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Phytohormones Producing Fungal Endophytes Enhance Nutritional Status and Suppress Pathogenic Fungal Infection in Tomato

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ABSTRACT

Two endophytic fungi, namely, *Curvularia lunata* and *Nigrospora sphaerica*, were isolated from *Melia azedarach*, an exotic tree introduced in Egypt from Asia. The fungal endophytes were identified by microscopic examination and molecular identification of nucleotide sequence by DNA sequencing of the purified PCR product. Their antagonistic activities against phytopathogenic fungi and their ability to produce important growth hormone and providing some necessary nutrients for plant growth were also evaluated. Both endophytes exhibited antagonistic activities: *C. lunata* caused 56 and 50% growth inhibition of *Alternaria solani* and *Fusarium oxysporum*, while *N. sphaerica* suppressed both pathogenic fungi by 63.4 and 56.6%, respectively. *N. sphaerica* was able to dissolve insoluble phosphorus, produce ammonia, and secrete 40 µg mL^{-1} of IAA. In contrast, *C. lunata* failed to dissolve phosphorus, secreted less amount of IAA (3 µg mL^{-1}), but produced ammonia. A greenhouse pot experiment was conducted using phosphorus deficient soil to find out the ability of both endophytes to improve growth of tomato plants. *N. sphaerica* significantly increased shoot fresh weight by 13 and 22% over *C. lunata* and the control, respectively. Concerning the nutritional status of tomato plants, both endophytes led to significant increase in nitrogen concentration in shoots when applying 50% of the recommended mineral fertilizer. *N. sphaerica* enhanced phosphorus concentration in shoots by 13% over the control. Finally, the antifungal activities of both endophytes against *F. oxysporum* in tomato plants were tested under glasshouse conditions. *N. sphaerica* was more potent than *C. lunata* in suppressing 40% of *F. oxysporum* infection and had positive impact on tomato plant growth. Our study results highlight the potential use of *N. sphaerica* endophytic fungi as plant biofertilizers and bio-control agent under glasshouse conditions.

Keywords: Antifungal activity, *Curvularia lunata*, *F. oxysporum*, *Nigrospora sphaerica*, Plant growth promotion.

INTRODUCTION

Searching for new, safe, and eco-friendly alternatives of synthetic pesticides and chemical fertilizers is a major concern for clean environment and sustainable agriculture. To minimize environmental pollution and health problems, bio-control microorganisms and their metabolites could be a good alternative for different synthetic agrochemicals. Many microorganisms have the ability to promote plant growth and reduce disease and pest attack by different mechanisms. These modes of action include increasing nutrients availability by fixing, solubilizing, and mobilizing micro and macro elements and producing active secondary metabolites antagonistic to large scales of pest

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and disease (Akholiya and Khunt, 2015; Egamberdieva *et al*., 2016; Rybakova, 2016).

Endophytes are microorganisms that spend the whole or part of their lifecycle colonizing inter-and/or intracellular parts of their host plants tissues without causing any symptoms of disease (Azevedo and Araújo, 2007). Endophytes have a great impact on host plant growth and yield. It promotes plant growth, suppresses pathogens, increases tolerance to drought, and solubilizes nutrients. However, the endophyte-plant interaction relationship is not fully understood as it ranges from mutualism to latent pathogenesis depending on plants and microbes genotypes, environmental conditions, and the dynamic interactions between the plant microbiome (Redman *et al*., 2001; Tan and Zou, 2001). Therefore, the objectives of the present study were: (1) To isolate endophytic fungi from an important medicinal tree, namely, *Melia azedarach* L. (Meliaceae), which is known for its richness in limonoids and terpenoids compounds, and (2) To study the role of the isolated endophytic fungi in plant defense against phytopathogenic fungi *in vitro* and under greenhouse condition and their effects on plant growth and mineral nutrient uptake in phosphorus deficient soil.

MATERIALS AND METHODS

Isolation of Endophytic Fungi

Samples of healthy leaf of *M. azedarach* were collected from Faculty of Agriculture Garden, Alexandria (31° 12' 56.3" N, 29° 57' 18.97" E.) Egypt. Leaves were washed by tap water, followed by distilled water, then, surface sterilized by submerging the whole leaf in 90% ethanol for 1 minute, followed by 3.0% sodium hypochlorite for 3 minutes. Then, the leaves were rinsed in three changes of sterile/distilled water for 1 minute each. The sterilized samples were cut into 5 mm^2 pieces and placed in Petri plates containing Potato Dextrose Agar (PDA) media with 50 mg L^{-1} of ampicillin to suppress the bacterial growth. Petri plates were sealed and incubated at 25±2°C and checked on alternate days until the endophytes started to emerge from the leaf samples. The growing endophytes were sub-cultured onto plates containing PDA (Sunitha *et al*., 2013).

