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Impact of arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast on root rot/ wilt disease and growth parameters of date palm offshoots in New Valley Governorate, Egypt

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Abstract

Root rot and wilt complex were the important diseases in offshoots and new orchards of date palm growing in different Oases in New Valley Governorate. The ability of arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) individually and in combination used as soil drench to induce resistance against root rot / wilt disease complex and their effect on growth parameters, mineral and pigment contents in leaves was examined. The obtained data show that AMF and BY individually and/or in combination were able to protect offshoots against root rot /wilt disease complex compared with check treatment (control)in pots under artificial infection with *Fusarium oxysporum*, *F. solani* and *F. moniliform* and naturally infection under nursery conditions in both locations (El-Kharga and El-Dakhla Oasis). The combination between AMF and BY was better than used individually of them for controlling root rot /wilt disease severity.

On the other hand, all treatments increased growth parameters viz. number of roots offshoot-1offshoot height (cm), number of leaves offshoot-1, leaflet number leaf-1, nick leaf thickness (cm) and leaf area (cm^2) in both locations. The combination between AMF and BY significantly increased all growth parameters compared with used individually. Also, all treatments increase leaf contents of nitrogen (N), phosphorus (P), potassium (K), sodium (Na), magnesium (Mg), calcium (Ca) and Chlorophyll a, b and carotenoid compared with control in both locations (El-Kharga and El-Dakhla).

In biochemical studies, activity of defense-related enzymes, including peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and catalase as well as total phenolic and flavonoids compounds were increased in offshoots treated with AMF and/or BY individually or in combination in both locations (El-Kharga and El-Dakhla). The combination between AMF and BY recorded the highest all oxidative enzymes, total phenolic and flavonoid compounds compared with AMF or BY alone.

Key Words: Date palm offshoots, Root rot and wilt diseases, AMF, BY, Growth parameters, Mineral and pigment contents, Defense-related enzymes, Phenols, flavonoids.

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Introduction

Date palm (*Phoenix dactylifera* L.) is considered as one of the most commercial and popular crop, so in some religious occasions, this crop occupies a special status in many countries of the world, especially in Egypt as a result of the nutritional value of fruits and due to its uses. In addition, date palms thrive well under drastic environmental conditions of many desert areas.

Date palm trees and offshoots are attacked by several soil borne pathogenic fungi causing severe losses and deterioration of trees worldwide (El-Morsi *et al*., 2012). *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *F. equiseti*, *F. semitectium*, and *Rhizoctonia solani* have been reported in different countries to cause root rot and wilt diseases in young and adult date palm trees (Rashed and Abdel Hafeez, 2001). Mansoori and Kord (2006) reported a serious disease of date palm caused by *F. solani* associated with yellowing and death of the fronds. The disease occurred in date palm groves in Kazeron district, west of Fars province in Iran. The causal pathogen was isolated from the crown and xylem rays sampled from the trunk 1.5 m above soil level. Pathogenicity test was performed by planting 1-year old date palm seedlings in artificially infested soil with an isolate from the trunk of diseased palm tree as well as seedlings planted in naturally infested soil. Similar symptoms were obtained in both procedures, distal portions of the roots and crown were affected. Sarhan (2001) found that *F. oxysporum* and *F. solani* were the most frequent and most abundant in the roots of date palm trees showing decline in middle of Iraq.

Agricultural practices of soil borne pathogens management in the field include fungicide applications, methyl bromide fumigation, soil solarization and the use of resistant or tolerant varieties (ref.). At present, no single method provides an adequate control of soil borne diseases (Maitlo *et al*., 2013).

Using chemicals to control soil borne pathogens causes several negative effects, such as: i) development of pathogen resistance, ii) harmful effects on humans, iii) bad impact on beneficial organisms and iv) environmental pollution. Moreover, many chemicals will be banned in the near future (ref). However, for sustainable agriculture, pathogens still need to be controlled in order to ensure healthy plant growth and productivity (Gerhard son, 2002). Therefore, finding of various beneficial micro-organisms are urgently needed in order to provide an alternative strategy to chemical control.

Biological control of different plant diseases was focused primarily using bacteria or filamentous fungi (Whipps, 2001). So, application of yeasts as bio control agents acts as a new trend against different pathogens. Potential use of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters were recent investigated by El-Tarabily and Siva it hamparam (2006). El-Tarabily (2004) reported that the fungal activities of *R.solani* diseased sugar beet plants were well suppressed by using different yeasts. Wide variety of yeasts has been used extensively for the biological control of post-harvest diseases of fruits and vegetables (Zheng *et al*., 2003) against moulds of stored grains (Petrsson *et al*., 1999) and to control powdery mildews (Urquhart and Punja 1997).

