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Arbuscular mycorrhiza and environmentally biochemicals enhance the nutritional status of *Helianthus tuberosus* and induce its resistance against *Sclerotium rolfsii*

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ABSTRACT

Chemical fungicides are effective tools in controlling plant pathogens; however, these chemicals can, on the other hand, distress the ecosystem. Accordingly, the current research investigates the potentiality of substituting traditional chemical fungicides by inducing plant resistance against infection with soil-born pathogens i.e. *Sclerotium rolfsii* in the presence of mycorrhizae (AMF) as plant inoculants and one of the following amendments: humic acid, sulphex (a mixture of canola oil and diluted sulphuric acid) and paclobutrazol (ABZ). To attain the abovementioned objective, a field (mildly infected with *S. rolfsii*) was cultivated with *Helianthus tuberosus* (a perennial plant belongs to the Asteraceae family) for two successive seasons (2014 and 2015) and the abovementioned treatments were tested for their feasibilities in controlling *S. rolfsii* infection against the chemical fungicide "Vitavax-200" either solely or in combinations in a complete randomized block design. Inoculating plants with AMF or amending soils with either humic acid, Sulphex or ABZ solely increased significantly the activities of plant defense enzymes by approximately 1.5–2.1 folds higher than the control treatment. These treatments also improved NPK availability in soil and; hence, increased their contents within plant tubers. Consequently, these treatments decreased the disease incidence and severity caused by *S. rolfsii* while improved shoot biomass and tuber yield. In spite of that, these results stood below the prospective of the fungicide treatment. The integrated treatments i.e. "humic acid + AMF", "Sulphex + AMF" and "ABZ + AMF" caused further significant improvements in both NPK availabilities in soil and plant areal bio-masses. This probably induced further plant resistance against the investigated soil-borne pathogen while recorded insignificant variations in disease incidence and severity when compared with the fungicide treatment. Moreover, the integrated treatments increased the tuber yields beyond those attained for the fungicide treatment. Accordingly, such integrated strategies can completely substitute the chemical fungicides; thus, minimize their negative impacts on the ecosystem.

1. Introduction

The Jerusalem artichoke (*Helianthus tuberosus*) is a perennial plant (Mehmood et al., 2019) that belongs to the Asteraceae family (Jantaharn et al., 2018; Zhong et al., 2019). Its tubers are rich in inulin, natural prebiotic (Nizioł-Łukaszewska et al., 2010; Lv et al., 2019) and phenolic compounds (Díaz et al., 2019); besides, being good sources of low energy diet (Knudsen and Hessov, 1995). Thus, these tubers are recommended as substitutes for potatoes (Mehmood et al., 2019), because their characteristic flavor and functional ingredients are acceptable for food consumption (Takeuchi and Nagashima, 2011). Moreover, the stems of *Jerusalem artichoke* can be used in cellulose production (Prusov et al., 2019) and their waste foliage can be used as bio-fuel (Gao et al., 2019; Mehmood et al., 2019). On the other hand,

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soil borne pathogens may cause considerable losses in the outcome yield of this vegetable crop (Lachman et al., 2008). Probably, chemical fungicides are effective in controlling plant pathogens; however, these chemicals can negatively distress the ecosystem (Dias, 2012; Mohamed et al., 2019). Accordingly, sustainable agriculture using integrated safe strategies has become an obligation to overcome such negative impacts (Mukerji and Ciancio, 2007).

Three approaches of no environmental hazards were considered for controlling soil borne pathogens e.g. *Sclerotium rolfsii*. The first approach depends on inoculating plants with arbuscular mycorrhizal fungi (AMF). Its external hyphae are several times greater than the host roots (Gahan and Schmalenberger, 2014), thereby AMF increases the absorbing surface areas of nutrients uptake (Cooper, 2018). Moreover, AMF can compete with soil borne pathogens on soil nutrients (Brimner and Boland, 2003; Mohamed et al., 2019) besides; AMF has the capability of lignifying plant cell walls (Sharma et al., 2004). Thus, AMF is considered an effective bio-fertilizer and, at the same time, an active bio-control agent that can induce the resistance of grown plants against soil borne pathogens especially *S. rolfsii* (Mohamed et al., 2019).

The second approach counts on improving the nutritional status of the grown plants to increase their resistance against the attack of soil pathogens (Dordas, 2009). In this concern, amending soils with humic acid increases significantly nutrient uptake by plants (Bernstein et al., 2019; Zanin et al., 2019); and also improves some key physico-biochemical attributes (Kaya et al., 2018). Moreover, spraying plants with sulphex (a mixture of canola oil and diluted sulphuric acid) can improve the nutritional status of the grown plants and, therefore, induces the plant resistance against *S. rolfsii* invasion (Eid and Abbas, 2014).

The third approach relies on stimulating the activities of plant defense enzymes. In this concern, peroxidase enzyme catalyzes the formation of reactive oxygen species (ROS) that damage cells of plant pathogens (Bienert et al., 2006). Likewise, polyphenol oxidase (PPO) enzyme suppresses the growth of pathogens (Mohamed et al., 2019). Moreover, it stimulates chitinase enzymes that can break down the cell wall of the soil borne pathogens (Natsir et al., 2002) into mono- and oligomers (Stoykov et al., 2015). It is then thought that the combination between the first and second approaches can promote the nutritional status of the grown plants while activate plant defense enzymes (third approach); hence, induce further plant resistance against soil borne pathogens especially *S. rolfsii*. This pathogen survives in soil for long time periods as sclerotia in absence of the host plant, and therefore its control seems to be very difficult (Mullen, 2001; Cilliers et al., 2003). Thus, introducing efficient safety amendments that are capable of inducing plant resistance against plant pathogens, together with inoculating plants with symbiotic AMF, might effectively control plant infection with pathogens throughout the different stages of plant growth.

Three combinations are considered in the current research. The first one is between humic acid and AMF. In this concern, application of humic acid improves mycorrhizal colonization (Abobaker et al., 2018) or at least slightly affects AMF colonization (Gryndler et al., 2005). Moreover, the cumulative effect of inoculating plants with AMF in presence of humic acid seemed to be superior on plant growth (Shajari et al., 2018) especially under environmental stresses (Naeeni et al., 2018). Moreover, this combination may control plant infection with pathogens e.g. root and stem crown rot in strawberry (Khafagi et al., 2018). The second combination is between sulphex and AMF. According to Casieri et al. (2012), sulphur (a component of sulphex) can stimulate the growth of AMF and probably results in further improvements in controlling soil borne pathogens. The third combination is between AMF and paclopetrazol. Probably paclobutrazol regulates the morphological and physiological characteristics of plant roots (Kamran et al., 2018) which might, in turn, improve root colonization with AMF.

