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Influence of Endophytic Fungi and Growing Media on the Growth and Some Chemical Constitutes in *Chrysophyllum oliviforme* Seedlings.

Ebeid AFA[1]* and Yasmin M Shebany[2].

[1]Timber Trees Dept., Hort. Res. Instit., ARC, Egypt.
[2]Dept. of Botany (Microbiology), Fac. of Science, Qena, South Valley Univ, Egypt.

ABSTRACT

The present study was carried out during 2015 and 2016 seasons in Kom- Ombo Tropical Farm, Aswan Botanical Garden, Hor. Res. Center, ARC, Egypt to determine the effects of endophytic fungi and growing media on Satinleaf (*Chrysophyllum oliviforme* L.) seedling growth. Three endophytic fungi i.e. *Alternaria alternata, Chaetomium globosum* and *Nigrospora oryzae* as well as three potting media namely peat moss, loamy sand and sand were used to study their effects on the growth attributes such as seedling length, root length, stem diameter, fresh and dry root weight, fresh and dry seedling weight as well as some of chemical compositions. The obtained results pointed out that all the used endophytic fungi had significant effects on the seedling parameters compared to the control. Applied of *A. alternata* resulted in the most effective on seedling growth, while applied *N. oryzae* resulted in the best result for chlorophyll a and nitrogen content for the seedlings of *C. oliviforme*. On the other side, among the used media, peat moss is the best for growing seedlings while, using sand as potting media resulted in the lowest values of growth characters.
Keywords: *Chrysophyllum oliviforme,* Endophytic Fungi, Growing media.

***Corresponding author**

INTRODUCTION

Chrysophyllum oliviforme L. (Satinleaf), Sapotaceae family, also known as wild star-apple and saffron-tree, is a beautiful, evergreen shrub or small tree usually 3 to 5 m in height. Under favorable conditions, older individuals occasionally reach 10 m or more in height and 30 cm in diameter at breast height. Growth is relatively slow at all stages, and the plants are relatively long-lived. The wood, which has a specific gravity of 0.9, is hard, heavy, and strong. It is used for construction in Cuba **(Little and Wadsworth, 1964).** Satinleaf has been extensively, but not intensively, planted as an ornamental tree, it makes a pretty addition to natural landscaping, a good foundation plant, and an attractive lawn, street, and parking lot tree **(Gilman and Watson, 1993).**

Endophytes are microorganisms that colonize and found asymptomatic infections in healthy plants **(Suryanarayanan and Kumaresan, 2000).** Fungal endophytes are considered as mycorrhizal associations among temperate-zone plants **(Carroll, 1988),** it has been found in conifers **(Legault *et al*., 1989),** and both monocotyledonous **(Frohlich *et al*., 2000)** and dicotyledonous angiosperms **(Rajagopal and Suryanarayanan, 2000).** Endophytic fungi have attracted great attention in the past few decades due to its ability to produce novel secondary metabolites for medical, agricultural and industrial use, and they are also considered as an outstanding source of bioactive compounds due to its ability to occupy any plants at any environments **(Strobel and Daisy, 2003).** Endophytic fungi are asymptomatic and may be mutualistic; plant protect and feed endophytes, which produce plant- growth- regulatory, antimicrobial, antiviral or insecticidal substances to enhance the growth and competitiveness of the host in nature **(Carroll, 1988).** Due to their capability of colonizing internal tissues of plants and their ability to promote plant growth and to control plant diseases, endophytic microorganisms can be used in agriculture as a tool to improve crop performance **.** In the past two decades, a great deal of information on the diversity and the role of endophytes have been collected **(Yuan *et al*., 2009).** In this respect, **Srivastava *et al.* (2011)** stated that inoculation of tomato seeds with *Fusarium pallidoroseum* improved proline content, acid and alkaline phosphomonoesterase and peroxidase activity. Moreover, the fungus enhanced shoot length and dry weight of wheat, maize, marigold, okra and brinjal over the control. However, **Anuar *et al.* (2015)** on their study of endophytic fungus *Phlebia Gano*EF3 on the growth of oil palm seedlings showed that seedlings treated with containing *Phlebia Gano*EF3 increased the seedling growth compared to untreated one. Also, these findings pointed out that *Phlebia Gano* EF3 is suitable to be used as biofertilizer for oil palm seedlings. Moreover, **Abou Alhamed and Shebany (2012)** study the impact of *Chaetomium globosum* isolated from *Althaea rosea* leaves on copper stress resistance of maize seedlings found that addition of the endophytic *C. globosum* alleviated the toxic effect of copper on maize growth. Also, treated of copper sulphate in combined with *Chaetomium* induced seedling dry weight, osmotic solute content and antioxidant enzyme.