Identification of Fungal Isolates

The endophytic fungi were stained with lactophenol cotton blue and examined in 40× light microscope and were identified on the basis of colony characteristics and microscopic characters of the spores using standard manual (Barnett and Hunter, 1998).

The isolated endophytic fungi were subjected to DNA extraction, using the Qiagen DNA extraction kit (Qiagen, Germany). These fungi were identified by amplification of *ITS1-4* gene using universal primers according to Hafez and Elbestawy (2009). ITS 1 (Forward primer: 5` TCC GTA GGT GAA CCT GCG G 3`) and ITS 4 (Reverse primer: 5` TCC TCC GCT TAT TGA TAT GC 3`). 25 µL of PCR reaction mixture contained 5 µL master mix, 1 µL forward primer, 1 µL reverse primer, 1µL DNA Template and 17 µL distilled water. The PCR amplified products were analyzed by gel electrophoresis at approximately 600–700 bp. The Internal Transcribed Spacer (ITS) of ribosomal DNA were amplified, sequenced, and the nucleotide sequences were compared with those sequences already deposited in the data bank of the National Center for Biotechnology and Information (NCBI) using the nucleotide Basic Local Alignment Search Tool (BLAST) to find the most closely related sequences. The identification of the species was determined based on the best sequence alignment score. The nucleotide sequences were deposited in NCBI nucleotide sequence databases to get accession numbers.

In Vitro Antagonistic Activity of Endophytic Fungi by Dual Culture

The antagonism between the isolated endophytic fungi to each other and to two phytopathogenic fungi was carried out by dual culture according to Fokkema (1978).

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Table 1. ITS-based identification of fungal isolates and their accession numbers.

Experimental genotypes			Reference genotypes		Identity
Isolate	Accession number	Sequence size (bp)	Strain	Accession number	
Saad1	MF113055	650	CRWL3	HG938367.1	100 %
Saad2	MF113056	700	AN 2	KY859790.1	100 %

Phytopathogenic fungi; *Alternaria solani* (EMCC 756), *Fusarium oxysporum* (EMCC 137) were obtained from Microbiological Resource Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. One mycelial plug (5 mm) of each endophyte and pathogen were placed on the same dish 5 cm from each other with three replicates of each treatment and incubated at 27ºC. The Percent of Growth Inhibition (PGI) was calculated using the following formula:

PGI (%)= (KR-R1)/KR×100

Where, KR is the average of three colony diameters (mm) from the point of inoculation to the colony margin on the control dishes, and R1 is the colony diameters from the point of inoculation to the colony margin in the direction of the antagonist (Korsten *et al*., 1995). The PGI was categorized on a Growth Inhibition Category (GIC) scale from 0 to 4, where 0= No growth inhibition; 1= 1-25% growth inhibition; 2= 26-50%; 3= 51-75%; and 4= 76-100%. Inhibition zone was recorded as the distance between the two fungal growths after seven days.

Characterization of Endophytic Fungi for Their Plant Growth Promoting Capability

The endophytic isolates were cultured in Potato Dextrose Broth (PDB) amended with 0.5 mg mL^{-1} tryptophan for 2 weeks at 28ºC in shaker incubator at 150 rpm. The ability of endophytes to produce IAA was determined by colorimetric method as preliminary test and then a more reliable HPLC method was performed to quantify the IAA produced (Glickmann and Dessaux, 1995; Szkop and Bielawski, 2013). For the colorimetric method, the development of pink or red color after adding Salkowski's reagent to the previous supernatant and incubation for 30 minutes indicates IAA production, and concentration of IAA produced by the isolates were calculated using the calibration curve of pure IAA as a standard. For HPLC method, 10 mL of endophytic culture broth was centrifuged at 15,000×g at 4ºC for 30 minutes. The supernatant was analyzed on HPLC using UV-detector at 245 nm and C-18 column. Methanol: water (75:25) mixture was used as mobile phase with flow rate of 1 mL min^{-1}. The growth hormone was identified on the basis of retention time of the standard IAA.