The current research aims at inducing *Jerusalem artichoke* resistance against *S. rolfsii* infection by inoculating plants with AMF in the presence of one of the following safe amendments i.e. humic acid, sulphex and paclobutrazol. The assumptions of this study are: single-type-treatments induce plant resistance against *S. rolfsii* attack and; therefore, minimize the negative consequences of this pathogen on the outcome yield (hypothesis one, H1). The integrated treatments are more efficient in controlling the negative consequences of *S. rolfsii* infection in Jerusalem artichoke than the single ones do (hypothesis two, H2). Moreover, such integrated treatments are thought to simulate the effects of the fungicide "Vitavax-200" on controlling this soil borne pathogen (hypothesis three, H3); thus, minimizing the negative impacts of the synthetic chemicals on soil ecology. To our knowledge, the combination of the biological and chemical approaches for controlling plant infection with *S. rolfsii* is not well investigated. Accordingly, we believe that the results of this study can improve our knowledge about the safe routes for controlling soil borne pathogens.

2. Materials and methods

2.1. Materials of study

The tuber seeds of *Helianthus tuberosus* (the Mammoth French White cultivar) were obtained from Horticulture Department, Faculty of Agriculture Moshtohor, Benha University (Egypt). Humic acid (purity 90.29%) was obtained from Fluka Chemika Company. This organic acid contained 405 g C kg^{-1}, 9.5 g N kg^{-1}, 10.4 g P kg^{-1}, 14.6 g K kg^{-1}, 4.8 g S kg^{-1}. A solution of 80 mg humic acid L^{-1} was then prepared using deionized water. Pure canola oil (100% pure under "Jenan" trade name) was purchased from Al-Ghurair Foods (LLC), Dubai. This oil was used in addition to analar sulphur (H_2SO_4) for the preparation of sulphated canola oil (sulphex 0.5%) as reported by Pohoreski (2004) and Eid and Abbas (2014). The outcome mixture was then left under the laboratory conditions for 18 h; after that, 3*M* NaOH was added gently to the mixture and then left for 24 h. Sulphex was taken from the top mixture; diluted with deionized water at a rate of 1:4 and underwent pH adjustment to 4 using NaOH (*3M*). A growth retardant, paclobutrazol (PBZ, 250 g L^{-1}), was purchased from syngenta under a commercial name "Cultur". This growth regulator underwent dilution with deionized water to prepare a solution of 40 mg ABZ L^{-1}. Surface soil samples were collected from the experimental vegetables Farm, Faculty of Agriculture, Benha University, Egypt before the first growing season i.e. 2014. These samples were air-dried, cursed, sieved to pass through a 2 mm sieve and then analyzed for their particle size distribution and chemical characteristics as outlined by Klute (1986) and Page et al. (1982) and the obtained results are presented in Table 1.

2.2. Identification of soil infection with S. rolfsii

The following features were observed on vegetable crops grown on the studied location before conducting the field experiment i.e. a white moldy layer with small, smooth and brown sclerotia. These features

Table 1
Particle size distribution and the chemical analyses of the investigated field prior to the first *growing season*.

Soil characteristic	Value
Sand (%)	22.3
Silt (%)	35.4
Clay (%)	42.3
Textural class[a]	Clay loam
$CaCO_3$ (g kg^{-1})	23.26
Organic matter (g kg^{-1})	2.13
pH [a]	7.73
EC[a] (dS m^{-1})	2.2

[a] Texture is estimated according to the International Soil Texture Triangle; EC was estimated in soil paste extract; pH was determined in soil: water suspension (1:2.5).

were identified according to Schwartz et al.(2005) as *S. rolfsii* infection. Further morphological identifications were characterized under the light microscope at The Plant Pathology Research Institute, Agriculture Research Centre (ARC), Giza - Egypt.

2.3. Propagation of AMF cultures

The AM fungi (*Glomus* spp) were supplied by the Agricultural Research Station, Agricultural Research Center (ARG), Egypt. This isolate was multiplied for 2 months under the greenhouse conditions using a Sudan grass (as a host plant) which was grown on a mixture of sand and loam soil (2:1, v/v previously steam-sterilized at 121 °C for three successive days (1 h daily)). Afterwards, plant roots were gently separated from soils, washed with sterilized deionized water and the heavily colonized roots were selected as inoculums under a light microscope at × 200 and × 400 magnification. These colonized Sudan grass root fragments, soil, spores and hyphal fragments were used later as inoculums at a rate of 20 g for each hill.

2.4. The field experiment

A field experiment was conducted for two successive seasons i.e. 2014 and 2015 in the experimental farm of the Faculty of Agriculture, Benha University, Egypt (30° 21′ 26″ N and longitude 31° 13′15″ E). This field was mildly infected with *S. rolfsii.* The experiment design was a complete randomized one (the plot area was 10.5 m^2, each comprised 3 rows × 5 hills per row) and the following treatments were considered: humic acid at a rate of 40 g per plot (equivalent to 38 kg ha^{-1}) after being mixed with a half kilogram of soil then broad-casted on soil surface (T1), humic acid + AMF (T2), Sulphex at a rate of 1 L per plot (equivalent to 980 m^3 ha^{-1}, T3), Sulphex + AMF (T4), paclobutrazol (40 mg L^{-1}) at a rate of 3 L $plot^{-1}$ (equivalent to 3 m^3 ha^{-1}, T5), paclobutrazol + AMF (T6), AMF (T7), the fungicide "Vitavax-200" applied at a rate of 5 g kg^{-1}dressing on tubers (T8) and the control treatment (T9). The flow chart of the experimental design is presented in Table 2.

Humic, sulphex and paclobutrazol treatments were added at two equal doses i.e. during tuber cultivation and three months after cultivation. Tubers of Jerusalem artichoke were cultivated at a rate of one tuber/hill (10 cm deep) in April. All plots received NPK fertilizers as recommended by the Egyptian Ministry of Agriculture i.e. 120 kg N, 26 kg P and 50 kg K ha^{-1} in the form of ammonium sulphate (205 g N kg^{-1}), calcium super phosphate (65.5 g P kg^{-1}) and potassium sulphate (400 g K kg^{-1}), respectively. All the agricultural practices were followed as usual e.g. irrigation every week to bring soil moisture near the saturation percentage beside of the periodical removal of plant weeds. The relative humidity ranged from 66.1 to 81.3% during the first growing seasons (2014) while ranged from 61.9 to 83.4% during the second growing season (2015). By November, plants reached their physiological maturity growth stage. After that, the tops were cut at the ground level, and their fresh weights were recorded (per plot). The tubers were dug, washed to remove stunted soil particles, air-dried and then their fresh weights were recorded (per plot). Disease incidence was also calculated according to Vidhyasekaran (2004) and Sennoi et al. (2013) as a percentage of the infected plants from the corresponding total numbers of the grown plants (infected plants/total plants × 100). Infection severity was estimated on a 0–5 scale according to Shahzad and Ghaffar, (1992) as follows (0 = 0% infection, l = l-10% infection, 2 = 11–25% infection, 3 = 26–50% infection, 4 = 51–75% infection and 5 = 75–100% root colonization with the pathogen. Afterwards, the disease severity percentage was calculated according to Liu et al. (1995) as follows:

$$\text{Disease severity} = (\Sigma\ (n \times r) / 5N) \times 100$$

Where: n = number of plants in each numerical rate (r0 … r5). r = numerical values of each category, r0 …..r5. N = the total number of plants multiplied by the maximum numerical rate r5. Soil samples were also sampled from the rhizosphere of each plot during the physiological maturity growth stage for estimating the availability of NPK within the soil rhizosphere.

