includes antibiosis, parasitism, inducing host-plant resistance and competition. Biological control using the arbuscular mycorrhizal fungi (AM) has special significance being an ecofriendly and cost-effective strategy for disease management, in addition to the positive effects on the plant growth and nutrition. Several researchers have studied the application of *Glomus mosseae*, *G. intraradices*, *G. clarum*, *Gigaspora gigantea*, and *G. margarita* on various crops. It was found that they 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[10, 11, 12, 9].

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[13-15].

The objective of this study was to: 1) isolate *S. rolfsii* that responsible for Jerusalem artichoke stem and tuber rots in Egypt, 2) evaluate the efficacy of AM, *Trichoderma* mixture and hydroquinone individually and in combinations in controlling stem and tuber rots diseases under greenhouse and field conditions and 3) study their role in enhancing growth, physiological activities, yield and improving tuber quality.

MATERIALS AND METHODS

Chemicals and Jerusalem artichoke tubers

Hydroqinone was obtained from Sigma Chemicals Co., USA. Benzothiadiazole (Benzo-(1, 2, 3) thiadiazole-7-carbothioic acid S-methyl ester wetta-ble granule 50% WG, Bion^R), was used in this experiment as positive control. Jerusalem artichoke tubers cultivar; Fuseau was obtained from Baramoon Horticulture Research Station, Dakahlia, Egypt.

Sampling

Soil and plant samples collected from different Jerusalem artichoke (JA) cultivated field sites including Dakahlia, Damietta, Beheira and Giza governorates, Egypt, were used in this study. At each study site, an area of 500 m^2 was chosen for sampling. Six diseased plants (exhibited typical symptoms of stem and tuber rots) were selected, and their stems and tubers samples were collected. Approximately, 2 kg of rhizosphere soil of healthy Jerusalem artichoke plants was collected in triplicates from each study site, and the samples were brought to Tag El-Ezz Research Station laboratory in sealed plastic bags, and stored at 4 ºC until use.

Isolation and identification of the pathogens and *Trichoderma* of JA

Rotten tubers and stems were separately washed, surface-disinfected for 3 min in 0.5% (v/v) sodium hypocholorite, rinsed by sterilized water, and sections (1 cm) were placed on potato dextrose agar (PDA) plates (Difco, USA), supplemented with antibacterial agent (L-chloramphenicol; 5mg/L and streptomycin sulphate; 5mg/L) and incubated at 25°C for 5-7 days. Hyphal tip or single spore technique was used to purify and obtaining pure cultures. The recovered isolates were then transferred into slant of potato carrot agar and kept at 4 °C for further studies. The isolated fungi were identified according to their cultural, morphological and microscopical characteristics as described by Booth[16], Domsch *et al.*,[17] and Watanable[18].

After isolation, the pathogenicity test was carried out following Koch postulates to determine the most aggressive pathogen, accordingly, *S. rolfsii* was found to be the main causal pathogen of stem and tuber rots, which was subjected to further investigations.

The collected rhizospheric soil samples were used to isolate *Trichoderma* species using a selective medium of Elad *et al.*,[19]. The developed colonies were transferred onto PDA slants and identified after growing them on malt extract agar for two days at 25 °C according to Bissett[20] and Kubicek and Harman[21].

Antagonistic activity and inoculum preparation of *Trichoderma* spp.

The antagonistic activity of ten isolates of *Trichoderma* spp. was carried out against *S. rolfsii* on PDA plates. The growth of the fungi

were recorded and interaction between both mycelia scored for degree of antagonism reaction using a scale of 1 to 5 [22], where 1= *Trichoderma* overgrowing pathogen and 5= pathogen overgrowing *Trichoderma*. *S. rolfsii* developed from plates of dual cultures were then microscopically investigated and the changes in the mycelium of the pathogen were recorded.

According to the results of the dual culture test, three *Trichoderma* strains were selected, and tested for possible antagonism between each other. A mixture consisted of spores of *T. atroviride* (Ta1 and Ta2) and *T. reesei* (Tr1) in equal proportions were used. The inoculum was prepared by growing each of antagonistic *Trichoderma* strain in bottles 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 of AM

A mixture of multi-arbuscular mycorrhizal fungi, kindly provided by Prof. Safwat El-Haddad, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt, was used. This mixture consists of equal proportions of spores of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *Glomus intraradices* Schenck & Smith, *Glomus clarum* Nicol. & Schenck, *Gigaspora gigantean* (Nicol. & Gerd.) Gerd. & Trappe, and *Gigaspora margarita* (Becker & Hall) in suspension form at concentration of 10^6 unit L^{-1}.