Phosphate Solubilization and Acids Production by Endophytes

Both fungal endophytes were examined for their ability to solubilize insoluble phosphate by using Illmer media according to Illmer *et al*. (1995) with a modification in $CaHPO_4$ concentration ($CaHPO_4$ 2.4 g). The presence of clearing zones around the fungal colonies was considered as indicator for positive solubilization activity. Acid production of the endophytic isolates was detected according to Louw and Webley (1958). The two endophytes were cultured in media containing bromocresol purple to improve the clarity and visibility of the yellow colored halo zone around the positive colonies.

Ammonia Production

Two mL from the PDB growth media were taken and distilled using Kjeldahl

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apparatus by adding 0.2 g MgO. The resulting ammonia was collected in boric acid with mixed indicator, then, the distillate was titrated with 0.01 molar H_2SO_4 and the ammonia concentration was calculated as µg ammonia per 1 mL from the PDB media.

Growth Promoting Activity by Endophytic Fungi and Mineral Analysis

A split plot experimental design with three replicates was conducted to assess the efficacy of the two fungal endophytes (*N. sphaerica* and *C. lunata*) as bio-inoculants in enhancing tomato growth in a Phosphorus (P) deficient soil. Main plots were assigned to test three mineral fertilizers levels: Nitrogen (N), P, and potassium (K) at three rates (zero, 50, and 100%) of the recommended dose, with or without the two fungal endophytes spore suspensions as subplots. All the pots contained one kilogram of unsterile calcareous soil (EC= 3.3 dS m^{-1}, $CaCO_3$= 10%, N= 10 mg kg^{-1}, P= 12 mg $kg^{-1}$and K= 250 mg kg^{-1}). The endophytes' spores were added to the soil around tomato seedlings two times at the rate of 10 mL pot^{-1}, each addition contained 10^7 fungal spores mL^{-1} of *N. sphaerica* or *C. lunata*. The first inoculation for each of the endophytic fungi was at the beginning of cultivation and the second one was in the middle of the plant growing period. The plants were harvested after 45 days of growth, fresh weight was measured, and then the whole plants were dried at 70°C for 48 hours until constant weight. Then, 0.1 g of dried ground plant was digested by using a mixture of concentrated sulfuric acid and hydrogen peroxide and then the total N was determined according to Bremner and Mulvaney (1982). Total P was determined calorimetrically according to Olsen and Sommers (1982), and K content was determined by flame emission spectrophotometry using a flame photometer according to Horneck and Hanson (1998).

Evaluation of Antagonistic Activities of Endophytes under Glasshouse Conditions

Endophytes and phytopathogenic fungi (*F. oxysporum)* were grown in PDA plates at 28°C for 1 week. The cultures were flooded with sterile distilled water, scrapped with surface sterilized spatula, and filtered through cheesecloth. Spore suspension concentration was determined and adjusted to 10^7 and 10^5 spore mL^{-1} for endophytes and pathogenic fungi, respectively. One-week old tomato seedling was planted in 9 cm pots containing 1 kg of unsterile soil in a greenhouse under natural sunlight at 30±2°C. At the beginning of the experiment, ten mL of endophytes suspension were applied to soil surface. Ten days later, the soil was re-inoculated by endophyte suspension before adding pathogenic fungi to encourage plant–endophyte association. Two types of control treatments were used. In the first control treatment, neither endophytes nor phytopathogenic fungi were added to the soil, while in the second control treatment, 10 mL of *F. oxysporum* spore suspension (10^5 spore ml^{-1}) was added. Pots were arranged in a randomized design in glass house at 30±2°C with 12 hours photoperiod (3.3 µmol m^{-2} s^{-1}) and were irrigated with 100% nutrients as per required for 45 days. Plants were daily observed and disease symptoms were recorded. Then, the plants were uprooted to measure seedling growth parameters and to calculate disease severity index. Disease severity was evaluated for each plant on a zero to 4 rating scale according to percentage of shoot and roots affected by necrosis, wilt, and dark brown colors (Zhang *et al*., 2012): 0= Healthy plants, 1= 1 to 33%, 2= 34 to 66%, 3= 67 to 97%, 4= Dead plants.

Disease severity (%)= {Σ (No. infected plants×Their infected degree)/(Total examined tested plants×Upper infected degree)}×100.