Table 2
Flow chart of the experimental design.

Non-inoculated treatments with AMF	Inoculated treatments with AMF
First replicate	
humic acid (T1)	humic acid + AMF (T2)
Sulphex (T3)	Sulphex + AMF (T4)
paclobutrazol (T5)	paclobutrazol + AMF (T6)
Fungicide treatment (T8)	AMF (T7)
control treatment (T9)	
Second replicate	
control treatment (T9)	AMF (T7)
paclobutrazol (T5)	paclobutrazol + AMF (T6)
humic acid (T1)	humic acid + AMF (T2)
Sulphex (T3)	Sulphex + AMF (T4)
Fungicide treatment (T8)	
Third replicate	
Fungicide treatment (T8)	
paclobutrazol (T5)	paclobutrazol + AMF (T6)
control treatment (T9)	AMF (T7)
Sulphex (T3)	Sulphex + AMF (T4)
humic acid (T1)	humic acid + AMF (T2)

2.5. Soil and plant analyses

2.5.1. Soil analyses

Soil samples were collected from the rhizosphere of each treatment by the end of each growing season. These samples were analyzed for their NPK available contents according to the standard methods reported by Page et al. (1982) as follows: (1) Available-N (NH_4^+ and NO_3^-) were extracted by K_2SO_4 (10 g kg^{-1}), reduced to NH_4^+ using MgO and Devarda alloy. Afterwards, NH_4^+ was trapped in boric acid (40 g L^{-1}) then titrated against 0.1*M* HCl. (2) Available-P was extracted by $NaHCO_3$ (0.5 *M*, pH 8.5), then determined using Spectrophotometer (JENWAY 6405 UV/Vis) after being reduced using ammonium molybdate and ascorbic acid reagents. (3) Available-K was extracted by ammonium acetate (1 *M*, pH 7) then measured by the flame photometer (JENWAY PFP7).

2.5.2. Plant digestion and NPK analyses

Representative portions of tubers were taken from each treatment, cut into pieces; oven-dried at 70 °C for 72 h, then ground and digested using a mixture of concentrated sulphuric acid and perchloric acid (prepared at a ratio of 2:1). Total-N in plant digests was measured by micro-Kjeldahel apparatus and total-P in the digests was reduced using ammonium molybdate and ascorbic acid then measured spectrophotometrically. Total K was measured using the flame photometer.

2.5.3. Extraction and determination of enzymes

Tubers portions (equivalent to 5 g each) were sampled, ground in presence of purified sand and sodium phosphate buffer (4 mL, 0.1*M*, pH 7.1). Afterwards, samples were filtered through cheesecloth, centrifuged at 3000 rpm for 20 min and then the supernatants were accessed for their activities of polyphenoloxidase (PPO), peroxidase and chitinase enzymes at 420, 425 and 540 nm, respectively according to Tuzunet al. (1989) and Mohamed et al. (2019) using Spectrophotometer (Spectronic 20-D).

2.5.4. Scanning structural colonization of AMF in root cell by electron microscopy

Root colonization with AMF was estimated under the light stereo microscope (Leica type 020–518.500) according to Phillips and

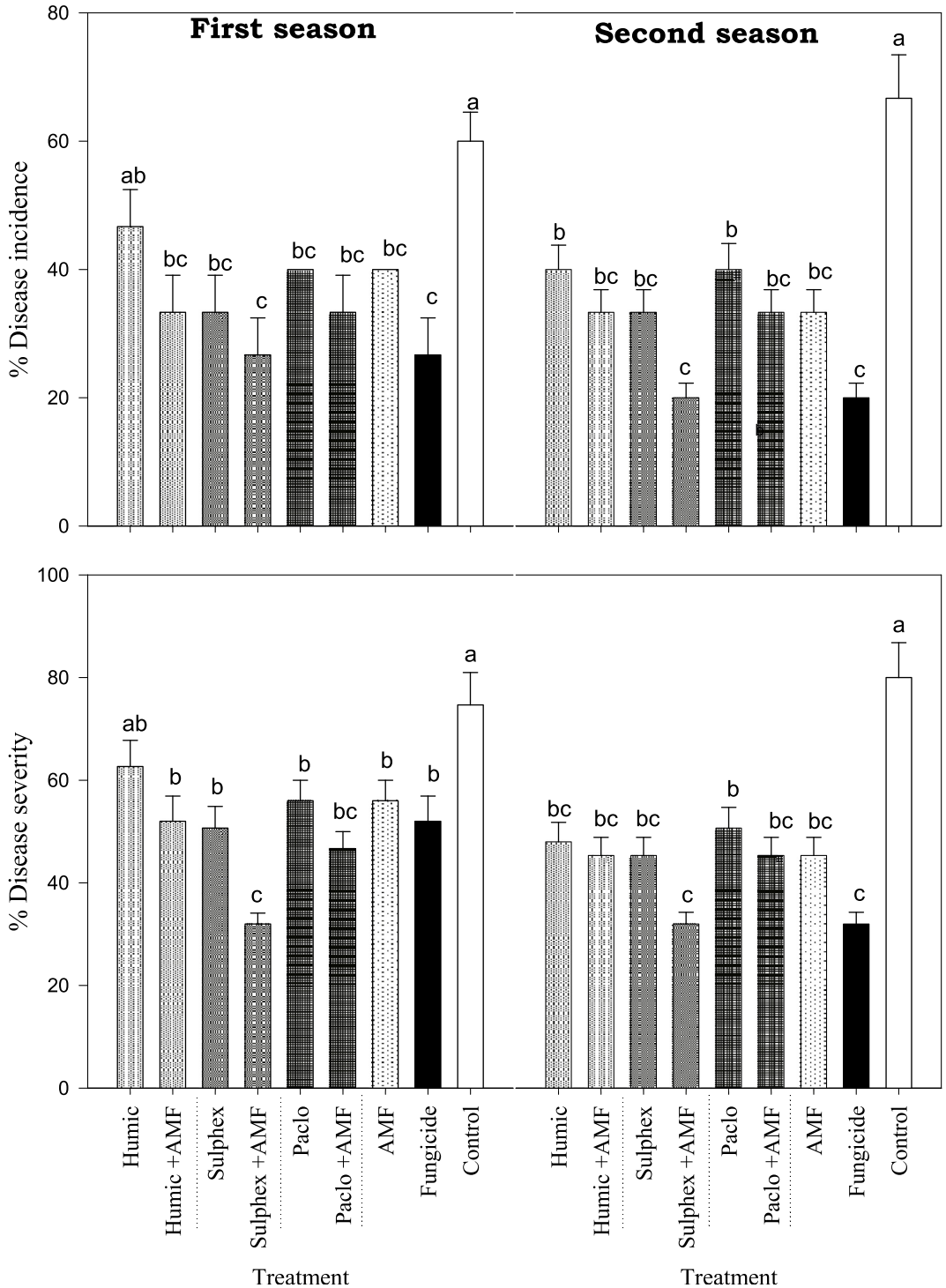

Fig. 1. Disease incidence and severity (mean + SD) as affected by amending plants (+/− AMF) with humic acid, sulphex and PBZ.