On the other hand ,arbus cular mycorrhizae fungi (AMF) also protect host plants against soil pathogens and also promote synthesis of plant growth promoting hormones in particular auxins, cytokinins and gibberellins and increased nutrient uptake (Matloob, *et al*., 2017). Under experimental conditions commercial crops inoculated with AMF have showed increased growth and yield (Semra *et al*., 2015).

The aim of this study was to suppress the soil-borne pathogenic fungi by using AMF and/or BY individually or in combination soil drench as bio-control agent and its effect on plant growth promoter, mineral and pigment contents in leaves of data palm offshoot. In addition, defense-related enzymes, phenols and flavonoids in leaves of date palm offshoots were determined.

Materials and methods

Source of fungal pathogens

A pathogenic isolates of *Fusarium oxysporum, F. equiseti, F. solani, F. semitectium* and *F. moniliforme* isolated from diseased date palm offshoots (cv. Saidy) collected from New Valley governorate (El-Morsi*et al*., 2012) was used in this study.

Preparation of fungal inocula

The inocula of pathogenic fungi were prepared from one week old culture grown on 50 mL potato dextrose broth (PDB) medium in conical flask (250 mL) and incubated at 25 ± 1°C. The content of the flask was homogenized in a blender for one min. Plastic pots were filled with sterilized soil mixed with the fungus inocula at the rate of 100 mL homogenized culture per pot, seven days before planting (AbdelMonaim, 2017).

Disease assessments

The severity of root rot/wilt complex was determined after 90 days (Abdou *et al*., 2001) using a rating scale of 0-5 on the basis of root discoloration or leaf yellowing: 0: no root discoloration or leaf yellowing; 1: 1-25% root discoloration or one leaf yellowed; 2: 26-50% root discoloration or more than one leaf yellowed; 3: 51-75% root discoloration plus one leaf wilted; 4: up to 76% root discoloration or more than one leaf wilted; and 5: completely dead plants. For each replicate a disease severity index (DSI) similar to that described previously (Liu *et al*., 1995) was calculated as follows:

$$DSI= \frac{\sum d}{d\ max \times n} \times 100$$

Whereas: d is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

Effect of Arbuscularmycorrhizal fungi (AMF) and/or Baker's yeast (BY) as biological control agents (BCAs) on root rot / wilt complex under greenhouse conditions

Evaluation the efficacy of approaches of arbuscularmycorrhizal fungi (AMF) and Baker's yeast (BY) against root rot /wilt complex incidence of date palm offshoots was carried out in pot experiment in the open greenhouse. The evaluated treatments were applied as soil drench. Seedlings of date palm at six months age were inoculated with tested fungi viz. *F. oxysporum*, *F. solani* and *F. moniliform* spore suspensions at 100 ml per pot (1×10^6 spores/ml) for each fungus prepared as above in pathogenicity tests. After one week of incubation, spore suspension of AMF at 100 ml/pot (1×10^3 spores/ml) and 100 ml of BY at concentration 2.5 gm/l of BY was added per pot individually and/or in combination (Arafat *et al*., 2012). In control treatment, pots were drenching with water alone. Incubation was continued for further 90 days. Five pots were used for each treatment as replicate. Disease severity percentage was visually recorded at 90 days after inoculation as above.

Effect of Arbuscularmycorrhizal fungi (AMF) and/or Baker's yeast (BY) as biological control agents (BCAs) on root rot / wilt and growth parameters under field conditions

Field experiments was carried out at New Valley Agric. Res. Station Farm and in special nursery in El-Dakhla during 2016 season, to evaluate the efficiency of the tested biological control agents arbuscularmycorrhizal fungi (AMF) and/or Baker's yeast (BY) individually and its combination for controlling root rot / wilt complex of date palm offshoots (cv. Saidy) as well as its effect on growth parameters. The chosen field test area was naturally infested with the causal organisms of root rot and wilt pathogens. The experimental design was a complete randomized block with four replicates. The experimental unit area was 2 m^2 (1 × 2 m). Each unit included 2 date palm offshoots (3- years-old). Soil of the planting with offshoots were drenched three time at 15 – day intervals with inocula of AMF (1×10^3 spore/ml) and/or BY (2.5 g/l) at rate 3 L per offshoot. Untreated soil was used for control. The disease severity was assessed for each treatment after 6 months from the least of application treatments. After the end of this experiment the following estimation were take:

- Root numbers $plant^{-1}$
- Plant height (cm)

- Number of leaves plant $^{-1}$
- Leaflet numbers leaf $^{-1}$
- Leaf thickness (cm)
- Leaf area (cm^2) (length × maximum width × 0.84) (Zagzog and Salem, 2017)

Estimation of chlorophyll and carotenoid contents

Chlorophyll and carotenoid were extracted from the leaves and estimated by the method of Arnon (1949). Chlorophyll content was calculated using the formula of Arnon (1949) and expressed in milligram per gram fresh weight. Carotenoid content was estimated using the formula of Kirk and Allen (1965) and expressed in milligrams per gram fresh weight.