Production of high quality tree seedlings is the main objective of tree nurseries but the slow growth of seedlings as *Chrysophyllum oliviforme* limits the high quality seedling production. However, using the appropriate growth media is an alternative to soil in which physical properties as aeration, drainage and water holding capacity are lacking. Also, applied microorganisms as biofertilizers in agriculture is considered to be a way to maintain the environment and ensure pollution. Nutrient value of the growth media can be enhanced by addition of beneficial microbes like plant growth promoting microorganisms (**Murugesan *et al*., 2016).** Therefore, the objective of this study was to examine three types of endophytic fungi namely; *Chaetomium globosum, Nigrospora oryzae* and *Alternaria alternata* on the growth and some chemical composition of *Chrysophyllum oliviforme* seedlings grown in different soil media at the nursery level**.**

MATERIALS AND METHODS

The experiment was conducted at the Tropical Farm of Kom-Ombo, Aswan Botanical Garden, Horticulture Research Institute, ARC, Egypt during the two seasons of 2015 and 2016 to investigate the effect of endophytic fungi and some growing media on the growth and chemical composition of *Chrysophyllum oliviforme* L.

Seed collection, preparation and planting:

Seeds of *C. oliviforme* were collected from mature trees in the Tropical Farm of Kom- Ombo, Aswan in February of the two seasons. Seeds were soaked in sterile water, for approximately about 48 hr under room temperature in order to speed up germination. The water was changed frequently to avoid fungal contamination. Thereafter, the seeds were sown were in 10th February of 2015 and 2016 in a seedling trays filled with river sand and after developing four true leaves (60 days after sowing), healthy, uniform transplants (7–10 cm tall) were used in the trial.

Fungal isolation, identification and cultivation:

Ocimum basilicum leaves were collected from different sites of South Valley University campus at Qena, Egypt. Samples were washed carefully by tap water, then by sterilized distilled water. Samples were surface sterilized by sequential immersion in 75% ethanol for 1 min, 4 % sodium hypochlorite for 3 min finally, 75% ethanol for 30 s. The samples were then rinsed by using sterilized distilled water, and shade dried **(Filip *et al*., 2003).** Leaves were cut for small pieces and placed on prior poured glucose-Czapek's agar plates according to **Smith and Dawson (1944)** then incubated at 28± 2 °C for 2 - 3 weeks in a microbiological incubator until the desired (*Chaetomium globosum*, , *Nigrospora oryzae* and *Alternaria alternate*) fungi growth.

Transplanting in the growth media and Inoculation:

One vigorous seedling of *C. oliviforme* was transplanted into each plastic pot of 5-L volume filled with different media according to treatments. The seedlings were planted on 10th April of the two seasons in plastic pots filled with loamy sand soil (from the top surface of land from the Tropical Farm) or sand or peat moss. The chemical properties of the used media are shown in Table (1a &b). Transplants were planted into a pots filled with the prepared substrate where the inoculum had been application by endophytic fungi or the control (non-inoculated plants). Treatments was commenced in 20th April and continued until 20th August of seasons; five doses in this period, every dose was 50 cm^3 / plant every 30 days, added as spray to the aboveground biomass. Plants were kept in a glasshouse with a 16/18 h light/dark photoperiod, 26/22°C light/dark thermoperiod. Each polythene bag was watered and weeded as at when due.