Hayman (1970) i.e. root hairs were cut into pieces (equivalent to 0.5 cm), dipped in KOH (10%) then heated on a water–bath (80–90 °C) for approximately 2 h. Afterwards, roots were washed up with tap water, acidified with HCl (10%) then stained with trypan blue and heated at 80–90 °C for 5–10min. Stained roots were then air-dried, immediately suspended in few drops of lactic acid on glass slides and scanned using the light stereo microscope. Fine root samples were collected from the lateral roots, dehydrated with a series of ethanol washes (30, 50, 70, 90, and 100%), critical point dried in CO_2, coated with gold, and examined with a JEOL JSM-5500LV Scanning Electron Microscope (SEM) at the Regional Centre for Mycology and Biotechnology (RCMB), Al- Azhar University, Egypt.

2.6. Data analyses and processing

Data were statistically analyzed using PASW Statistics software through the analysis of variance (ANOVA) and Dunken Test at 0.05 probability level. Figs. 1 and 3–6 were presented using SigmaPlot 10.0.

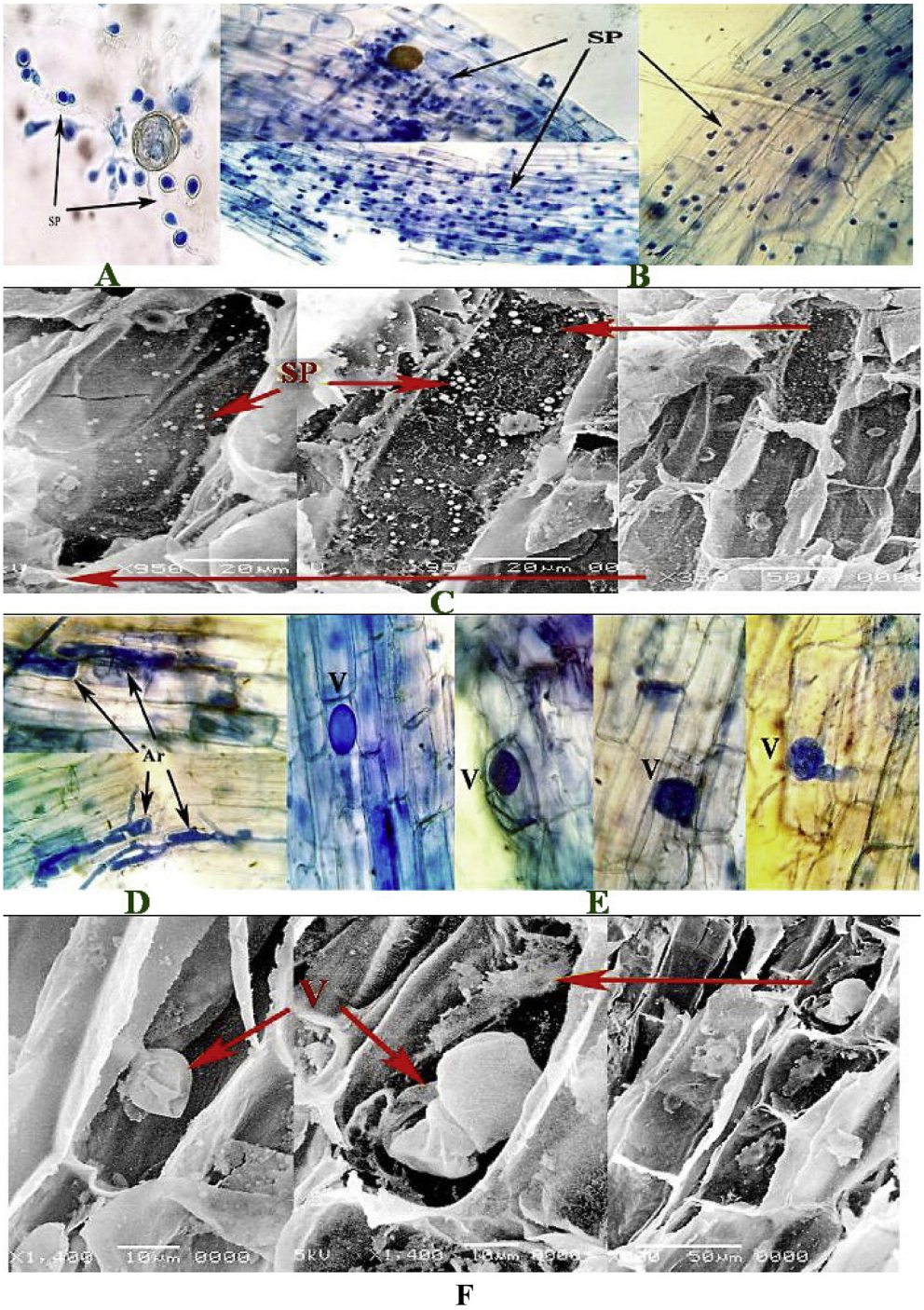

Fig. 2. (A–F) Photomicrographs of structural colonization of AMF in root cell. A. (SP) Spores of AMF(X400), B. (SP) Spore of AMF in root cell (X200) and C. Spore of AMF in root cell under scanning electro microscope.D. (AR) Arbuscule. E. (V) vesicles in root cell × 400 and F. (V) vesicles in root cell under scanning electro microscope.

3. Results

3.1. Disease incidence and severity as affected by amending plants (+/− AMF) with humic acid, sulphex and PBZ

Fig. 1 reveals that inoculating plants with AMF decreased significantly the disease incidence and severity of *S. rolfsii*. In this concern, the structural colonization of AMF in root cell is presented by photomicrographs in Fig. 2. Likewise, applying the investigated amendments/chemicals decreased significantly the disease incidence and severity. It seems that the integrated or combined treatments i.e. humic acid + AMF (T2), sulphex + AMF (T4) and paclobutrazol + AMF (ABZ) (T6) were more efficient in decreasing the disease incidence and severity than the single treatments did. Moreover, such integrated treatments recorded insignificant variations in disease incidence and severity with the fungicide treatment.