Leaf mineral contents

The leaf samples were washed with tap water, rinsed twice in distilled water and air dried in an oven at 70°C. The dried leaves were ground and digested with H_2O_2 and H_2SO_4 according to Evanhuis and De Waard (1980). Suitable aliquots were taken for the determination of the mineral content. Nitrogen was determined by the Kjeldahl method (AOAC, 1995). Phosphorus was determined by the ascorbic acid method according to Murphy and Riley (1962). Potassium and sodium were determined with a flame photometer. The Ca, Mg contents were measured using atomic absorption spectrophotometer. The concentrations of N, P, K, Ca, Na and Mg were expressed as percentages.

Antioxidant enzyme activity assays

After four months from planting, fresh samples were taken from roots of offshoots treated and untreated with AMF and/or BY individually and/or in combination and extracted according to Maxwell and Bateman (1967). Then the extracts were used for assaying biochemical change associated with the tested treatments of organic compounds, the activities of peroxidase enzyme (Hammerschmidt *et al*., 1982), polyphenoloxidase enzyme (Gauillard*et al*., 1993), phenylalanine ammonia lyase enzyme (Cavalcanti *et al*., 2007) and catalase activity was measured by hydrogen peroxide assay based on formation of its stable complex with ammonium molibdate (Goth, 1991).

Protein concentration

Total protein content of the samples was quantified according to the method described by Bradford (1976).

Determination of total phenol and flavonoid contents

A powder of each material (10 g) was extracted by 100 ml of 60% ethanol with continuous shaking at 30°C for 24 h. The extracts were filtered through Whatman No. 1 filter paper and the liquid portions were analyzed for phenolic, flavonoids contents. Samples were analyzed in triplicate.

Total phenol content determination

The total phenol contents were quantified by the Folin-Ciocalteau phenol reagent according to the protocol of Xu and Chang (2007). The method is based on the colored reaction of phenolics with the reagent. Folin-Ciocalteau reagent is reduced to a blue colored oxide at pH 9.0 upon the reaction with phenols. Intensity of the resulting color was measured in a spectrophotometer at 765 nm. Aliquots of test samples (50 µl, 20 mg sample/ml 60% ethanol) were mixed with 3.0 ml distilled water, 750 µl 7% Na_2CO_3, and 0.5 ml Folin-Ciocalteau reagent. The absorbance was measured at 765 nm after 30 min incubation at room temperature. The standard curve was prepared using known concentrations of gallic acid (GAE). The total phenol content in the test samples was calculated from the Gallic acid standard curve and expressed as mg GAE/ 1g sample.

Determination of Total Flavonoid Content

Colorimetric aluminum chloride method was used for flavonoid determination using a method by Xu and Chang (2007). Aliquots of extract were taken and the volume made to 2 ml with distilled water and then mixed with 75 µl of 5% $NaNO_2$. After 6 min, 150 µl $AlCl_3$ (10%) solution was added and allowed to stand for 5 min before adding 0.5 ml of 1.0 M NaOH and 2.5 ml distilled water. The solution was mixed and the absorbance was measured at 510 nm. A standard calibration curve was prepared using known concentrations of quercetin (QE). The total flavonoid in the test samples were calculated and expressed as mg QE/1g sample.

Statistical analysis

Analyses of variance were carried out using MSTAT-C, 1991 program version 2.10. Least significant difference (LSD) was employed to test for significant difference between treatments at P≤0.05 (Gomez and Gomez, 1984).

Results

Isolation, Identification of the causal organism (S) and pathogen city tests

Five Fusarium species were isolated from date palm offshoots showed root rot and wilt symptoms. These species were identified as *F. oxysporum*, *F. equiseti*, *F. solani*, *F. semitectium* and *F. moniliforme*. The pathogen city tests indicated that all , tested fungi significantly caused root rot and wilt diseases in date palm offshoots var. Saidy (Fig. 1). *Fusarium oxysporum* was the most pathogenic fungi as they recorded percentage (89.26 %)root rot/wilt severity (89.26%) followed irrespective orders by *F. solani* and *F. moniliforme* where they caused 82.18% and 73.26% disease , respectively. On the contrary, *F. equiseti and F. Semitectium* recorded the lowest percentages of these criteria.