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Statistical Analysis

All treatments were performed in triplicate and all the values were reported as average of triplicate determination. Data of the nutrition experiment was analyzed using split plot design. According to Naessens *et al*. (1986), the difference among the treatments at the 0.05 significant level was determined by Least Significant Difference (LSD) test using SAS procedures. Inhibition percent of phytopathogenic fungus by the endophytic fungi was subjected to one-way analysis of variance followed by Student–Newman–Keuls test (Cohort Software Inc. 1985) to determine significant differences between mean values at the probability level of 0.05.

RESULTS

Identification of Endophytic Fungi

Under the light microscope, fungal isolates (Figure 1) were identified to genus level as *Curvularia* sp. and *Nigrospora* sp, by their sporulation structures on PDA growing medium.

In the present study, PCR amplification of the ITS regions resulted in fragments ranged in length between 650 to 700 bp (Figure 2). The NCBI nucleotide database matching results of the sequences obtained in this study indicated that the two examined isolates belonged to two different genera. The observed high identity results (100%) to database sequences allowed the authentication of the isolates at the species level as *C. lunata* (GenBank accession no. MF113056) and *N. sphaerica* (GenBank accession no. MF113055).

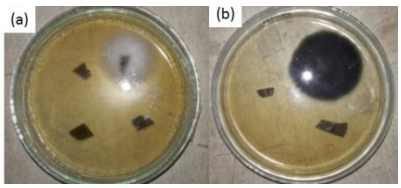

Figure 1. The endophytic fungi emerging from *Melia azedarach* leaves samples: (a) The white growth to the left is *Nigrospora sphaerica* and (b) The dark growth to the right is *Curvularia lunata*.

Antagonistic Activities of Endophytic Fungi by Dual Culture

Dual culture bioassay showed antagonistic activities of both endophytic fungi to each other and to the phytopathogenic fungi. *C. lunata* antagonistic activity was characterized by the existence of inhibition zone (Figure 4) with *A. solani*, *F. oxysporum* and *N. sphaerica*. The antagonistic effects of *C. lunata* ranged from intermediate antagonism of both *Alt. solani* and *F. oxysporum* to high antagonism of *N. sphaerica* (Table 6). *N. sphaerica* showed medium antagonism of both *F. oxysporum* and *A. solani* with no inhibition zone (Figure 3).

The Ability of Endophytic Isolates to Produce IAA and Ammonia and to Solubilize Phosphorus

N. sphaerica showed significant production of IAA 40 µg mL^{-1} and had the

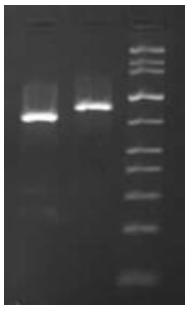

Figure 2. Agarose gel electrophoresis of the ITS regions amplified using ITS1 and ITS4 PCR primers. The gel shows (from right to left) DNA Ladder and the 700,650 bp fragments of *Curvularia lunata* and *Nigrospora sphaerica* respectively.

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ability to dissolve insoluble phosphate and produce acids. In contrast, *C. lunata* failed to solubilize phosphate or produce acid, and produced lower amount of IAA 3 µg mL^{-1} (Figure 3). Both fungal endophytes were able to produce ammonia at nearly the same rate.

Effect of Mineral Fertilizer and Endophytes on Tomato Growth

Analysis of variance presented in Table 2 shows the effect of mineral fertilizer levels and the tested fungal endophytes on both fresh and dry weight of shoot and root of tomato plant. The data revealed that shoot and root weights were highly significantly affected by the two main studied factors, except in case of root fresh weight. On the other hand, the interaction between the factors was not significant for all the tested parameters, except root dry weight. Mean values of shoot and root fresh weight and shoot dry weight were affected by mineral fertilizer levels and increased significantly by increasing NPK rates in soil, reaching 30.76, 7.85, and 4.99 g per plant, respectively (Table 4). Table 5 shows the mean values for shoot fresh and dry weight as affected by the two tested endophytes; the superiority of *N. sphaerica* compared to *C. lunata* was observed.

Nutrients Content in Tomato Plants

Table 2. Means squares and level of significance of fresh and dry weight (g) of tomato plant as affected by mineral fertilizers levels and endophytes treatments.