3.2. Plant defense enzymes as affected by amending plants (+/− AMF) with humic acid, sulphex and PBZ

Inoculating plants with AMF improved significantly the activities of plant defense enzymes by approximately 1.5–2.1 folds higher than the

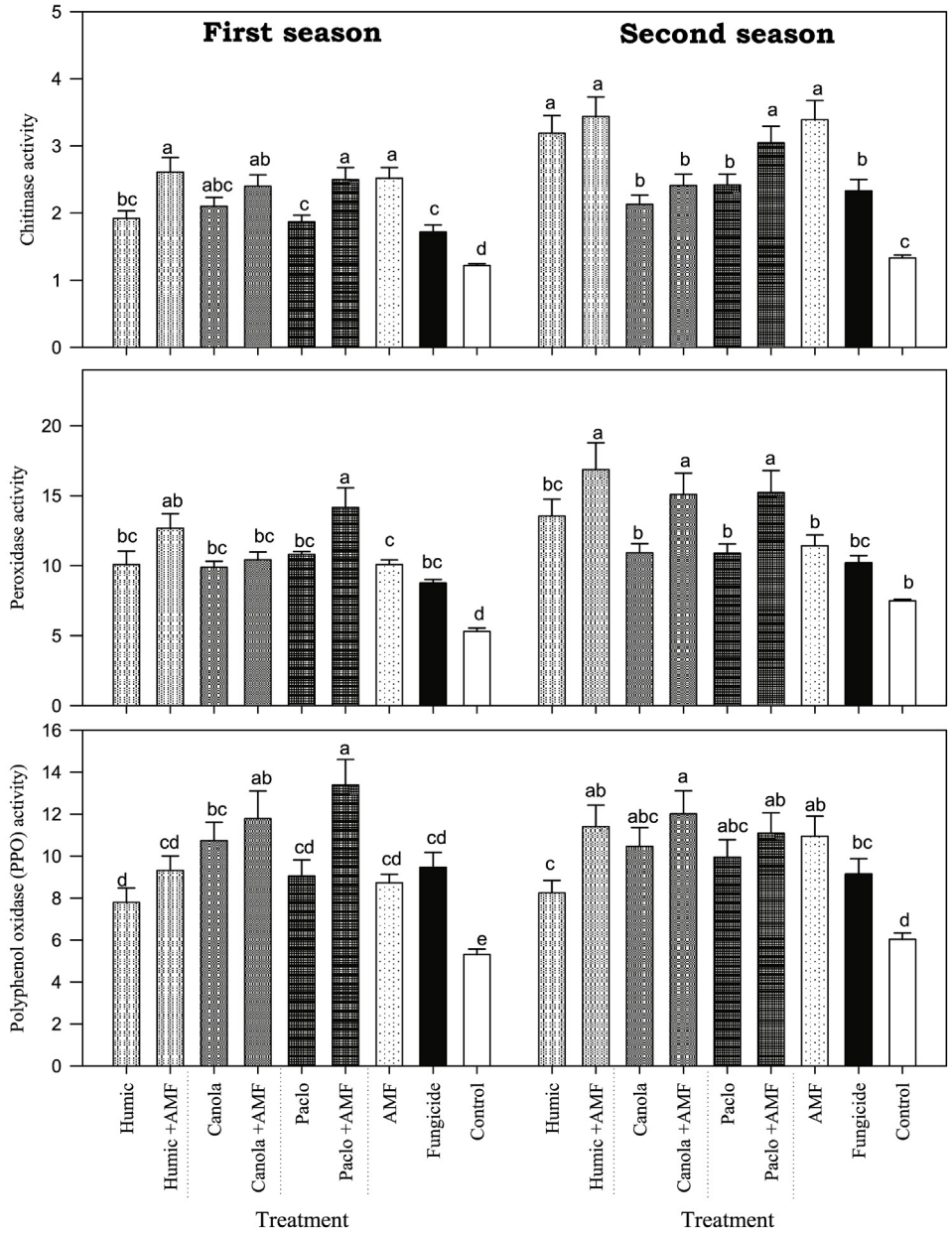

Fig. 3. Effects of amending plants (+/− AMF) with humic acid, sulphex and PBZ on the activities of plant defense enzymes (mean + standard deviation). (1) Chitinase activity (mM N-acetyl glucose amine equivalent released/gram fresh weight tissue/60 min), peroxidase activity (the change in absorbance (O.D)/minute/gram fresh weight), PPO: Polyphenoloxidase activity (change in absorbency (O.D)/minute/gram fresh weight). Different letters on bars indicate significant difference between treatments at $P < 0.05$.

non-inoculated control ones (Fig. 3). Such increases were even higher than the corresponding ones of plants treated with the fungicide "Vitavax-200" (except for the peroxidase enzyme whose activity did not vary significantly between these two treatments). On the other hand, inoculating plants with AMF in presence of either humic acid, sulphex or paclobutrazol did not elaborate further significant increases in activities of plant defense enzymes beyond those attained owing to the inoculation with AMF solely; in spite of that, applying these amendments solely improved significantly the activities of plant defense enzymes when compared with the control treatment.

3.3. Availability of NPK in soil as affected by amending plants (+/− AMF) with humic acid, sulphex and PBZ

Fig. 4 reveals that the investigated amendments i.e. humic acid, sulphex and paclobutrazol improved significantly the availability of NPK in soil during both seasons of study. Moreover, inoculating plants with AMF resulted in further significant increases in the availability of soil-P (during the two seasons of study) and soil-K (during the first season only). In case of N availability, inoculating plants with AMF increased significantly this fraction of soil N by approximately 1.2 folds higher than the non-inoculated control plants; however, the combination between any of the used chemicals and AMF did not improve significantly N availability in soil when compared with their sole applications.

3.4. NPK content within the tubers of Jerusalem artichoke as affected by amending plants (+/− AMF) with humic acid, sulphex and PBZ