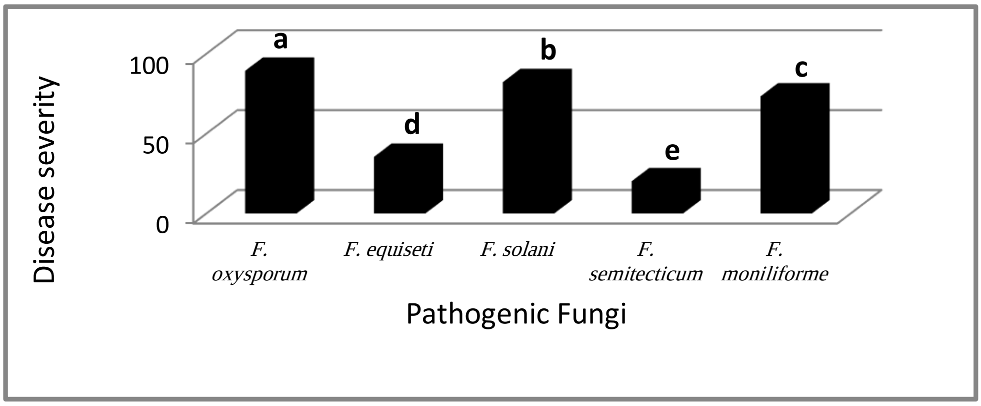

Fig 1. Pathogenic ability of five Fusarium species on root rot and wilt severity of date palm seedlings under greenhouse conditions. Different letters indicate significant differences among pathogenic fungi within the same column according to least significant difference test (p≤0.05).

Effect of arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) as biological control agents on root rot and wilt severity under greenhouse conditions:

Data present in Table 1 show that all tested treatments significantly and reduced disease severity caused by tested pathogenic fungi (*F. oxysporum*, *F. solani*, *F. moniliform*) compared with control. The combination between AMF and BY recorded the lowest diseases severity in all tested pathogenic fungi followed by AMF alone then BY treatment. On the other hand, all treatments more effective for suppressed *F. oxysporum* followed by *F. moniliform*, while these treatments recorded the lowest protection against *F. solani* in this respect.

Table (1) Effect of arbuscularmycorrhizal fungi (AMF) and/or Baker's yeast (BY) on disease severity of root rot / wilt disease of date palm seedlings under greenhouse conditions.

Treatments	*F. oxysporum*		*F. solani*		*F. moniliform*	
	% disease severity	% Protection	% disease severity	% Protection	% disease severity	% Protection
Arbuscular mycorrhizal fungi (AMF)	30.67c	64.06	36.67c	54.54	27.33c	58.38
Baker's yeast (BY)	38.00b	55.47	40.33b	50.01	33.67b	53.81
AMF+BY	20.33d	76.17	25.00d	63.22	18.67d	71.57
Control	85.33a	0.00	80.67a	0.00	65.67a	0.00

Different letters indicate significant differences among treatments within the same column according to least significant difference test (p≤0.05).

Effect of arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) as biological control agents on root rot / wilt complex severity under field conditions

Data present in Fig. 2 showed that all treatments significantly decreased root rot/wilt disease complex caused by soil borne pathogens under naturally infection in nursery cultivated in El-Kharga and El-Dakhla. Efficiency of the tested AMF and BY for controlling this disease varied.However, the combination between AMF and BY was the most effective for decreasing root rot and wilt severity compared with individually wage. Also, AMF was the most effective one for controlling root rot and wilt disease complex than BY in both locations (El-Kharga and El-Dakhla).

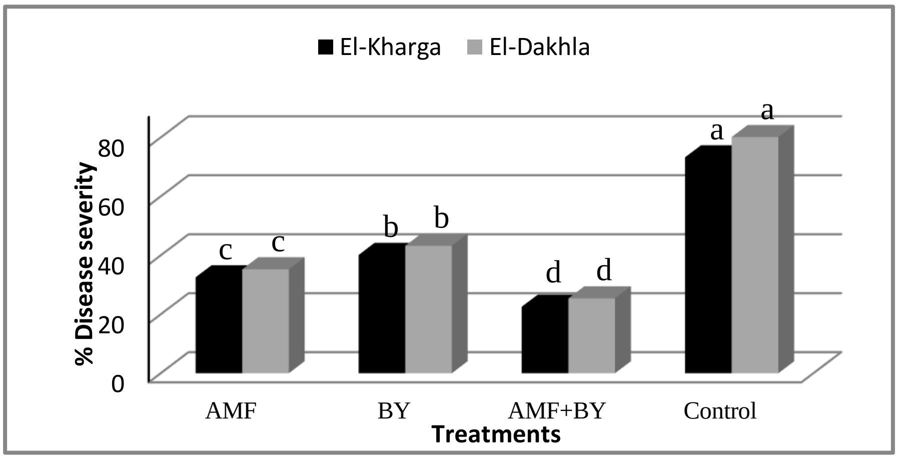

Fig 2. Effect of arbuscular mycorrhizal fungi (AMF) and Baker's yeast (BY) individually or in combination on disease severity of root rot /wilt diseases complexof date palm offshoots var. Saidy under nursery conditions cultivated in El-Khargaand El-Dakhla during season 2016. Different letters indicate significant differences among treatments within the same column according to least significant difference test (p≤0.05).