Effect	df	Mean squares			
		Fresh weight (g)		Dry weight (g)	
		Shoot	root	Shoot	root
Mineral fertilizer levels (A)	2	222.08 ***	4.90 *	8.52 **	.83 ***
Added endophytes (B)	2	60.48 ***	2.38 ns	4.26*	0.72 ns
A×B	4	12.31 ns	2.25 ns	0.81 ns	0.18ns

* Significant at 0.05 level of probability; ** Significant at 0.01 level of probability; *** Significant at 0.001 level of probability; ns: Non-significant, df= Degree of freedom.

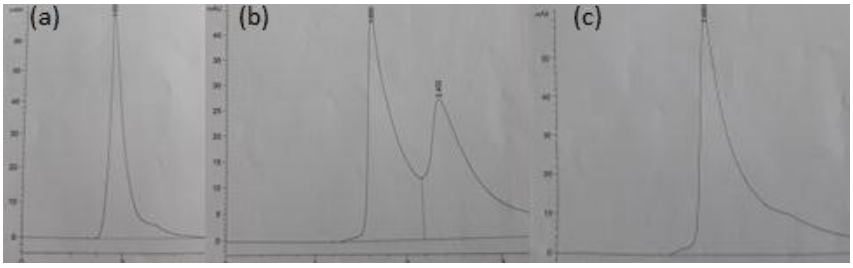

Figure 3. HPLC chromatogram of: (a) Standard Indole acetic acid, (b) *Nigrospora sphaerica* filtrate, (c) *Curvularia lunata* filtrate.

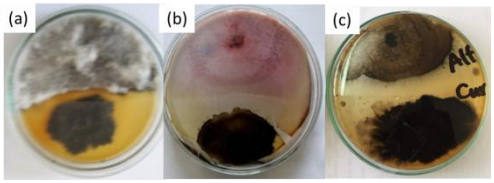

Figure 4. Antagonistic effect of endophytic fungi by dual culture: (a) *Curvularia lunata* and *Nigrospora sphaerica*; (b) *C. lunata* and *Fusarium oxysporum*; (c) *C. lunata* and *A. solani*.

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The effect of mineral fertilizer and the two tested endophytes on the concentration of N in the shoot and root of tomato plant is presented in Table 3. Analysis of variance revealed highly significant effect with using the increasing rates of NPK fertilizers. The use of endophytes also gave highly significant effect on N concentration. It is clear that *N. sphaerica* was able to increase the N content of the tomato plant when compared to the control (no endophytes) and this increase was clearly evident in plant that obtained the fully recommended mineral fertilization. The interaction between the effect of mineral fertilization and the endophytes inoculation produced positive results with tomato leaf and root N content. This is clearly shown in Figure 5, which indicates that the inoculation of *N. sphaerica* with 50% of the recommended mineral fertilization has led to a significant increase in N content of leaves and root of tomato plants.

Phosphorus concentration was significantly increased in leaves and roots of tomato plants, when inoculated with the *N. sphaerica*, and the same result as for N was obtained when we added the full dose of the recommended mineral phosphorus fertilization. Our results showed that *N.*

Table 3. Means squares and level of significance of total nitrogen, phosphorus, and potassium concentration in tomato plant as affected by mineral fertilizers levels and endophytes treatments.

Effect	df	Mean squares					
		Nitrogen (%)		Phosphorus (%)		Potassium (%)	
		Shoot	Root	Shoot	Root	Shoot	Root
Mineral fertilizer levels (A)	2	8.65***	2.38***	0.30***	0.08***	21.25***	4.15***
Added endophytes (B)	2	0.37***	0.47***	0.01*	0.01*	0.05 ns	0.46***
A*B	4	0.05*	0.09*	0.003 ns	0.003 ns	0.05 ns	0.18ns

* Significant at 0.05 level of probability; ** Significant at 0.01 level of probability; *** Significant at 0.001 level of probability; ns: Non-significant, df= Degree of freedom.

Table 4. Means of fresh and dry shoot weights, phosphorus and potassium concentration in shoot of tomato plant as affected by mineral fertilizers levels.[a]

	fresh weight (g)		Shoot dry	Phosphorus (%)		Potassium (%)	
	Shoot	Root	Weight(g)	Shoot	Root	Shoot	Root
0 (%)	21.12 c	6.59 b	3.10 b	0.23 c	0.18 c	1.65 c	1.07 c
50 (%)	23.86 b	6.56 b	3.65 b	.33 b	0.27 b	3.42 b	1.73 b
100 (%)	30.76 a	7.85 a	4.99 a	0.58 a	0.38 a	4.71 a	2.42 a
LSD (0.05)	2.4	1.11	1.09	0.03	0.02	0.12	0.06

[a] Means in the same column followed by the same letters are not significantly different at 0.05 level of probability. $LSD_{0.05}$= Least Significant Difference at 0.05 level probability.