Mass production of AM inoculum was carried out using the pot culture technique and Sudan grass as a host plant[9]. Spores of the previous formula were inoculated on surface-sterilized (10% sodium hypochlorite for 30 min) Sudan grass seeds, which were sown in plastic pots (40 cm diameter) containing twice-sterilized sandy loam soil. The plants were grown in greenhouse (25-30 ºC) with a transparent plastic roof and open sides. No fertilizer or any chemical was applied to the soil. Thirty days after planting Sudan grass in the pot culture, the plants were cut above the soil surface and the soil was allowed to dry out in the pot, then crushed by hand and used as AM inoculum.

Greenhouse Experiment

The study was under taken during August to October 2011 at Tag El-Ezz Research Station, Dakahlia, Egypt. *S. rolfsii* was cultured on PDA plate 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 for 10 days; the inoculum was then ready to use.

A healthy-looking tubers of Jerusalem artichoke with 3 to 4 active buds, were incubated for one week in a moist peat moss to facilitate germination under open-side greenhouse and regularly watered. 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 fungi. Apparently healthy tubers were surface sterilized in 1% sodium hypochloride followed by soaking in BTH (1 g/1000 ml) (as a positive control) or HQ (20 mM) for two hours and then dried using sterilized paper. The negative control treatment was prepared by soaking healthy tubers in sterilized water. Two tubers were planted in each pot, one week after inoculation with *S. rolfsii*. Pots were regularly watered to near field capacity with tap water.

The treatments applied in this study were; 1) control, (2) BTH, (3) *T*, (4) AM, (5) HQ, (6) *T*+AM, (7) *T*+HQ and (8) AM+HQ. Pots that were inoculated with AM and/or *T* received 40 g of the inoculum per pot, as seed-bed before planting. In the case of infection, *S. rolfsii* was used to infest another group containing the same previous treatments. All pots were kept under greenhouse conditions with average temperature ranged from 20 to 25 °C.

Disease assessment and biochemical testes under greenhouse conditions

The disease severity of the tubers was rated after 30 days of sowing, while stem rot (defined as wilting of all leaves on a plant) was followed up by observing the plants daily until 90th day after sowing. The 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, the total phenol content was determined using Folin

Ciocalteau reagent according to the method described by Maliak and Singh[23], whereas, extraction and assay of polyphenoloxidase (PPO) and peroxidase (POD) were carried out according to the methods described by Maria *et al*.[24] and Maxwell and Bateman[25], respectively.

Field Experiment

The experiments were carried out under field conditions at Baramoon, Agric. Res. Station, Dakahlia, Egypt during 2012 and 2013 summer seasons. Soil is clay loam in texture containing 41.65 % clay, 31.9%silt and 26.45% sand. The EC dSm^{-1} in soil past = 0.85. pH in water suspension (1:2.5) = 7.9, organic matter = 1.3% and $CaCO_3$ = 3.1%. Available N, P and K were 35, 11.65 and 309mg kg^{-1}, respectively. The average temperature of the two seasons ranged between a minimum of 12.5°C and a maximum of 37.3°C. The tubers were used as seeds, within the weight range of 20 to 25 g each. Sown was on April, 1st in both seasons.

The previous treatments in greenhouse experiments were applied under field conditions, to study their effect on the incidence of tuber and stem rots, under natural infection as well as, on physiological aspects, growth and yield of JA plants.

Plots (each 3×6 m^2) were ploughed well, all weeds were removed, and soil was leveled, dissected to lines. Ammonium nitrate (33.5%, N), mono-superphosphate (15.5%, P_2O_5) and potassium sulphate (48%, K_2O) were applied at recommended doses. 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.

Monitoring of disease incidence, tuber yield and tuber quality

The disease incidence values of JA tuber and stem rots were assessed at 40 and up to 160 days from sowing for pre-and post-emergence damping-off, respectively. At flower initiation stage (120 days after planting), three JA plants were randomly selected and plant height and shoot fresh and dry weights $plant^{-1}$ was determined. The leaf area $plant^{-1}$was determined according to Koller[26]. Chlorophyll content was estimated in the leaves using a Minolta SPAD chlorophyll Meter as described by Yadava[27].