Effect of arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) on growth parameters:

Data present in Table 2 showed that all tested treatments significantly increased all growth parameters *viz.* root numbers plant^{-1}, plant height, number of leaves plant^{-1}, leaflet number leaf^{-1} and nick leaf thickness of date palm offshoots (var. Saidy) compared with control in both growing seasons. The combination between AMF and BY recorded the highest growth parameters than used of them individually, where increased of root numbers plant^{-1} from 5.33, 6.67 in control to 40.67 and 44.67, plant height from 102.36, 106.33 in control to 255.36 and 231.67 cm and number of leaves plant^{-1} from 1.33 and 1.67 to 6.67 and 6.25 in both growing seasons, respectively. Also, both treatments increased leaflet number leaf^{-1} from 24.55, 26.67 in control to 122.68 and 120.0, nick leaf thickness from 0.63, 0.72 in controls to 1.67, 1.56 cm and leaf area from 524.85 and 625.42 cm^2 to 3859.65 and 3958.05 cm^{2}in both locations, respectively. On the other hand, AMF improved of plant growth than BY in both locations.

Table (2) Effect of arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) on growth parameters of date palm offshoots var. Saidy under nursery conditions in El-Kharga and El-Dakhla during season 2016.

Treatments	Root numbers plant^{-1}	Plant height (cm)	Number of leaves plant^{-1}	Leaflet number leaf^{-1}	Nick leaf thickness (cm)	Leaf area (cm^2)
El-Kharga						
Arbuscular mycorrhizalfungi(AMF)	26.00b	202.00b	5.00b	96.33b	1.42b	3055.36b
Baker's yeast (BY)	22.33c	189.36c	4.67c	72.33c	1.25c	2515.42c
AM+BY	40.67a	255.36a	6.67a	122.68a	1.67a	3859.65a
Control	5.33d	102.36d	1.33d	24.55d	0.63d	524.85d
El-Dakhla						
Arbuscularmycorrhizal fungi (AMF)	29.67b	210.36b	5.67b	99.67b	1.55a	3256.36b
Baker's yeast (BY)	21.33c	175.69c	4.67c	68.67c	1.39b	2986.36c
AM+BY	44.67a	231.67a	6.25a	120.00a	1.56a	3958.05a
Control	6.67d	106.33d	1.67d	26.67d	0.72c	625.42d

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$).

Mineral contents

Analysis of leaf mineral contents showed a significant increase in nitrogen (N), phosphorus (P), potassium (K) sodium (Na), calcium (Ca) and magnesium (Mg) in date palm offshoot leaves grown in soli treated with AMF and/or BY individually and/or in combination when compared with control plants in El-Kharga and EL- Dakhla locations (Table 3). The highest increase was obtained when soil treated with AMF+ BY followed by AMF alone. While, BY treatment recorded the lowest leaves contents from all tested minerals except sodium content.

Photosynthetic pigment contents

The photosynthetic pigment contents in date palm offshoot leaves cultivated in soil treated with AMF and/or BY individually and/or in combination were measured after 180 days of treatments application. Data listed in Table (4) show that AMF and BY led to increase in chlorophyll a, b and carotenoids in leaves either applied individually or in combination in both location (El-Kharga and El-Dakhla) compared with control. The combination between

AMF and BY recorded the highest offshoots leaves from contents chlorophyll a, b and carotenoids more than used AMF or BY individually in both experiential locations. On the other hand, BY treatment recorded the lowest offshoot leaves content of chlorophyll a, b and carotenoids compared with the other tested treatments.

Table (3) Effect of arbuscularmycorrhizal fungi (AMF) and/or Baker's yeast (BY) on nitrogen (%), phosphorus (%), potassium (%), sodium (%) and calcium (%) of date palm offshoots var. Saidy under field conditions in El-Kharga and El-Dakhla.

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Sodium (%)	Calcium (%)	Magnesium (%)
El-Kharga						
Arbuscular mycorrhizalfungi(AMF)	1.33b	1.65b	0.39a	0.12c	0.012b	0.56ab
Baker's yeast (BY)	1.05c	1.32c	0.28b	0.20b	0.009c	0.51b
AM+BY	1.56a	1.82a	0.43a	0.31a	0.019a	0.64a
Control	0.62d	0.75d	0.28c	0.06d	0.004d	0.33c
El-Dakhla						
Arbuscularmycorrhizal fungi (AMF)	1.52b	1.77a	0.42a	0.15c	0.014b	0.62b
Baker's yeast (BY)	1.26c	1.25b	0.33b	0.23b	0.011b	0.53c
AM+BY	1.75a	1.78a	0.42a	0.35a	0.021a	0.72a
Control	0.73d	0.82c	0.32b	0.08d	0.005c	0.29d

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$).