Application of either humic acid, sulphex or paclobutrazol increased

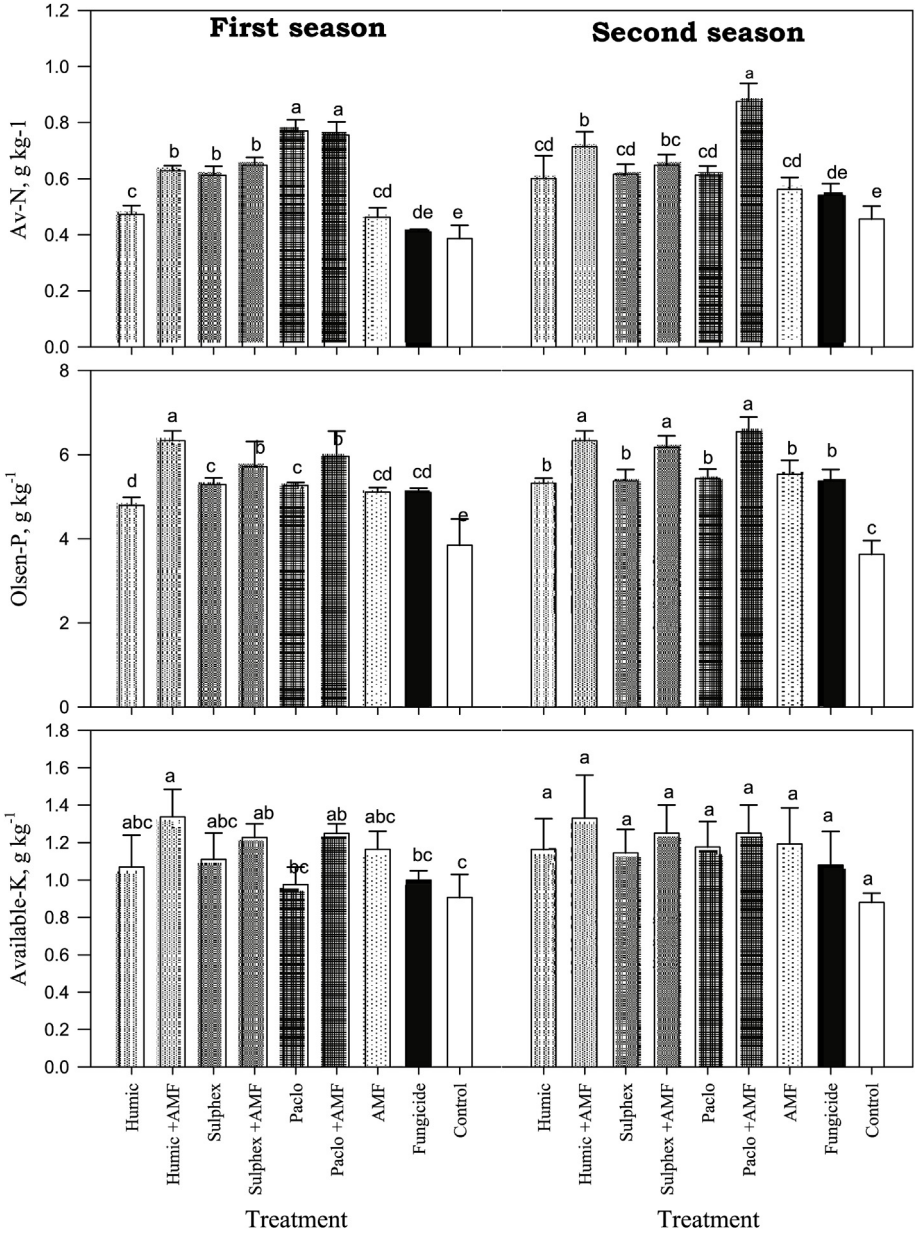

Fig. 4. Effect of amending plants (+/− AMF) with humic acid, Sulphex and PBZ on the availability of NPK in soils.

significantly NPK contents within the plant tubers (Fig. 5). Likewise, inoculating plants with AMF improved significantly NPK contents within tubers. Such increases were significantly confirmed during the two seasons of study. Moreover, the combinations between AMF and these chemicals resulted in further significant improvements in the nutritional status of plants (as shown in the tuber contents of NPK). In this concern, the combination between humic acid + AMF recorded the highest significant increases of both P and K contents in tubers. Although, treating plants solely with any of the investigated treatments did not increase significantly NPK contents within the plant tubers when compared with the fungicide treated plants; however, the combination between these treatments resulted in further significant increases in the nutritional status of plant tubers.

3.5. Shoot fresh weight and the tuber yield of Jerusalem artichoke as affected by amending plants (+/− AMF) with humic acid, sulphex and PBZ

Inoculating plants with AMF increased significantly shoot biomass as well as the tuber yield by approximately 1.8 and 1.9 folds higher than the control treatment, respectively (Fig. 6). Such a treatment recorded insignificant variations with the corresponding ones attained for the fungicide treatment during the first season of study; however, the fungicide treatment seemed to be superior during the second season. Likewise, application of the investigated chemicals or amendments improved significantly shoot biomass and tuber yields; yet, such increases stood below those attained due to the fungicide treatments. The combination between AMF and the investigated chemicals recorded significantly higher increases in both shoot biomass and tuber yield than those attained due to the fungicide treatment.

3.6. Disease incidence and severity in Jerusalem artichoke as affected by NPK contents in tubers and the activities of plant defense enzymes

Table 3 reveals that both the disease incidence and severity in Jerusalem artichoke were correlated significantly and negatively with the nutritional status of the grown plants (as exhibited by NPK contents within plant tubers). The investigated disease markers were also

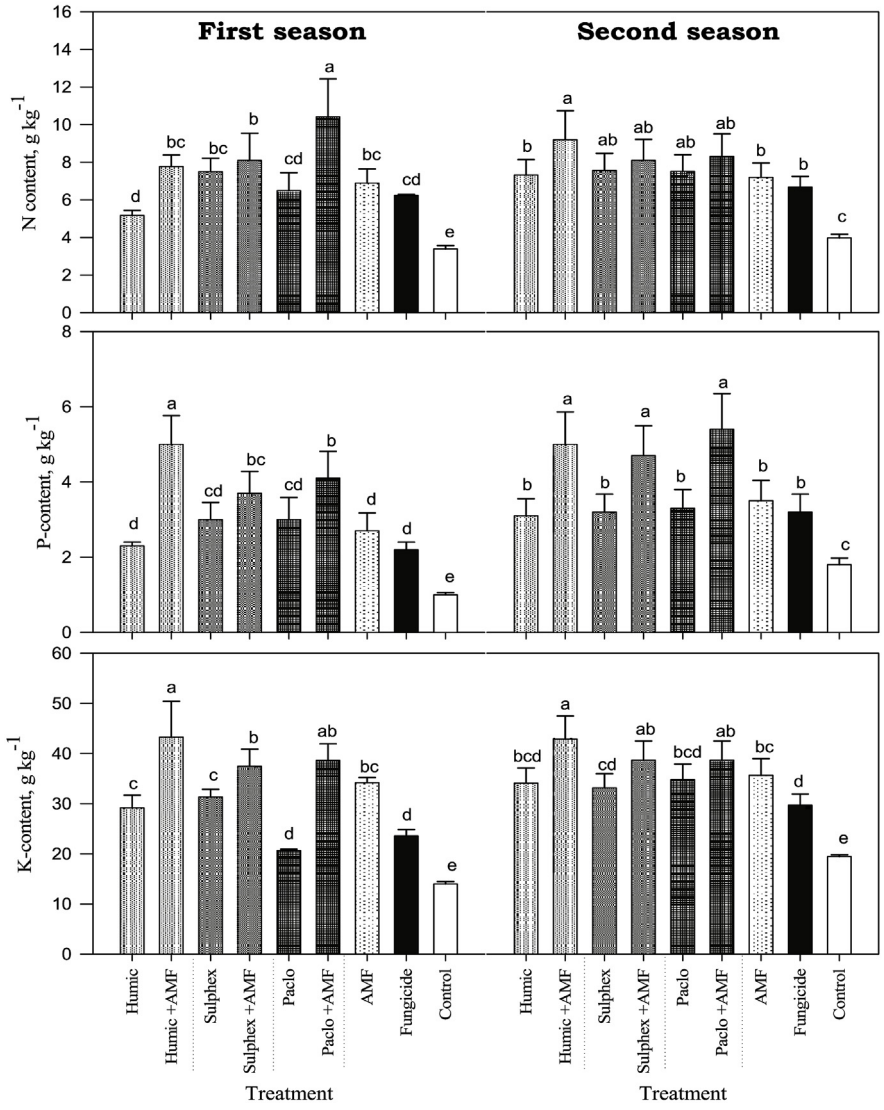

Fig. 5. Effect of amending plants (+/− AMF) with humic acid, sulphex and PBZ onNPK contents within the tubers.