Tubers were harvested on October, 1st in both seasons; the total tuber yield was recorded. Marketable yield was recorded using good shapes healthy tubers weighted more than or equal to 40 g. Unmarketable yield was estimated by weight of yield of culls (off-shape, blemished, green, and diseased) and less than 40 g.

A weight of 100 grams of fresh tubers was oven dried at 70°C till a constant weight and ground to a fine powder with electric grinder. The dried ground tubers was used for the determination of tuber dry matter percent, inulin content by the method of Winton and Winton[28], protein according to Robinson[29] and total carbohydrate following the method of Dubois *et al*.[30].

Statistical analysis

Data were analyzed with the statistical analysis software CoStat (version 6.4), by the one-way completely randomized blocks design. Duncan's multiple range test was used to compare means at probability (*P*) level ≤ 0.01 or 0.05.

RESULTS AND DISCUSSION

Isolation of pathogenic fungi

Concerning the isolation trials (Fig. 1), 21 fungi belonging to 14 genera were isolated from infected JA tubers. The primary screening of the isolated fungi shows that there were significant differences in the frequency of the native fungi over the infected tubers. Some of them were found in high frequencies (*S. rolfsii*, 67.9% and *R. solani*, 46.0%) and others were present in low frequencies (*F. moniliforme*, 3.2% and *Trichoderma* spp., 2.0 %). Under the given environmental conditions, the pathogenic fungus; *S. sclerotiorum* recorded moderate frequency. Among isolated *Fusarium* spp., *F. rosum* and *F. oxysporum* was most common. Anther isolates including *Alternaia* sp., *Trichoderma* sp. and *Oedocephalum* sp. were recorded.

Isolation trails from rotten Jerusalem artichoke tuber by McCarter and Kays[8] showed that *Sclerotium rolfsii* was the most important pathogens. Also, AbdAl-Aziz *et al*.,[31] isolated 24 fungi belonging to more than five genera from rotten Jerusalem artichoke tubers, *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium* and *Trichoderma* were the most common genera, they added that most of these fungi are plant pathogens and cause several diseases.

The pathogenicity test was carried out on the different isolates to select the most aggressive strain causing the ideal symptoms of rots. The results declare that *S. rolfsii*, was the causative pathogen (data not shown). So, this fungus was selected for further investigation.

Isolation of *Trichoderma* and antagonism with *S. rolfsii*

Ten *Trichoderma* species were isolated from rhizosphere soil samples of different districts

Table 1. Growth of *S. rolfesii* as affected by *Trichoderma* spp. in dual culture test

Trichoderma isolate	Growth of *S. rolfesii* (%)*		Antagonism
	3 day	5 day	reaction**
T. atroviride Ta1	24.3 bc	40.0 de	1
T. atroviride Ta2	20.5 c	38.0 e	2
T. harizanum Th1	35.9 ab	58.0 a	3
T. harizanum Th2	32.4 ab	52.7 ab	3
T. harizanum Th3	36.8 a	46.3 b-e	3
T. koninige Tk2	32.2 ab	50.1 a-c	3
*T. reesei*Tr1	28.6 a-c	43.4 c-e	2
T. viride Tv1	37.9 a	48.2 b-d	2
T. viride Tv2	33.0 ab	54.0 ab	3
T. viride Tv3	32.4 ab	54.0 ab	3

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

*Growth of *S. rolfsii*(%) = $\frac{\text{Radius growth of } S.\ rolfsii \text{ in the direction } Trichoderma}{\text{Radius of growth in the absence of } Trichoderma} \times 100$

**The antagonism reactions of *Trichoderma* with *S. rolfsii*was recorded based on the antagonism scale of Bell *et al*. [22] after the 5th day of dual growth, using a scale of 1 to 5, where 1= *Trichoderma* overgrowing *S. rolfsii* and 5= *S. rolfsii* over growing *Trichoderma*.