Table (4) Effect of arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) on chlorophylls a, b and carotenoids (mg/g f.w.) of date palm offshoots var. Saidy under field conditions in El-Kharga and El-Dakhla during season 2016.

Treatments	Chlorophyll-a (mg/g fresh weight)	Chlorophyll-b (mg/g fresh weight)	Carotenoids (mg/g fresh weight)
El-Kharga			
Arbuscular mycorrhizal fungi (AMF)	0.85b	0.81b	0.60b
Baker's yeast (BY)	0.69c	0.67c	0.55b
AM+BY	0.98a	0.93a	0.75a
Control	0.57d	0.42d	0.32c
El-Dakhla			
Arbuscular mycorrhizal fungi (AMF)	0.89b	0.85b	0.77b
Baker's yeast (BY)	0.75c	0.72c	0.61c
AM+BY	1.02a	0.95a	0.88a
Control	0.62d	0.52a	0.39d

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$).

Biochemical changes associated with arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) treatments:

The accumulation of peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and catalase (CA) as well as content of each of phenolic and flavonoids compounds in date palm offshoot leaves after soil drench with AMF and/or BY individually and/or in combination in both locations were studied.

Oxidative enzymes

Data present in Table (5) show that all treatments significantly increased oxidative enzymes *viz.* peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and catalase (CA) in offshoots grown in soil treated with AMF and/or BY either individually or in combination compared with control in both locations. In deed Also, activity of all tested oxidative enzymes was highly in offshoots treated with AMF and BY combination combined AMF and BY than used of them individually in both locations. AMF recorded highly activities of PO, PPO and CA more than BY treatment. On contrary, the activity of PAL was highly in BY treatment more than AMF in both locations. Also, the obtained data indicate that activities of all oxidative enzymes were highly in offshoots grown under El-Dakhla more than El- Kharga locations.

Total phenolic compounds

Results presented in Table (6) show that all treatments significantly increased total phenolic compounds in leaves of date palm offshoots when applied as soil drench individually and/or in combination compared with control. The combination between AMF and BY recorded the highest levels of total phenolic compound contents in both locations followed by AMF alone, while BY treatment gave the lowest value of total phenolic compounds in this respect.

Total flavonoid compounds:

The total flavonoid compounds contents were significantly increased in date palm offshoots leaves when grown in soil treated with arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) individually or in combination compared with control (Table 6). Also, the obtained data show the application AMF+BY gave highly total flavonoid compounds in leaves of date palm offshoots more than AMF or BY individually in both locations. The treatment of AMF recorded highly levels of total flavonoids more than treatment BY.

Table (5) Effect of arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) on oxidative enzymes of date palm offshoots var. Saidy under nursery conditions in El-Kharga and El-Dakhla during season 2016.

Treatments	PO activity	PPO activity	PAL activity	CA activity
El-Kharga				
Arbuscular mycorrhizalfungi (AMF)	0.852b	0.925b	1.698b	3.865b
Baker's yeast (BY)	0.658c	0.812c	1.754b	3.286c
AM+BY	0.968a	1.025a	2.356a	4.128a
Control	0.325d	0.396d	0.714c	2.002d
El-Dakhla				
Arbuscularmycorrhizal fungi (AMF)	0.825b	0.986a	1.854c	4.012b
Baker's yeast (BY)	0.785c	0.852b	1.985b	3.965b
AM+BY	0.986a	1.002a	2.256a	4.568a
Control	0.365d	0.421c	0.896d	2.124c

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$)

Discussion

Date palm offshoots under the New Valley conditions are subjected to infection with different root diseases caused by many soil-borne pathogenic fungi causing considerable root rots and wilt in the orchards. Soil-borne pathogenic fungi *viz. F. oxysporum*, *F. Equisetai F. solani F. semitectium* and *F. moniliforme* caused root rots of date palm trees in both locations of New Valley governorate under study, El-Kharga and El-Dalkla Oasis (El-Morsi *et al.*, 2009, Arafat *et al.*, 2012 and El-Morsy *et al.*, 2015)**.** Pathogeni city tests showed that *Fusariu moxysporum, F. solani* and *F. Moniliforme* were the causal agents of root rot and wilt diseases on date palm seedling. Synthetic fungicides are helpful to sustain crop production by protecting plants from fungal diseases, but resistance to fungicides is one of critical causes of poor disease control of agriculture. Therefore developing alternative agents for the control of pathogenic fungal diseases in plants (Aguin *et al.*, 2006).