correlated significantly and negatively with the activities of plant defense enzymes (chitinase, peroxidase and polyphenol oxidase). On the other hand, plant defense enzymes were correlated significantly and positively with NPK contents within tubers. It seems that the significant increases that occurred in both shoot fresh weights and tuber yields owing to the application of the investigated treatments were correlated significantly and negatively with the concurrent reductions that occurred in both the diseases incidence and severity while the corresponding correlations with both the increases in PK contents in tubers and activities of plant defense enzymes were significant and positive.

4. Discussion

The current research investigates the potentiality of substituting traditional chemical fungicides with the induction of plant resistance against *Sclerotium rolfsii* infection in presence of mycorrhizae (AMF) as a plant inoculant and one of the following amendments: humic acid, sulphex and paclobutrazol (ABZ). These treatments are thought to improve the nutritional status of the grown plants; hence improve their potentiality to control soil-borne pathogens. In this study, NPK were considered because these macro-nutrients are essential for plant growth besides being critical for the development of plant defense mechanisms against pathogens. Nitrogen, for example, is involved in the synthesis of proteinases (Oliveira et al., 2003) which probably suppresses the secretion of fungal enzymes that "are involved in plant cell wall penetration" (Vidhyasekaran, 2004). Phosphorus (P) is utilized by infected plants to repair damaged cells (Mohamed et al., 2019). Potassium (K) potentiality probably arises because of its high permeability through the membrane as compared with the other ions; thus, it has the greatest impact on the membrane potential of plants (Amtmann et al., 2008).

Results obtained herein reveal that inoculating plants with AMF improved significantly the availability of NPK in soil and consequently raised their concentrations within the plant tubers. This, in turn, improved significantly shoot fresh weight as well as the tuber yield per hectare. These results agree with those of Azcón-Aguilar and Barea (1997), Janczura et al. (2006) and Wehner et al. (2010) who recommended inoculating plants with AMF to suppress their infection with soil borne pathogens. It is thought that the fungal pathogens invade host plants to get nutrients (Howlett, 2006). In AMF inoculating plants, AMF can compete with the soil borne pathogens on soil nutrients thus reduce their subsistence in soil (Mohamed et al., 2019), besides, AMF is capable of increasing the solubility of soil nutrients i.e. P (Chien et al., 2011) and K(Casieri et al., 2012) Moreover, inoculating plants with AMF improves biological nitrogen fixation in soil

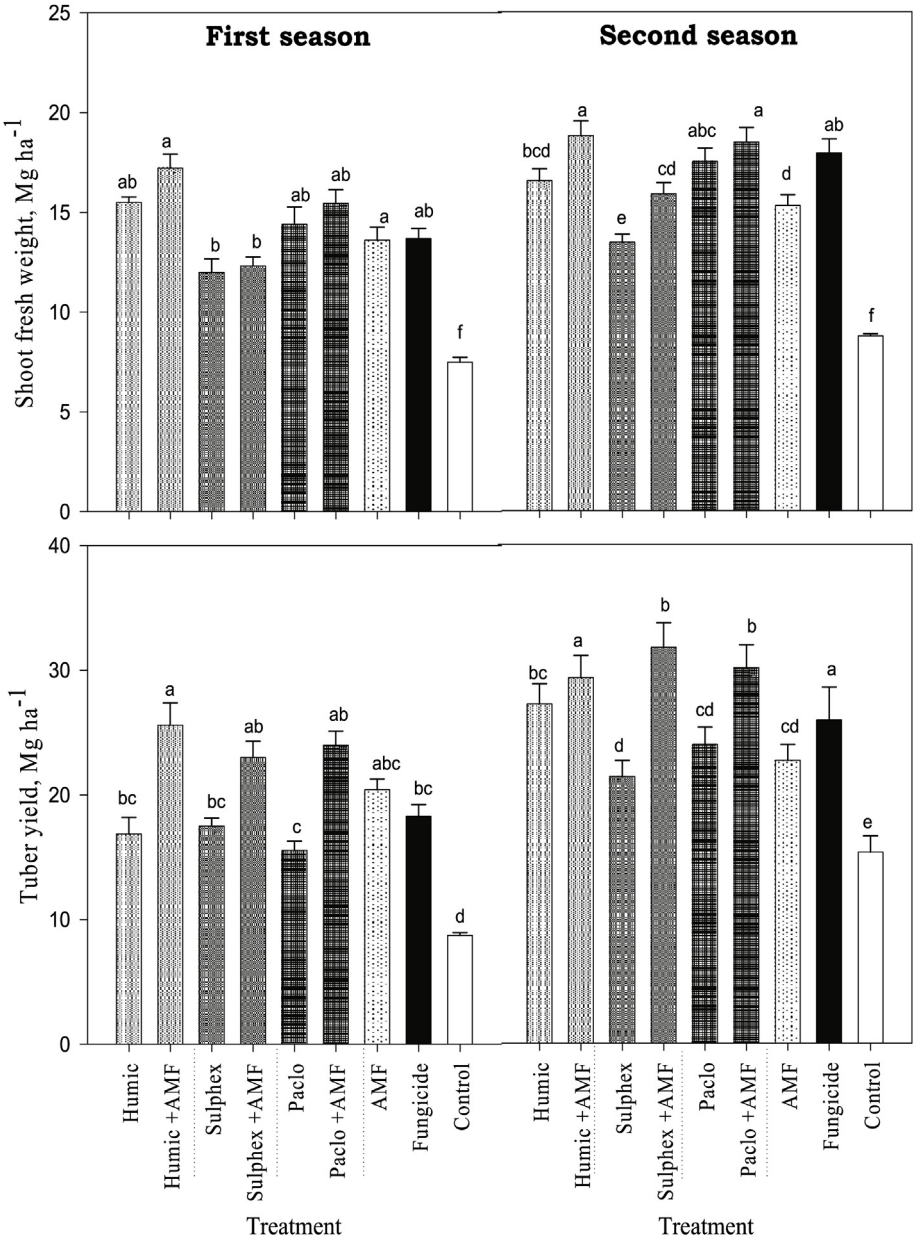

Fig. 6. Effect of amending plants (+/− AMF) with humic acid, sulphex and PBZ on the shoot biomass and tuber yield of *Helianthus tuberosus*.