Table (1a): Some of the chemical properties of the used sand and loamy sand media.

Soil	PH	$CaCO_3$ %	OM %	EC dSm^{-1}	Soluble cations and anions (meq /100 g)							
					Cations				Anions			
Sand	7.75	2.73	0.03	0.86	Na^+	K^+	Ca^{++}	Mg^{++}	$SO4^-$	Cl^-	HCO_3^-	CO_3^-
					0.12	0.03	0.36	0.11	0.30	0.13	0.11	0
Loamy sand	8.50	0.45	0.41	0.25	0.04	0.12	0.43	0.25	0.06	0.03	0.09	0

Table (1b): Some of the chemical properties of the used peatmoss media.

O.C.%	Total N%	C/N ratio	PH 1:2.5	EC dSm^{-1}	Some of minerals in soil (ppm)					
					P	K	Fe	Mn	Zn	Cu
44.35	0.54	82.13	6.11	0.39	22	665	8.76	4.70	4.55	0.53

Experimental design and statistical analysis:

The polythene bags were arranged in a randomized complete design (RCD) as twelve (3 endophytic fungi plus control x 3 growth media treatments) replicated five times to give a total of 60 .The means of the different treatments were compared by using L.S.D test at 5% probability, according to **Snedecor and Cochran (1980).**

Data collection:

The effects of the treatments were evaluated five months later (in 20th September) based on seedling height, stem diameter, fresh and dry weights of shoots and roots, number of leaves / plant. Chemical determinations: Chlorophyll a, b and total carotenoids were determined according to **Saric *et al.* (1967),** total carbohydrates in dried leaves were determined according to **Herbert *et al.* (1971).** Minerals contents of N, P and K were determined in the dried sample of leaves at 65 C^O for 48 hrs using method described by **(Cotteine, 1980).**

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RESULTS

Shoot parameters:

Data presented in Tables 2 &3 pointed out that the transplants treated by *Alternaria alternate* were significantly increasing values of plant height and stem diameter (24.3 and 0.31 cm respectively) in the mean of seasons compared to the other treatments. Data in the same Table disclosed that transplants grown in peat moss medium gave the highest values of plant height and stem diameter (22.1, and 0.24 cm, respectively) in the mean of seasons. The differences of interaction between the endophytic fungi and growth media treatments were significant. Plant height and stem diameter of *C. oliviforme* transplants were improved as a result for using *A. alternate* and planting in peat moss medium in the first and second seasons.

Table (2): Means of plant height (cm) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Plant height (cm)							
Control	14.83	12.50	16.89	**14.74**	15.16	12.85	16.88	**14.96**
C. *globosum*	18.19	15.56	18.98	**17.58**	17.16	14.84	19.09	**17.03**
N. *oryzae*	22.75	20.10	25.21	**22.69**	21.26	19.93	25.97	**22.39**
A. *alternata*	24.20	20.90	26.39	**23.83**	25.56	21.38	27.23	**24.72**
Mean (B)	**19.99**	**17.27**	**21.87**		**19.79**	**17.25**	**22.29**	
LSD 5%	**A: 0.95 B:0.59 Ax B:1.17**				**A:0.31 B:0.60 AxB:1.19**			

Table (3): Means of stem diameter (cm) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Stem diameter (cm)							
Control	0.15	0.13	0.17	**0.15**	0.15	0.12	0.19	**0.15**
C. *globosum*	0.20	0.19	0.22	**0.20**	0.21	0.19	0.23	**0.21**
N. *oryzae*	0.19	0.24	0.21	**0.21**	0.19	0.25	0.21	**0.22**
A. *alternata*	0.31	0.24	0.35	**0.30**	0.31	0.27	0.34	**0.31**
Mean (B)	**0.21**	**0.20**	**0.24**		**0.22**	**0.21**	**0.24**	
LSD 5%	**A: 0.02 B:0.24 AxB:0.49**				**A:0.01 B: 0.25 AxB:0.50**			