Table 2. Disease parameters of JA as affected by the tested treatments under greenhouse conditions

Treatment		Tuber	Stem	Survivals
Pathogen	Biotic and/or abiotic	rot, %	rot, %	%
Control (none)	None	1.4 c	1.4 d	97.2 a
	BTH	1.4 c	0.0 d	98.6 a
	T	1.4 c	0.0 d	98.6 a
	AM	1.4 c	1.4 d	97.2 a
	HQ	0.0 c	0.0 d	100.0 a
	T+AM	1.4 c	0.0 d	98.6 a
	T+HQ	0.0 c	0.0 d	100.0 a
	AM+HQ	0.0 c	0.0 d	100.0 a
S. rolfsii	None	40.3 a	22.2 a	32.0 d
	BTH	26.4 b	16.7 a-c	57.0 bc
	T	27.8 b	16.7 a-c	55.6 bc
	AM	30.6 ab	20.8 ab	48.6 c
	HQ	22.2 b	16.7 a-c	61.1 bc
	T+AM	22.2 b	11.1 c	66.7 b
	T+HQ	27.8 b	13.9 bc	58.3 bc
	AM+HQ	20.8 b	9.7 c	69.5 b

Different letters within a column indicate significant difference ($P \leq 0.05$).

of study sites. Five species of *Trichoderma* viz., *T. atroviride*, *T. harzianum*, *T. koninige*, *T. reesei* and *T. viride* were identified. *Trichoderma* is a genus of fungi that is present in all soils, where they are the most prevalent cultivable fungi. That is why, all *Trichoderma* isolates were tested against the previous three aggressive pathogens. The advantage of isolation of antagonistic fungi from nature habitats over the genetically manipulated or ones that have been isolated from a different environmental set-up is the easier adaptation and succession when incorporated into biological control program, such isolate can be effectively used for controlling several diseases.

In dual culture test, 10 *Trichoderma* isolates were tested, of them; three *Trichoderma* isolates strongly inhibited the growth of *S. rolfsii* (Table 1).The highest reduction in the growth of *S. rolfsii* (60.0, 62.0 and 56.6 %) were recorded by *T. atroviride* Ta1, *T. atroviride* Ta2 and *T. reesei* Tr1, respectively. *T. atroviride* (Ta1 and Ta2) and *T. reesei* Tr1 showed high degree of antagonism reaction after five days of dual culturing. The best antagonism reaction (1) was recorded by *T. atroviride* Ta1 on *S. rolfsii*, which means the occurrence of strong mycoparasitism. Plates were then, microscopically investigated for testing the overgrowth. The light microscope 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 species of *Trichoderma* (Ta1, Ta1 and Tr1) were found to produce inhibition halos and sporulated over the colonies of *S. rolfsii*, with different degree. Melo and Faull[32] recorded similar observations. Once the fungal pathogen come into contact, *Trichoderma* spp. attach to and can coil around it. In some cases, form appressoria on the host surface, wherein *Trichoderma* produce several cell wall degrading enzymes and probably 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[32, 33].

It was found to use a mixture of *T. atroviride* Ta1, *T. atroviride* Ta1 and *T. reesei* Tr1, therefore, the antagonism among the three *Trichoderma* strains was clarified. However, no visible antagonism could be observed. On contrary, a clear compatibility had been confirmed. Hence, the triple inoculation of *Trichoderma* was used as a mixture (*T*).

Table 3. Physiological characteristics of JA as affected by the tested treatments under greenhouse conditions

Treatment		Total phenol	Polyphenoloxidase	Peroxidase
Pathogen	Biotic and/or abiotic	(mg catechol g^{-1} fresh weight)	($Unit^{-1}$ min. g^{-1} fresh wt.)	($Unit^{-1}$ min. g^{-1} fresh wt.)
Control (none)	None	0.305 d	0.87 b-e	1.37 b-d
	BTH	0.325 d	1.13 a-d	3.07 a
	T	0.325 d	1.73 a	1.37 b-d
	AM	0.319 d	0.93 b-e	1.20 b-d
	HQ	0.312 d	1.23 a-c	0.93 cd
	T+AM	0.285 d	0.80 b-e	1.90 bc
	T+HQ	0.325 d	0.70 b-f	1.20 b-d
	AM+HQ	0.412 b	1.27 ab	1.13 cd
S. rolfsii	None	0.292 d	0.47 d-f	1.33 b-d
	BTH	0.339 cd	0.53 b-f	0.67 d
	T	0.332 d	0.33 ef	2.13 b
	AM	0.339 cd	0.87 b-e	1.50 b-d
	HQ	0.652 a	0.57 b-f	1.30 b-d
	T+AM	0.405 bc	0.03 f	1.03 cd
	T+HQ	0.412 b	0.63 b-f	0.53 d
	AM+HQ	0.592 a	0.50 c-f	1.20 b-d