Table 3
Correlation coefficients among the disease incidence and severity in Jerusalem artichoke, shoot fresh weights, tuber yields, NPK contents within tubers and activities of plant defense enzymes (n = 54).

		Disease		Plant growth		Plant defense enzymes			Nutritional status		
		Incidence	Severity	Shoot fresh weight	Tuber yield	Chitinase	Peroxidase	Polyphenol oxidase	N in tubers	P in tubers	P in tubers
Disease	incidence										
	severity	0.869[a]									
Plant growth	Shoot fresh weight	−0.502[a]	−0.473[a]								
	Tuber yield	−0.610[a]	−0.630[a]	0.645[a]							
Plant defense enzymes	Chitinase	−0.421[a]	−0.486[a]	0.677[a]	0.591[a]						
	Peroxidase	−0.462[a]	−0.506[a]	0.638[a]	0.519[a]	0.674[a]					
	Polyphenol oxidase	−0.553[a]	−0.615[a]	0.418[a]	0.387[a]	0.460[a]	0.665[a]				
Nutritional status	N in tubers	−0.536[a]	−0.529[a]	0.591[a]	0.512[a]	0.662[a]	0.662[a]	0.727[a]			
	P in tubers	−0.476[a]	−0.496[a]	0.680[a]	0.609[a]	0.677[a]	0.755[a]	0.667[a]	0.753[a]		
	K in tubers	−0.466[a]	−0.541[a]	0.624[a]	0.568[a]	0.764[a]	0.727[a]	0.672[a]	0.772[a]	0.820[a]	

[a] Correlation is significant at the 0.01 level (2-tailed).

(Miransari, 2011) and increases nitrogen utilization by plants under stress conditions (Karagiannidis et al., 2002).

Likewise, the application of humic acid, sulphex or paclobutrazol decreased significantly the disease incidence and severity as compared with the control treatment. This might take place because such treatments increased significantly NPK availability in soil; hence, raised their contents within tubers and consequently increased plant resistance against pathogens. In this concern, the addition of humic acid in combination with the low-grade phosphate rock simulates the effect of the single superphosphate fertilizer on supplying P needed for the plant growth (Rosa et al., 2018). Also, humic acid improves plant photosynthetic pigments (Dawood et al., 2019). In case of sulphex, sulphur (S) (a component of this chemical) is considered a vital nutrient for increasing plant survival under biotic and abiotic stresses (Rausch and Wachter, 2005) e.g. it is involved in formation of sulphur-containing amino acids which are antioxidants against free radicals (Colovic et al., 2018) and; therefore, coincide with the synthesis of sulphur-containing defense compounds against fungal pathogens (Kruse et al., 2007). It can also increase the solubility of soil P (Kuhad et al., 2011) and probably micronutrients (Marschner, 2012). Moreover, vegetable oils can stimulate the growth of beneficial soil biota (Bonnett et al., 2012) and this might, in turn, suppress plant infection with soil born pathogens (Mohamed et al., 2019).

Paclobutrazol is a plant growth hormone that induces flowering (Singh and Bhattacherjee, 2005) by inhibiting the biosynthesis of gibberellins (Singh et al., 2005; Fonouni-Farde et al., 2019); thus, slow down cell division and elongation without harming cells (Mabvongwe et al., 2016). On the other hand, this chemical stimulates root elongation and enhances root physiology (d'Arêde et al., 2017; Kamran et al., 2018) with little or no effect on photosynthetic pigment contents or leaf gas exchanges (Ribeiro et al., 2019).

Based on the results of the single treatments, it can be deduced that inoculating plants with AMF or amending soils with either humic acid, sulphex or ABZ solely decreased the disease incidence and severity caused by *S. rolfsii* while, at the same time, improved shoot biomass and tuber yield and; hence, these results support the first hypothesis. In spite of that, the obtained results stood below the prospectives of the chemical fungicide effect. Probably, further enhancements in nutrients availability and uptake might; consequently, induce effective strategies for controlling soil borne pathogens. In this concern, three integrated treatments i.e. "humic acid + AMF", "Sulphex + AMF" and "ABZ + AMF" were considered in the current research.

The first combination is between humic acid and AMF. It was found that the integrated application of humic acid + plant growth-promoting rhizobacteria (PGPR) induced the biostimulants that effectively control pests (Sattari Nasab et al., 2018). Probably the combination between humic acid and AMF is as effective as the abovementioned treatment for inducing the plant resistance against *S. rolfsii*. Our records indicate that amending humic acid to the AMF inoculated plants recorded further significant improvements in NPK availability in soil than single treatments did and this, in turn, increased significantly shoot biomass and tuber yield. Moreover, this integrated treatment recorded superior effect to that of the fungicide treatment on the outcome yield without introducing health risk hazards to the ecosystem which are potentially associated with the fungicide treatment e.g. inhibition of the non-target microbial communities with negative consequences for health and productivity of grown plants (Shi et al., 2019).

The second integrated treatment is between paclobutrazol and AMF. According to Keramati et al. (2017), the integrated treatment between the symbiont fungi (e.g. *P indica*) and paclobutrazol improved the activities of superoxide dismutase, catalase and ascorbate peroxidase activity under stress conditions and these enzymes might induce plant resistance against soil born pathogens. Results obtained herein confirm the effectiveness of such an integrated treatment for controlling *S. rolfsii* infection by the increase of NPK contents within tubers, decreasing the disease incidence and severity; therefore, improving the tuber yield. Such increases exceeded those attained by either the single treatments or the fungicide.

The third combination is between sulphex and AMF. This integrated treatment also improved significantly NPK contents within the tubers of Jerusalem artichoke *(Helianthus tuberosus* L.), and increased shoot biomass as well as the tuber yield of Jerusalem artichoke to exceed the corresponding increases recorded for either the single treatments or the fungicide treated plants. Accordingly, these three integrated treatments can entirely substitute the chemical fungicides in controlling plant infection with *S. rolfsii* and; therefore, we accept the second and third hypotheses.

5. Conclusion

Application of biochemicals i.e. humic acid, sulphex or paclobutrazol to AMF inoculated plants increased significantly NPK availability in soil and; hence, improved their contents within the grown plants to levels exceeding those attained due to the application of either of the single treatments. Moreover, such integrated treatments increased significantly the activities of plant defense enzymes which are thought to be the markers pointing out towards induction of plant resistance against pathogen attack. Thus, these integrated treatments induced considerably plant resistance against *S. rolfsii* invasion and furtherly increased the areal biomass and tuber yield of *Helianthus tuberosus*. It is worthy to mention that such integrated treatments were also more efficient than the fungicide treatment in controlling plant infection with *S. rolfsii* and; therefore, these integrated strategies are recommended to substitute totally the chemical fungicides; hence minimize the negative impacts of these chemicals on soil ecology.

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