Applied of the different endophytic fungi as spraying treatments on *C. oliviforme* transplants was significantly increased shoot fresh and dry weight compared to the control in the two seasons as shown in Tables 4&5. However, *A. alternate* followed by *C. globosum* were the most effective treatments for the previous characteristics in the mean of seasons. Growing of *C. oliviforme* transplants in peat moss as media resulted in significantly increasing of the shoot fresh and dry weight compared to the other growth medium in the two seasons. Regarding the interaction treatments, it is clear from the same Table that the differences were significant. Transplants that applied with *A. alternate* and grown in peat moss medium gave the highest values of the shoot fresh weight compared to the other treatments in the first and second seasons. Meanwhile, the treatment of *A. alternate* in combination with the loamy sand soil was more effective for shoot dry weight in the tow saeasons.

Table (4): Means of shoot fresh weight (gm) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Shoot fresh weight (gm)							
Control	2.99	2.37	4.55	**3.30**	2.90	2.60	4.39	**3.30**
C. *globosum*	4.42	3.58	5.29	**4.43**	4.52	3.72	5.59	**4.61**
N. *oryzae*	3.58	5.13	4.14	**4.28**	3.72	5.54	4.36	**4.54**
A. *alternata*	6.28	4.51	6.57	**5.79**	6.16	4.61	6.68	**5.82**
Mean (B)	**4.32**	**3.90**	**5.14**		**4.33**	**4.12**	**5.26**	
LSD 5%	**A: 0.28**	**B: 5.37**	**Ax B:10.73**		**A: 0.20**	**B: 5.59**	**AxB:11.18**	

Table (5): Means of shoot dry weight (gm) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Shoot dry weight (gm)							
Control	1.37	1.08	2.40	**1.62**	1.07	1.17	2.30	**1.51**
C. *globosum*	2.62	2.78	3.03	**2.81**	2.73	1.89	2.77	**2.46**
N. *oryzae*	2.78	3.00	2.22	**2.67**	1.89	3.04	2.61	**2.51**
A. *alternata*	3.58	2.26	3.34	**3.06**	3.38	2.28	3.36	**3.01**
Mean (B)	**2.59**	**2.28**	**2.75**		**2.27**	**2.10**	**2.76**	
LSD 5%	**A:0.08**	**B:3.36**	**AxB:6.72**		**A: 0.39**	**B: 2.97**	**AxB:5.94**	

It was apparent from Table 6 that the application of endophytic fungi had significant positive effect on the number of leaves of satinleaf tree seedlings. In most cases the highest numbers of leaves were recorded with *A. alternate* (9.6/ seedling) in the mean of seasons. A tendency of increasing leaf numbers with the vegetative growth was observed. The highest number of leaves was recorded as a result of cultured in peat moss compared to the other growing medium. The differences of interaction between the endophytic fungi and growth media treatments were significant. The highest leaf number was observed at *A. alternate* treatment in peat moss media.

Table (6): Means of number of leaves/plant of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Number of leaves/plant							
Control	7.50	6.61	8.15	**7.42**	7.72	6.73	8.60	**7.68**
C. *globosum*	8.89	7.74	9.59	**8.74**	9.08	7.97	9.67	**8.91**
N. *oryzae*	7.74	9.51	8.11	**8.45**	7.97	9.18	8.11	**8.42**
A. *alternata*	10.06	8.37	11.32	**9.92**	9.79	8.37	11.15	**9.77**
Mean (B)	**8.55**	**8.06**	**9.29**		**8.64**	**8.06**	**9.38**	
LSD 5%	**A: 0.23**	**B:10.47**	**Ax B:20.94**		**A: 0.33**	**B: 10.65**	**AxB:21.30**	

Root parameters:

It was found that root fresh and dry weights were varied significantly with different endophytic fungi treatments as well as within the selected media. Tables 7&8 represent the average root fresh and dry weights (gm) of the treated seedlings. A trend of increases in root weight with using of *A. alternate* for transplants was observed. However, the highest root fresh and dry weight was recorded for *C. oliviforme* transplants grown in peat moss medium whereas the lowest one was observed as a result of growing in loamy sand soil. Regarding the interaction treatments, it is clear from the same Table that the differences for the root weight were significant. The heavy weights of fresh and dry root were due to applied *A. alternate* on transplants cultured in peat moss media in the two seasons.