Table 5. Means of shoot fresh and dry weight and phosphorus content of tomato plant as affected by endophytes treatments.[a]

Endophytes treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Endophytes treatments	Phosphorus (%)	
				Shoot	Root
No endophytes	22.98 b	3.45 b	0.35 b	0.24 b	No endophytes
C. lunata	24.70 b	3.59 b	0.37 b	0.28 ab	C. lunata
N. sphaerica	28.07 a	4.07 a	0.42 a	0.31 a	N. sphaerica
LSD (0.05)	1.98	1.06	0.04	0.039	LSD (0.05)

[a] Means in the same column followed by the same letters are not significantly different at 0.05 level of probability. $LSD_{0.05}$= Least Significant Difference at 0.05 level probability.

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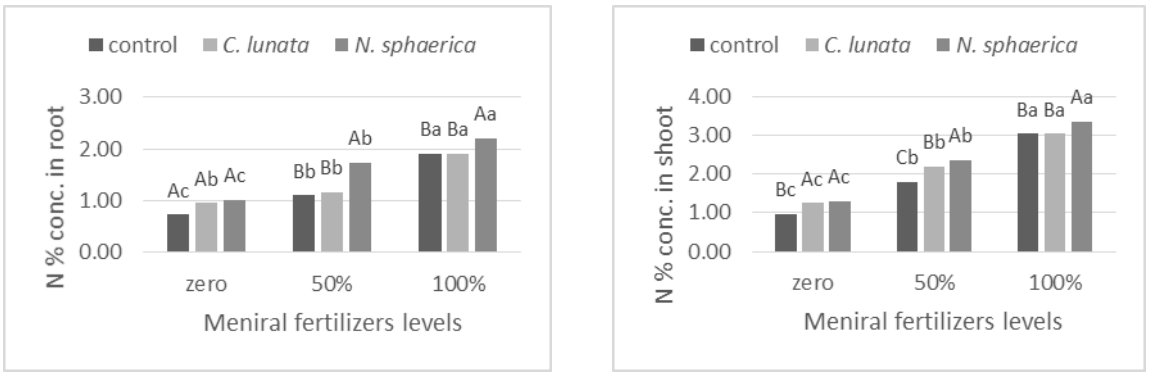

(a) (b)

Figure 5. Effect of three mineral fertilizers levels and the two tested endophytes on: (a) Nitrogen concentration in root, (b) N concentration in shoot. Bars marked with the same capital letter indicate no significant difference among the tested endophytes treatments under the same NPK level. Bars marked with the same small letter indicate no significant difference among the three mineral fertilizers levels for the same endophytes treatments.

sphaerica had the highest phosphate solubilization capability.

Concerning K, addition of the recommended complete mineral dose resulted in a significant increase in the concentration of the plant tissues; 37% higher than the plants fertilized with half the dose and three fold of the plants that were not fertilized by this element. In spite of the positive effect of the addition of K fertilization on the tomato plant, inoculation with both fungal endophytes did not have any positive or significant effect on its concentration in tomato leaves (Table 3).

Antagonistic Activities of Endophytic Fungi against *F. oxysporum* (EMCC 137) under Greenhouse Condition

Under pots condition, phytopathogenic fungi *F. oxysporum* caused significant reduction in shoot and root lengths, older leaves turned brown, and the plant frequently wilted. A cross section of the root showed that plants inoculated with *F. oxysporum* alone were infected with the pathogen and vascular tissue was brown and disease severity percent was 50%.

Table 6. Antagonistic activities of endophytic fungi to each other and to phytopathogenic fungi by dual bioassay.

Fungus	*Curvularia lunata* (MF113056)				*Nigrospora sphaerica* (MF113055)			
	Phytopathogenic fungi		*C. lunata*		Phytopathogenic fungi		*N. sphaerica*	
	PGI (%)	GIC	PGI (%)	GIC	PGI (%)	GIC	PGI (%)	GIC
Alternaria solani (EMCC 756)	56	3	15	1	63.4	3	25	1
Fusarium oxysporum (EMCC 137)	50	2	25	1	56.6	3	30	2
Nigrospora sphaerica (MF113055)	70.2	3	14.3	1	-	-	-	-

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Interestingly, treatment of soil by *N. sphaerica* endophyte suppressed the infection and had 10% disease severity and increased root and shoot lengths of tomato plants (Table 7). *C. lunata* was less antagonistic to *F. oxysporum* under glass house conditions, causing disease severity of 30%, although it exhibited high antagonistic activity against *F. oxysporum* under *in vitro* dual culture bioassay.