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

Table 4. Vegetative growth and tuber dry matter of JA as affected by the tested applications in 2012 and 2013 seasons

Biotic and/or abiotic	Plant height (cm)		Shoot fresh weight plant^{-1} (g)		Shoot dry weight plant^{-1} (g)		Leaf area plant^{-1} (m^2)		Chlorophyll content in leaves (SCMR)		Dry matter of tuber (%)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
None	190.3 e	210.3 f	1982 e	2028 d	500.2 g	471.3 f	3.06 e	2.90 f	28.8 e	28.8 c	17.35 e	17.16 e
BTH	210.7 d	200.7 ef	2120 c	1966 e	518.4 fg	462.8 f	3.07 e	2.76 g	30.7 e	28.6 c	19.00 c-e	18.04 de
T	225.3 cd	223.0 de	2036 d	2143 c	527.1 ef	496.8 e	3.18 d	3.08 e	33.2 de	30.4 c	18.46 de	19.23 c-e
AM	230.0 bc	233.7 cd	2218 b	2231 b	560.2 cd	536.3 cd	3.58 c	3.19 d	38.6 bc	30.6 c	20.07 b-d	20.83 a-c
HQ	227.7 bc	230.3 cd	2163 c	2167 c	542.3 de	518.4 d	3.62 c	3.10 de	36.4 cd	34.8 b	19.21 c-e	19.90 b-d
T+AM	243.7 ab	251.3 ab	2411 a	2384 a	586.2 b	573.7 b	3.85 b	3.64 b	42.3 ab	38.8 a	21.76 ab	22.510 a
T+HQ	235.3 bc	240.7 bc	2390 a	2250 b	575.7 bc	553.0 c	3.81 b	3.40 c	40.3 a-c	36.8 ab	20.88 bc	21.30 a-c
AM+HQ	252.0 a	260.0 a	2418 a	2382 a	610.9 a	596.3 a	3.94 a	3.80 a	44.8 a	38.6 a	23.24 a	21.81 ab

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

Table 5. Yield and its components as well as quality of JA tubers as affected by the tested applications in 2012 and 2013 seasons

Biotic and/or abiotic	Tuber yield (ton fed.$^{-1}$)						Total carbohydrates (mg g^{-1} dry weight)		Protein (%, dry matter)		Inulin (mg g^{-1} dry weight)	
	Marketable		Unmarketable		Total							
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
None	17.35 e	17.16 e	2.19 a	2.12 a	19.54 d	19.28 d	41.70 e	36.86 g	12.08 a	10.21 f	11.92 c	12.76 d
BTH	19.00 c-e	18.04 de	1.17 cd	1.72 b	20.17 cd	19.76 cd	40.65 f	38.00 f	12.10 a	11.34 d	12.08 c	13.50 c
T	18.46 de	19.23 c-e	1.35 b	1.33 c	19.81 d	20.57 b-d	43.11 c	39.76 e	12.14 a	10.88 e	12.30 c	13.00 d
AM	20.07 b-d	20.83 abc	1.31 bc	1.13 cd	21.38 b-d	21.96 a-c	42.28 d	40.73 d	12.38 a	11.76 c	13.10 b	13.81 bc
HQ	19.21 c-e	19.90 b-d	1.35 b	1.14 cd	20.56 cd	21.04 a-d	42.03 de	38.11 f	12.16 a	12.08 bc	12.36 b	14.03 ab
T+AM	21.76 ab	22.51 a	1.41 b	0.94 d	23.17 ab	23.45 a	45.60 a	42.18 c	12.80 a	12.56 a	14.22 a	14.42 a
T+HQ	20.88 bc	21.30 a-c	1.27 bc	1.12 d	22.15 abc	22.42 ab	44.43 b	44.80 a	12.67 a	12.21 ab	13.90 a	14.11 ab
AM+HQ	23.24 a	21.81 ab	1.03 d	1.05 d	24.27 a	22.91 ab	44.82 b	43.76 b	12.80 a	12.38 ab	14.30 a	14.26 ab

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

Biocontrol capacity of T, AM and/or HQ in greenhouse

S. rolfsii was tested against individual or combination of *T*, AM and/or HQ under greenhouse conditions. In addition, plants inoculated with AM were investigated to insure the succession of mycorrhizal infection, which was confirmed on the roots of the inoculated JA tubers.