Table (7): Means of root fresh weight (gm) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Root fresh weight (gm)							
Control	0.87	1.02	1.23	**1.04**	0.92	1.07	1.32	**1.10**
C. *globosum*	1.78	2.24	2.40	**2.14**	1.62	2.14	2.28	**2.01**
N. *oryzae*	2.24	1.82	2.33	**2.13**	2.14	1.67	2.25	**2.02**
A. *alternata*	1.90	2.37	2.62	**2.30**	1.78	2.33	2.43	**2.18**
Mean (B)	**1.70**	**1.86**	**2.15**		**1.62**	**1.80**	**2.07**	
LSD 5%	**A: 0.06**	**B:2.57**	**Ax B:5.13**		**A: 0.09**	**B: 2.42**	**AxB:4.84**	

Table (8): Means of root dry weight (gm) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Root dry weight (gm)							
Control	0.43	0.46	0.67	**0.52**	0.46	0.61	0.77	**0.61**
C. *globosum*	0.73	1.25	1.54	**1.17**	0.72	1.00	1.14	**0.95**
N. *oryzae*	1.25	0.95	1.37	**1.19**	1.00	0.82	1.09	**0.97**
A. *alternata*	1.08	1.38	1.63	**1.36**	0.93	1.16	1.32	**1.14**
Mean (B)	**0.87**	**1.01**	**1.30**		**1.04**	**1.20**	**1.08**	
LSD 5%	**A:0.12**	**B:0.14**	**AxB:2.88**		**A:0.09**	**B:1.16**	**AxB:2.32**	

Biochemical analysis:

Results in Table 9 showed that the highest content of chlorophyll (a) was recorded with *N. oryzae* in the mean of seasons. Moreover, the best result of chlorophyll (a) was found in case of the growing seedlings in peat moss media, while the lowest was observed in sand soil in the two seasons. Regarding the interaction treatments, the highest values of chlorophyll (a) were recorded by the transplants treated with *A. alternate* in peat moss medium.

Significant difference was observed between the three endophytic fungi species, used media as well as the interaction between treatments (Tables 10&11). Over all, *A. alternate* treatment showed higher in chlorophyll (b) and total carotenoids of *C. oliviforme* followed by *N. oryzae* in the mean of seasons. The previous characteristics were increased in peat moss based medium treatment than the other medium. From the analysis of data observed that the peat moss based media with *A. alternate* were found to be the most effective in increasing the chlorophyll (b) content of seedlings, while the most effective treatment for the total carotenoids was pea tmoss plus *C. globosum.*

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Table (9): Means of chlorophyll a (mg/ gm FW) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Chlorophyll a (mg/ gm FW)							
Control	0.21	0.18	0.27	**0.22**	0.27	0.24	0.35	**0.29**
C. *globosum*	0.22	0.27	0.40	**0.30**	0.22	0.29	0.38	**0.30**
N. *oryzae*	0.36	0.31	0.43	**0.37**	0.37	0.30	0.41	**0.36**
A. *alternata*	0.34	0.31	0.41	**0.35**	0.34	0.31	0.41	**0.35**
Mean (B)	**0.28**	**0.27**	**0.38**		**0.30**	**0.29**	**0.39**	
LSD 5%	**A: 0.10**	**B:0.05**		**AxB:0.10**	**A: 0.08**	**B: 0.05**		**AxB:0.09**

Table (10): Means of chlorophyll b (mg/ gm FW) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Chlorophyll b (mg/ gm FW)							
Control	0.21	0.18	0.23	**0.21**	0.18	0.15	0.22	**0.18**
C. *globosum*	0.24	0.21	0.28	**0.24**	0.26	0.19	0.28	**0.24**
N. *oryzae*	0.28	0.24	0.33	**0.28**	0.29	0.25	0.33	**0.29**
A. *alternata*	0.31	0.27	0.37	**0.32**	0.32	0.26	0.35	**0.31**
Mean (B)	**0.26**	**0.23**	**0.30**		**0.26**	**0.21**	**0.30**	
LSD 5%	**A: 0.01**	**B:0.02**		**Ax B:0.04**	**A: 0.02**	**B: 0.03**		**AxB:0.03**