DISCUSSION

To the best of our knowledge, this is the first isolation of *Curvularia lunata* as an endophytic fungus from *M. azedarach.* However, *C. lunata* was isolated before from *Azadirachta indica* by Verma *et al*. (2011) and from *Cymbopogon caesius* grass by Avinash *et al*. (2015). *N. sphaerica* was isolated from *Moringa oleifera* by Zhao *et al*. (2012), from *Melia azedarach* by Dos Santos and Rodrigues (2003) and from *Indigofera suffruticosa* by Santos *et al.* (2015).

This study showed the importance of using *N. sphaerica* and its ability to increase tomato plant biomass and its capability to reduce the infection rate of wilt disease caused by *F. oxysporum* in tomato plants under glasshouse conditions. The positive effect of *N. sphaerica* on fresh and dry weights of tomato might be attributed to its ability to produce plant growth promoters such as IAA, producing ammonia and acids, and solubilizing phosphorus, which can increase plant growth and minerals uptake. This result was supported by the findings of Glick *et al*., (2007), who found that increasing IAA and decreasing ethylene level increased root and shoot length as well as weight of the tested plant. In addition, Saad El-Din (2017) studied the effect of endophytes isolated from the medicinal plant (*Teucrium polium* L.) as bio-inocula for maize plant. The result showed higher fresh and dry weight for the inoculated plant and concluded that endophytes directly promote plant growth through the production of plant hormones, particularly IAA. Fungal endophytes can also help plants to access insoluble P through excretion of protons or enzymatic production that solubilize insoluble P and significantly improve plant growth (Oteino *et al*., 2015; Taktek *et al*., 2017). This may be due to the close linkage of endophytes inside plant tissues, which facilitates nutrients exchange and enzymes activity (Matsuoka *et al*., 2013; Murphy *et al*., 2014).

Many studies suggest that endophytes can mitigate disease symptoms of phytopathogenic fungi and improve plant growth by numerous modes (Shahzad *et al*., 2016; Egamberdieva *et al*., 2017). Although the mechanism is not fully understood, results of those studies suggest that pre-inoculation of plant root system by endophytes can interfere with the early infection processes and disease development by pathogenic fungi. Moreover, production of ammonia by fungal endophytes not only provides plants with N or decreases the cost of crop production but also intensifies the plant defense against phytopathogens colonization (Li *et al*., 2016). Endophytes can also increase P solubilization and nutrients uptake, which help the host plant to

Table 7. Effects of antagonistic endophytes (*Curvularia lunata and Nigrospora spherica*) on seedling growth of tomato plant inoculated with *F. oxysporum* in greenhouse experiment.[a]

Fungi treatments	Shoot length (cm)	Root length (cm)
Control	45.0±1.00 a	20.0±0.00 a
F. oxysporum	41.5±1.50 b	10.5±2.50 c
C. lunata + *F. oxysporum*	41.5±1.50 b	13.5±2.50 c
N. spherica+ *F. oxysporum*	44.5±1.50 a	16.5±1.50 b

[a] Data are expressed as means±SE from experiments with three replicates. Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

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resist disease infection (Servin, 2004; Ongena and Jacques, 2008; Mei and Flinn, 2010; Zhao *et al*., 2014; Lecomte *et al*., 2016; Torres *et al*., 2016). This was the case with *N. sphaerica*, which had the ability to solubilize phosphorus, introducing acids and producing good amount of ammonia and IAA, so, all this may help tomato plant to resist *F. oxysporum* infection.