Data in Table (2) indicate that, considerable difference in the disease incidence and severity symptoms on JA seedlings was observed on the various treatments. The control treatment (*S. rolfsii*) showed the ideal disease severity of tuber (40.3 %) and stem (22.2 %) rots. The dual inoculation of the tubers by AM+HQ or *T*+AM were the most effective treatments in reducing symptoms of tuber and stem rots incidence on JA seedlings caused by *S. rolfsii* as compared to inoculation by *S. rolfsii* only. On the other hand, the single inoculation with *T*, AM or BTH recorded lower efficiency on disease incidence.

Leta and Selvaraj[12] reported that a greater suppressive effect on white rot (*Sclerotium cepivorum* Berk) of onion bulbs observed in plants inoculated with combination of AM (*Glomus aggregatun* or *G. aggregatum*) plus *Trichoderma* (*T. harzianum* or *T. viride*), than single inoculation. These results was attitude by working of Sennoi *et al*.,[9] who reported that combination of *T. harzianum* and *G. clarum* in JA was most effective in controlling southern stem rot caused by *S. rolfsii*. This may be due to synergistic effects of *T*, and AM in suppression of the fungal pathogen. Synergism between *T* and AM could be due stimulation of spore production in AM[34]. Gryndler[35] documented that saprophytic fungi could stimulate the growth of *Glomus mosseae* mycelium, and subsequent formation of small vegetative spores on mycelium was induced. Another mechanism

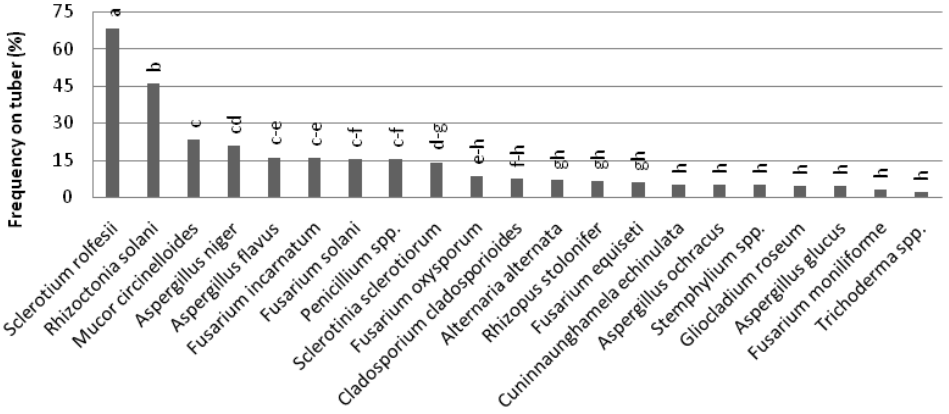

Fig. 1. Frequency of the different isolated fungi on JA tuber. Columns designated with different letter(s) differ significantly($P \leq 0.05$)

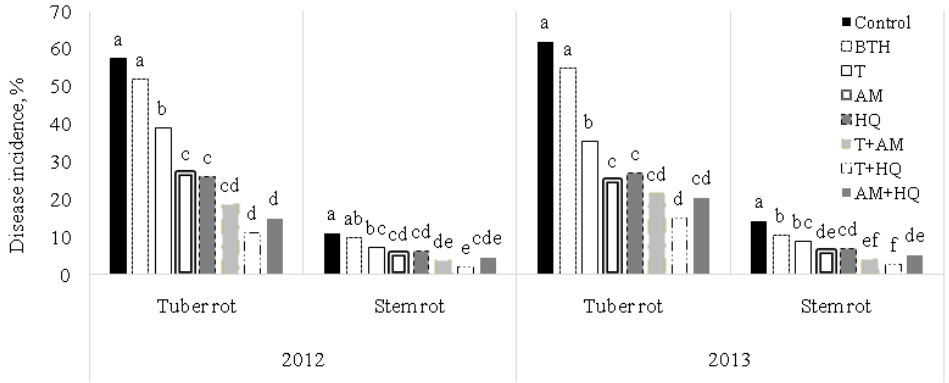

Fig. 2. Disease incidence of JA as affected by the tested treatments under field conditions during two growing seasons. Columns of each group superscripted by the same letter (s) do not significantly differ ($P \leq 0.05$)

suggesting enhanced resistance and/or tolerance of plants against the pathogen[9]. Strullu and Plenchette[36] reported that the symbiosis between mycorrhizae and roots of many crops had a positive influence on the plant's nutrition and on protection against some diseases. Mycorrhizae may raise plant tolerance to pathogens by enhancement of plant nutrition, competition for nutrients and infection sites, and changes in root morphology.