Table (11): Means of total carotenoids (mg/ gm FW) of *Chryzphylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Total carotenoids (mg/ gm FW)							
Control	0.30	0.28	0.34	**0.31**	0.28	0.24	0.35	**0.29**
C. *globosum*	0.38	0.35	0.42	**0.38**	0.35	0.31	0.42	**0.36**
N. *oryzae*	0.35	0.37	0.32	**0.35**	0.40	0.34	0.45	**0.40**
A. *alternata*	0.41	0.33	0.42	**0.39**	0.41	0.32	0.40	**0.38**
Mean (B)	**0.36**	**0.33**	**0.38**		**0.36**	**0.30**	**0.41**	
LSD 5%	**A: 0.02**	**B: 0.46**		**AxB:0.92**	**A: 0.02**	**B: 0.02**		**AxB:0.04**

Total carbohydrates and nitrogen contents as affected by the endophytic fungi and growth media treatments are shown in Tables 12&13. It was noticed that, the best means of total carbohydrates, registered significant increments by seedlings treated with *A. alternate* while, nitrogen contents were recorded great increment by using *N. oryzae* in the mean of seasons as compare to the untreated plants. Referring to the data observed in the same Tables, revealed that the highly means of total carbohydrates and nitrogen contents *C. oliviforme* leaves were recorded due to the application of peat moss as growing media in both seasons. On the other hand, the best content in carbohydrate was recorded in the treatment combination consisting of peat moss media + foliar application *A. alternate*, while the lowest content of the two parameters was in the control treatment (without fungi application) + sand soil as media. Meanwhile, seedlings grown in peat moss as media with *N. oryzae* application developed the largest content of nitrogen compared to the other treatments in the mean of seasons.

Table (12): Means of total carbohydrate (mg/gm) of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Total carbohydrate (mg/gm)							
Control	1.44	1.16	1.61	**1.40**	1.37	1.23	1.59	**1.40**
C. *globosum*	1.71	1.48	1.81	**1.67**	1.61	1.40	1.76	**1.59**
N. *oryzae*	1.48	1.78	1.54	**1.60**	1.40	1.78	1.60	**1.59**
A. *alternata*	1.71	1.49	1.76	**1.65**	1.68	1.54	1.80	**1.67**
Mean (B)	**1.56**	**1.48**	**1.68**		**1.52**	**1.49**	**1.69**	
LSD 5%	**A:0.10**	**B:1.99**	**AxB:3.98**		**A:0.05**	**B:1.91**	**AxB:3.82**	

Table (13): Means of nitrogen (%) content of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Nitrogen (%)							
Control	0.88	0.74	1.10	**0.91**	0.90	0.68	1.19	**0.92**
C. *globosum*	1.28	0.85	1.36	**1.16**	1.38	0.94	1.62	**1.31**
N. *oryzae*	1.46	1.21	1.60	**1.42**	1.59	1.32	1.72	**1.54**
A. *alternata*	1.45	1.19	1.57	**1.40**	1.51	1.24	1.66	**1.47**
Mean (B)	**1.27**	**1.00**	**1.41**		**1.35**	**1.05**	**1.55**	
LSD 5%	**A: 0.06**	**B: 0.05**	**AxB:0.10**		**A:0.06**	**B: 0.05**	**AxB:0.10**	

Data in Tables 14&15 revealed that differences among different endophytic fungi species on the phosphorus and potassium content were significant at 5% level. *C. oliviforme* seedlings treated by *A. alternate* showed the most phosphorus and potassium contents as mean of seasons. Also, analysis of data showed that differences among different growing media on the previous characteristics were significant. Phosphorus and potassium in plants grown in peat moss was also suitable. The differences between interactions of treatments were significant. The largest amount of P and K content was observed in seedlings cultivated in peat moss as growing media + *A. alternate*, while minimum of these elements was obtained in *C. oliviforme* seedlings cultivated in sand soil without fungi application.