Although the laboratory tests proved that *C. lunata* had antagonistic activity against phytopathogenic fungi and it can produce ammonia and IAA hormone, we conclude that its poor results as a bio-control agent, or as plant growth promoter, when used in soil may be due to the lack of adaptation to high pH and lack of P availability in the calcareous soil used in this study. Concerning this issue, we propose testing *C. lunata* on different soil types to determine the optimum conditions for more efficient uses of this fungus. In contrast, *N. sphaerica* proved to be more adaptable and well suited as a bio-control agent and vital fertilizer for this type of soil. However, we cannot say by this study whether these endophytes isolated from *M. azedarach* colonized tomato plants and acted as endophytes or acted as rhizospheric organisms, thoug it enhanced tomato plant growth under these environmental conditions. As previous studies have found, some endophytes are able to colonize a wide range of host plants while others are specialized to one or a few hosts and some endophytes could colonize above- and below-ground plant tissues, i.e., the rhizosphere and aerial tissues (Zabalgogeazcoa, 2008; Rodriguez *et al*., 2009). This plant-endophyte interaction varies from mutualism to pathogenicity; it depends on a set of abiotic and biotic factors (Hardoim *et al*., 2015). Therefore, more studies are needed to focus on establishing the colonizing ability of these endophytes to understand the symbiotic relationship between endophytes and their host plant and other plant species under different environmental conditions. Further studies are required to isolate secondary metabolites, which may be responsible for the antifungal activities of these endophytic fungi, and to evaluate the potential of co-inoculation of the two endophytes and their effect on phytopathogenic microorganisms and plant growth under green house and field conditions.

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قارچ های درون رُست تولید کننده هورمون های گیاهی باعث بهبود وضعیت تغذیه و جلوگیری از عفونت قارچی گوجه فرنگی می شوند

م. م. گ. سعد، و ح. ه. بدری

چکیده

دو قارچ درون رُست به نام های *Curvularia lunata* و *Nigrospora sphaerica* از درخت نابومی *Melia azedarach* که از آسیا به مصر آورده شده بود جداسازی شد. قارچ های درون رُست با بررسیهای میکروسکوپی و شناساسی ملکولی توالی نئوکلئوتید به وسیله تعیین ترتیب دی.ان.ا. (DNA sequencing) مربوط به محصول خالص PCR شناسایی شد. سپس، فعالیت های آنتاگونیستی آنها علیه قارچ بیماریزای گیاهی و توان آنها در تهیه هرمونهای مهم گیاهی و تامین برخی عناصر غذایی لازم برای رشد گیاه مورد ارزیابی قرار گرفت. هردوقارچ درون رُست فعالیتهای آنتاگونیستی نشان دادند: *C. lunata* باعث ۵۶٪ و ۵۰٪ بازدارندگی رشد از، به ترتیب، *Alternaria solani* و *Fusarium oxysporum* شد در حالیکه *N. sphaerica* در حد ۶۳/۴٪ و ۵۶/۶٪ از رشد هر دو قارچ بیماریزا جلوگیری کرد. *N. sphaerica* قادر به حل کردن فسفر نامحلول، تولید آمونیاک، و تراوش $40\ \mu g\ mL^{-1}$ از ماده IAA بود. در مقایسه، *C. lunata* نتوانست فسفر را حل کند و مقدار کمتری IAA ($3\ \mu g\ mL^{-1}$) تراوش داشت ولی آمونیاک را تولید کرد. نیز، برای تعیین توانایی هردو قارچ درون رُست برای بهبود رشد گیاه گوجه فرنگی، یک آزمایش گلدانی در گلخانه با کاربرد خاک دارای کمبود فسفر اجرا شد. نتایج نشان داد که *N. sphaerica* باعث افزایش معنادار وزن تازه شاخسار در حد ۱۳٪ و ۲۲٪ در مقایسه با ، به ترتیب، *C. lunata* و تیمار شاهد شد. در ارتباط با وضعیت تغذیه گیاه گوجه فرنگی، در تیماری که ۵۰٪ کود معدنی توصیه شده مصرف شده بود، هر دو قارچ درون رُست منجر به افزایش معنادار غلظت نیتروژن در شاخسار شدند. *N. sphaerica* باعث ۱۳٪ افزایش غلظت فسفر در شاخسار در مقایسه با تیمار شاهد شد. در آخر، فعالیت های ضد قارچی هر دوقارچ درون رُست علیه *F. oxysporum* در گوجه فرنگی در شرایط گلخانه ای بررسی شد. قارچ *N. sphaerica* یا ۴۰ ٪ بازدارندگی آلودگی *F. oxysporum* قوی تر از *C. lunata* عمل کرد و اثرهای مثبتی روی رشد گیاه گوجه فرنگی داشت. نتایج پژوهش ما، پتانسیل استفاده از قارچ درون رُست *N. sphaerica* را به عنوان یک کود زیستی و کنترل کننده زیستی در شرایط گلخانه به خوبی نشان می دهد.

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