Hydroquinone recorded positive effect on disease parameters

HQ is synthesized naturally in the leaves, bark and fruit of a number of plants, especially the ericaceous shrubs such as cranberry, cowberry, bearberry and blueberry, and was reported to be a potential inhibitor for some seed-borne pathogenic fungi[13, 14].

Physiological evaluation of JA in greenhouse

Total phenol, PPO and POD are physiological parameter reflecting the health condition of plant. Data of physiological profile of JA plants as affected by the tested treatments under pathogen stress are presented in Table (3). The total phenol was induced in the treated plants even in the presence of the pathogen. In this respect, HQ, AM+HQ, *T*+HQ and *T*+AM were the most inducers for total phenol in the pants infected with pathogen. However, inoculation of the control plants with either *T* or treatment with BTH significantly induced PPO or POD, respectively, compared with all treatments.

Total phenols and PPO play important role in plant protection. The first step of the defense mechanism in plants involves a rapid accumulation of phenols at the infection site, which restricts or slows the growth of the pathogen because of its action as antioxidant, antimicrobial, and photoreceptor[37, 38]. The suggested mechanisms for the pathogen defense role of PPO, include; (1) general toxicity of PPO-generated quinones to pathogens[2] alkylation and reduced bioavailability of cellular proteins to the pathogen, (3) cross-linking of quinones with protein or other phenolics, forming a physical barrier to pathogens in the cell wall, and[4] quinone redox cycling leading to H_2O_2 and other reactive oxygen species, which are known to be important factors in plant pathogen interactions and defense signaling[39, 40]. That is why the levels of both total phenols and PPO are naturally high in resistant varieties[40].

The present data reveal that POD activity in the infected plants did not record marked variation. Conversely, Janki *et al.*,[41] reported that, in case of plant infection, there was immediate response by plants increasing PPO 5-fold on the first day, which starts decreasing from the 6th day indicating multiplication of fungus in the plant system. In this connection, Donald and Cipollini[42] suggested that POD activity is probably induced in plants to some degree at all times in the field due to the ubiquitous nature of such sources of mechanical perturbation as wind, rain, and the effect of gravity on plant tissues. However, the level of POD induction is likely to vary with the degree of exposure of plants to such mechanical perturbation as chronic winds of varying intensity. The roles that POD can play in cell wall toughening and in the production of toxic secondary metabolites and its simultaneous oxidant and antioxidant capabilities can make it an important factor in the integrated defense response of plants to a variety of stresses[42, 41].

***In vivo* evaluation of disease incidence**

The previous promising evidences of the greenhouse results encourage the performing of the next field trials to evaluate the proposed treatments under natural conditions. As depicted in Fig. (2), most treatments, (except BTH treatment) significantly reduced the incidence of tuber and stem rots of JA plants during the two successive seasons. The combination of HQ with *T* or AM was the most effective treatments, which reduced disease incidence in plants by more than 80.8, 71.5 % and 77.1, 66.3%, respectively in first and second seasons.

The site(s) and number of hydroxyl groups on the phenol compounds *e.g.*, hydroquinone are thought to be related to their 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[43]. 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. The main mode of protection is by scavenging oxygen-derived species or minimizing the formation of oxygen-derived species[44]. Elwakil[13] found that HQ not only

inhibited the seed-borne fungi but also improved the growth of peanut and raised the yield by up to 50 %.Also, Al-Askar *et al*.[14] reported that soaking alfalfa seeds in 12 mM HQ reduced seed rot and seedling mortality under greenhouse conditions. Sennoi *et al*.,[9] found that AM (*G. clarum*) alone gave better control of *S. rolfsii* on JA than did inoculation with *T. harzianum* alone.