Table (6) Effect of arbuscularmycorrhizal fungi (AMF) and/or Baker's yeast (BY) on total phenolic and flavonoids of date palm offshoots var. Saidy under field conditions in El-Kharga and El-Dakhla during season 2016.

Treatments	Total phenolic compounds (mg/ g dry weight)		Total Flavonoids (mg/ g dry weight)	
	El-Kharga	El-Dakhla	El-Kharga	El-Dakhla
Arbuscular mycorrhizalfungi(AMF)	0.968b	1.025b	0.447b	0.435b
Baker's yeast (BY)	0.865c	0.968b	0.321c	0.302c
AMF+BY	1.256a	1.425a	0.585a	0.524a
Control	0.425d	0.502c	0.124d	0.155d

Different letters indicate significant differences among treatments within the same column according to least significant difference test ($P \leq 0.05$).

Biological control of fungal plant pathogens appears as an attractive and realistic approach, and numerous microorganisms have been identified as bio-control agents. A considerable role in limiting the populations of these pathogenic fungi inhabiting the above ground parts of plants is played by antagonistic microorganisms. Such properties are first of all exposed by the arbus cular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) (El-Mehalawy, 2004, El- Morsi, *et al.*, 2009 and Ziedan *et al.*, 2011).

In this study, we evaluated the effect of AMF and/or BY when applied as soil drench for suppression of root rot/ wilt complex of date palm seedling under artificial infection in pots and natural infection under nursery conditions. The obtained data show that AMF and BY significantly reduced root rot and wilt incidence either applied as individually treatment or in combination. While, the combination between AMF and BY reduced disease incidence more than used AMF or BY individually under pots and/or nursery conditions.

Our studies showed that biological control agents such as AMF and BY caused higher reduction in root rot and wilt severity in many crops (Azcon -Aguilar and Barea , 1996, El-Tarabily and Sivasithamparam, 2006 and El- Morsi *et al*., 2015). Arras (1996) stated that antibioses, competition for colonization sites and nutrients and degradation of the pathogen toxins and enzymes by yeasts may be involved in the suppuration of the growth of different patho genic fungi *in vitro* and *in vivo*.

AM fungi are known to increase the resistance of plants to soil-borne pathogens (Bagyaraj, 2006) by modification of cell wall, production of antimicrobial compound and altered rhizosphere micro flora (Sampangi and Bagyaraj, 1989). The disease suppressive effects of *P. fluorescens* are also well established and may result from production of antibiotics, side rophores, hydrocyanic acid, salicylic acid and competition for nutrients (Sharma, 2006).

On the other hand, all treatments significantly increased all tested growth parameters viz. number of roots offshoot^{-1} , offshoot height (cm), number of leaves offshoot^{-1}, leaflet number leaf^{-1}, nick leaf thickness (cm) and leaf area (cm^2) and increased mineral contents *viz.*nitrogen (%), phosphorus (%), potassium (%), sodium (%) and calcium (%) of date palm offshoots var. Saidy under nursery conditions in El-Kharga and El-Dakhla during 2016. Also, these treatments increased chlorophyll a, b and carotenoids contents in date palm offshoots leaves. The combination between AMF and BY increased all above parameters, mineral, chlorophyll a, b and carotenoids.

Arbuscular mycorrhizal fungi (AMF) can stimulate plant growth especially in soil substrates with low fertility. The enhancement of growth by AMF is mainly due to improved phosphorus absorption (Smith *et al*., 1986). In addition, mycorrhizal infection results in increased uptake of other macro and micronutrients (George *et al*., 1992). Plants get benefit by enhancement of nutrient and water uptake and other benefits such as resistance to stress factors. Arbuscular mycorrhizal fungi also protect host plants against soil pathogens and also promote synthesis of plant growth promoting hormones in particular auxins, cytokinins and gibberellins (Wanjiru, 2009). Under experimental conditions commercial crops inoculated with AMF have showed increased growth and yield.

The enhancing effect of yeast on the vegetative growth parameters, photosynthesis increased, producing bioactive substances, such as hormones and enzymes and controlling soil diseases was strongly supported by Shalaby and El-Nady (2008) and Agamy *et al*. (2013).

The oxidative enzymes i.e. peroxidase (PO), polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL) and catalase (CA) as well as total phenolic and flavonoid compounds are important in the defense mechanism against pathogens, through their role in the oxidation of phenolic compounds to quinines, causing increasing in antimicrobial activity. Therefore, they may be directly involved in stopping pathogen development (Ngadze *et al*., 2012).

The present results showed that, the AMF and BY individually and/or in combination which tested as soil treatment as resistance inducer treatments increased activities of PO, PPO, PAL and CA enzymes and total phenolic and flavonoids compounds to different extents in leaves of date palm offshoots comparing to the untreated control. The combination between AMF and BY recorded the highest activities of all tested oxidative enzymes and total phenolic and flavonoid compounds.