Table (14): Means of phosphorus (%) content of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Phosphorus (%)							
Control	1.17	0.93	1.37	**1.16**	1.15	0.99	1.29	**1.14**
C. *globosum*	1.55	1.30	1.69	**1.51**	1.52	1.25	1.60	**1.46**
N. *oryzae*	1.86	1.59	1.88	**1.78**	1.70	1.50	1.80	**1.67**
A. *alternata*	1.91	1.66	2.11	**1.89**	1.82	1.61	1.92	**1.78**
Mean (B)	**1.62**	**1.37**	**1.76**		**1.55**	**1.34**	**1.65**	
LSD 5%	**A: 0.07**	**B: 0.04**	**Ax B:0.09**		**A:0.09**	**B: 0.04**	**AxB:0.09**	

Table (15): Means of potassium (%) content of *Chrysophylum oliviforme* as affected by endophytic fungi species, growing media and their interaction during 2015and 2016 seasons.

Endophytic fungi spp (A).	Growing media (B)							
	2015				2016			
	Loamy sand	Sand	Peat moss	Mean (A)	Loamy sand	Sand	Peat moss	Mean (A)
	Potassium (%)							
Control	0.67	0.64	0.91	**0.74**	0.60	0.47	0.82	**0.63**
C. *globosum*	0.90	0.74	1.06	**0.90**	0.94	0.70	1.30	**0.98**
N. *oryzae*	1.19	0.82	1.31	**1.11**	1.35	0.86	1.48	**1.23**
A. *alternata*	1.43	1.07	1.52	**1.34**	1.53	1.10	1.61	**1.41**
Mean (B)	**1.05**	**0.82**	**1.20**		**1.11**	**0.78**	**1.30**	
LSD 5%	**A: 0.09**	**B: 0.04**	**AxB:0.08**		**A:0.06**	**B: 0.04**	**AxB:0.09**	

DISCUSSION

The growth of *Chrysophyllum oliviforme* trees is relatively slows, especially in the early stages, and in order to increase production, nurseries use many agricultural operations; fertilization is considered the most common. This leads to extra costs for nursery operators and also leads to a negative impact to the environment if nitrogen leaches from nurseries situated in areas nearby water sources. The alternatives are inexpensive and environmentally safe, such as the use of plant growth-promoting microorganisms to enhance the productivity of newly-established seedlings of forest plantations (**Cram and Dumroese, 2012)**. Recently, **Murugesan *et al*. (2016)** pointed out that in nurseries, the vigorous of seedlings is very poor due to insufficiency of desired microorganisms and the rate of mineralization and nitrogen fixation is very low. This problem can be overcome by treating suitable bioinoculants to induce the growth and nutrient uptake in seedlings of *Eucalyptus.* Also, many researchers conducted to study the effect of growing media on the growth of woody seedlings. In this study, we use of three types of endophytic fungi namely, *Alternaria alternata, Chaetomium globosum* and *Nigrospora oryzae,* in addition to three potting media (sand, loamy sand and peat moss) on *C. oliviforme* seedlings.