It is of especial important to mention that the typical symptoms of rot diseases appeared on control plants, sclerotial stem rot symptoms appeared mainly at initiation stage of flowering and tubers production (90 to 120 days of sowing). Infected plants first showed a moist decay at or slightly below the soil surface, where infection initiated. Stem lesions expand up and down the stem, leading to 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, forming sclerotia (1 to 2 mm in diameter). Infected tubers (typically through stolons) showed profuse growth of robust white mycelium that caused a white to light brown soft rot, sometimes involving the entire cluster of tubers on a plant. The fungus quickly grew over the tuber surface and invaded, resulting in a moist cheesy decay. Portions of infected plant parts and nearby soil often were covered with the white, radiating mycelium of *S. rolfsii*.

***In vivo* evaluation of vegetative growth and tuber dry matter**

The plant height in 2012 and 2013 seasons, reached the maximum values of 252 and 260 cm, respectively, when AM+HQ were applied (Table 4). Additionally, inocluaton with AM combined with HQ or *T*. had significant effect on shoot fresh and dry weights per plant, leaf area, chlorophyll content and tuber dry matter, in comparison with the other treatments. This was true in both seasons of study.

The pronounced positive effects on the vegetative growth parameters of plants may be attribute to the fact that plants under inoculation with AM increased utilization of water and nutrients, particularly phosphorus, and that in turn, enhanced the vegetative growth and tuber dry matter. AM take up a significant fraction of all plant photosynthetically fixed carbon, while the mycorrhizal plant obtains nutrients, such as inorganic phosphate via the AM hyphae. The inoculation with AM can improve plant growth and biomass accumulation of bioenergy crops (*Galega orientalis* and *Helianthus tuberosus)* even in non-sterile soil containing naturally occurring AM, that is why more than 80% of vascular plant families are capable of forming the AM symbiosis[45].

The increment in chlorophyll content, which is a good parameter reflecting the health condition of plant, is by enhancing the efficacy of photosynthetic apparatus with a better potential for disease resistance and decrease in photophosphorylation rate usually occurring after infection[46]. 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[4].

In vivo evaluation: yield and its attributes as well as tuber quality

Considerable yield losses of control plants was observed, which caused by tuber and stem rots. Most of these losses returned to tuber rot phase by soil-borne pathogens. Inoculations with AM combined with HQ or *T*, significantly influenced marketable tuber yield and total tuber yield (Table 5), followed by *T*+HQ and sole inoculation with AM. Highest tuber yield (24.27 and 22.91 ton fed^{-1}), in both seasons, was obtained from application of AM+HQ recording 24.2 and 18.8 % increases over the control, respectively.

Tuber quality appeared to respond to seed tuber inoculation in a similar manner to total yield. Total tuber yield increase was due to primarily the increase in marketable tuber yield in larger grade and decrease of the unmarketable yield (Table 5) as well as the remarked improvement in vegetative growth characters and chlorophyll content (Table 4). Total carbohydrates, inulin content and protein of Jerusalem artichokeare are shown in Table 5. The application of *T*+AM produced the highest contents of total carbohydrates (1st season), inulin content and protein (both seasons). However, the difference between the former treatments and inoculation with AM+HQ was not reach the level of significance.

The tested treatment had great impact in decreasing root and stem rots of JA, hence increasing survivals under field conditions (Fig. 2). Mycorrhiza inoculation significantly improved

almost all measured parameters, *i.e.*, plant height, shoot fresh and dry weights, root fresh and dry weights and tuber yield as well as phosphorus content of many plants[47]. Pathologically, AM significantly decreased disease incidence and severity compared to *Rhizoctonia*-infected plants. This could be due to the direct antagonistic effect of AM on the pathogen and/or the improvement of the growth conditions of the host plant. Generally, application of AM improved the plant growth by its direct effect on improving the internal status of the plants or indirectly by reducing the harmful effect of the pathogen.

Production program of Jerusalem artichoke in Egypt is facing the problem of rot diseases, which is more severe at the end of production period (August and September). Using chemicals to control such diseases is discouraged owing to their toxic effects on non-target organisms, the undesirable changes they inflict upon the environment and due to development of resistant strains of pathogens against various chemical fungicides. Anyhow, rot diseases problem is limiting the commercial production as well as reducing the yield and quality of JA. There are several individual methods to achieve the target of the present investigation, but none of them introduces integrated solution. The present investigation is an integrated solution through the use of AM+HQ or AM+*T* for both controlling the stem and tuber rots and minimize the use of fungicides, as well as improving growth, physiological activities, yield and tuber quality.

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