Different mechanisms have been shown to play arole in plant protection by AM fungi and BY namely: (I) enhancement of plant nutrition, (ii) competition with the pathogen for resources and space, (iii) plant morphological changes and barrier formation, (iv) changes in biochemical compounds related with plant response, (v) alleviation of physical stresses, and (vi) changes in antagonist and/ or deleterious microbe populations in the mycorrhizosphere (Vierheilig, 2004).

Oxidative enzymes are important in the defense mechanism against pathogens, through its role in the oxidation of phenolic compounds to quinines, causing increasing in antimicrobial activity. Therefore, it is may be directly involved in stopping pathogen development (Melo *et al.*, 2006 and Wen *et al.*, 2005); accelerating the cellular death of cells close to the infection site, preventing the advance of infection and/or by generating a toxic environment which will inhibit the growth of the pathogen inside the cells (Bi and Felton, 1995). Many plant phenolic compounds are known to be antimicrobial, function as precursors to structural polymers such as lignin, or serve as signal molecules (Hammerschmidt, 2005). Beckman (2000) has pointed out the importance of phenolic compounds in reducing wilt diseases may be less in their direct toxicity to the pathogen and more in host defense pathways and in signaling for host defenses. Phenolic compounds are building blocks for many secondary metabolites, including those involved in host defense responses.

In conclusion, the obtained results of the present study could suggest that soil dranch with arbuscular mycorrhizal fungi (AMF) and/or Baker's yeast (BY) can be used as a safe control measure of the disease on date palm offshoot and as a stimulant of vegetative growth parameters. Also, these treatments increased some mineral, chlorophyll a, b and carotenoids contents in date palm offshoots. In addition, they increased defense-related enzymes, phenols and flavonoids in leaves of date palm offshoots.

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تأثيرفطريات الميكروهيزا والخميرة على مرض عفن الجذور والذبول وصفات النمو لفسائل نخيل البلح في محافظة الوادي الجديد- مصر

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الملخص العربى

يعتبر مرض عفن الجذور والذبول من أهم الأمراض التى تصيب فسائل نخل البلح المنزرعة في المشاتل والبساتين الحديثة في محافظة الوادي الجديد- مصر. وفى هذا البحث تم دراسة تأثير اضافة لقاح فطر الميكروهيزا والخميرة الى التربة أما منفردين أو مجتمعين معا على شدة الإصابة بمرض عفن الجذور والذبول تحت ظروف العدوى الصناعية فى الاصص والعدوى الطبيعية فى المشتل وصفات النمو وتركيز العناصر المعدنية وصبغات التمثيل الضوئى فى أوراق فسائل نخيل البلح (صنف صعيدي)وتبين من النتائج المتحصل عليها أن كل هذه المعاملات أدت إلى خفض شدة الإصابة بهذا المرض وكانت المعاملة بفطريات الميكروهيزا مجتمعة مع الخميرة أكثر فاعلية من إستخدام كلا منهما على حده سواء فى تجارب الاصص او فى المشتل.

من ناحية أخرى تبين إن كل المعاملات المستخدمه تؤدى إلى زيادة صفات النمو المتمثلة في طول النبات وعدد الأوراق لكل فسيلة وعدد الوريقات لكل ورقة وسمك الورقة وكانت المعاملة بفطريات الميكروهيزا والخميرة مجتمعة معا أفضل من استخدام كلا" منها على حده. ايضا ادت المعاملة بكل من فطريات الميكروهيزا والخميرة الى زيادة محتوى اوراق فسائل النخيل من العناصر المعدنية ومنها النتروجين والفوسفور والبوتاسيوم والصوديوم والكالسيوم وصبغات الكلورفيل والكاروتين وكانت المعاملة بفطر الميكروهيزا مع الخميرة افضل من استخدام اى منهما بمفرده فى موقعى الدراسة (الخارجة والداخلة).

تبين من الدراسات البيوكيمائية تأثر نشاط الانزيمات المرتبطة بالمقاومة مثل انزيم البيروكسيديز والبولى فينيل اوكسيديز والفنيل الانين امونيا ليز والكتاليز بالاضافة الى الفينولات والفلافونيدات الكلية فى اوراق فسائل نخيل البلح المعاملة بفطريات الميكروهيزا والخميرة منفردة او مجتمعة معا. وكان إستخدام الميكروهيزا والخميرة معا أعطى أعلى نشاط لتلك الانزيمات والفينولات والفلافونديات مقارنة بإستخدام أى منهما بمفرده.

الكلمات الدالة: فسائل نخيل البلح، أمراض عفن الجذور والذبول، قياسات النمو، محتويات المعادن والصبغة، والإنزيمات المتعلقة بالدفاع، الفينولات، الفلافونويدات.