The results from this study pointed out those endophytic fungi can promote the growth of the *C. oliviforme* seedlings. Applied of the different endophytic fungi as spraying treatments on the transplants was significantly increased plant height, stem diameter, shoot and root fresh and dry weights as well as number of leaves/plant compared to the control in the two seasons. However, *A. alternata* was the most effective treatments for the previous characteristics in the mean of seasons. *Alternaria alternata* has often been isolated as an endophyte in different endophyte studies **(Taylor *et al*., 1999; Romero *et al*., 2001; Kumaresan and Suryanarayanan, 2002).** Based on the results from this study, we can conclude that a significant effect of *A. alternata* as endophyte infection was found on chlorophyll (b), carotenoides, total carbohydrates and phosphorus and potassium contents compared to the other treatments. In our study, it has been appeared that endophytic fungi especially *A. alternata* can induce host plant growth and nutrient uptake, which is in agreement with the field results of **(Malinowski *et al.,* 2005).**Meanwhile, applied *N. oryzae* resulted in the most effective on chlorophyll (a) and nitrogen content for the seedlings of *C. oliviforme*. *Alternaria* includes saprophytic, endophytic and pathogenic species; at least 268 metabolites from *Alternaria* fungi have been reported and they include nitrogen-containing metabolites, steroids, terpenoids, pyranones, quinones, and phenolics **(Lou *et al*, 2013).** It has been summarized that endophytic fungi can promote host plant growth, nutrient uptake and increase tolerance to different abiotic factors **(Malinowski *et al.,* 2005).** However, it provides induced resistance to pathogens and pests with the production of different alkaloids **(Shankar *et al.,* 2007). Ren *et al.* (2008)** study the influence of nitrogen fertilizer and endophyte infection on the mineral element content of perennial ryegrass found that endophyte infection affected mineral concentrations in roots more than in shoots. Similar to the result reported in tall fescue **(Malinowski *et al.* 2000; Strobel *et al.,* 2004).**

On the other hand, *Chaetomium* species are among the biologically active endophytes. It is reported to be involved in the production of chemically diverse metabolites, such as anthraquinones, xanthones, terpenoids, and steroids **(Shankar *et al.,* 2008; Zhang *et al.,* 2012).** These metabolites possess antimicrobial, antioxidant, and cytotoxic properties. **Abou Alhamed and Shebany (2012)** on their study the impact of *Chaetomium globosum* on copper stress resistance of maize seedlings revealed that combination of copper sulphate and *Chaetomium* increased seedling dry weight and antioxidant enzyme activity compared to copper sulphate alone. Also, the fungal scavenger system might be important for strengthening the maize ability to resist copper toxicity. However, the previously endophyte infection has been reported to increase carbohydrate concentrations in host plant **(Cheplick and Cho, 2003; Hunt *et al*., 2005; Rasmussen *et al*., 2007).**

In the present study, using peat moss as a growing media found to be the best one, while using sand resulted in the lower values of the growth characters of *C. oliviforme* seedlings. Of the reason for this increase in the growth characters of seedlings in medium of peat moss along with *A. alternata* may be due to the combined effect of potting media and endophytic fungi. Peat moss helped in providing good physical and chemical characteristics, helped in water uptake, served as nutrient source and provided good media for seedling growth. *A. alternata* helps in uptake of nutrients or due to the production of growth promoting substances. These results are in conformity with those of **Murugesan *et al.* (2016)** on their study of the effect of growing media with bioinoculants on the quality seedlings of *Eucalyptus tereticornis*. **Aklibasinda *et al.* (2011)** study the effect of different growing media on *Pinus sylvestris* pointed out that in the seedling production, the best media were peat alone and mixtures of rice hulls added to peat up to 30%. Peat is commonly used in the production of pot plants and seedling as growing media either in its pure form or mixture with other materials. **Ercisli *et al.* (2005)** on strawberry stated that, in general peat gave the best results in terms of above and underground parts of plants in both cultivars compared to the other media. On the other hand, **Mehwish *et al.* (2007)** on their study of different growing media on *Dahlia pinnata* summarized that using of sand alone as media resulted in the least response and provided unsatisfactory results for all measurements.

CONCLUSION

Through this research, it is clear that the use of *A. alternata* as enndophytic fungi gives the best results for the growth and quality of *C. oliviforme* seedlings i.e. plant height, stem diameter, shoot and root fresh and dry weights, number of leaves/plant, chlorophyll b, carotenoides, total carbohydrates, phosphorus and potassium contents. On the other side, applied *N. oryzae* as endophytic fungi resulted in the best results for chlorophyll a and nitrogen content for the seedlings of *C. oliviforme*. However, minimum values for the growth characters were recorded with the comparison treatment. Planting seedlings in peat moss as media is conducive to improving growth compared with loamy sand or sand soil.

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