

## Improving Rooting Characteristics in Air-layers of *Ficus elastica* var. *decora* Using Indole-3-butyric Acid (IBA) in the Presence of *Bacillus subtilis* and Arbuscular Mycorrhizal Fungi



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### Abstract

The current experiment was conducted to study the response of *Ficus elastica* var. *decora* air-layers to seven treatments of IBA (control, 50 and 100 ppm in rooting substrate, 1500 and 3000 ppm by painting girdled zone, and 1500 and 3000 ppm in talc paste) in the presence of root promoting microorganisms including *Bacillus subtilis* and arbuscular mycorrhizal fungi (AMF). The obtained results indicated that 3000 ppm IBA applied by painting girdled zone gave a significant increase in rooting percentage and the best root and growth characteristics (number of days for root appearance, number, length, fresh and dry weights of roots per rooted air-layer, survival percentage, plant height and number of leaves and shoots) as well as total content of carbohydrates and phenolics, and showed the shortest period required for root appearance in comparison with the other treatments. Air-layers treated with *B. subtilis* were superior to those treated with AMF. The best results concerning rooting percentage, root characteristics, total contents of carbohydrates and phenolics, survival percentage, increment in growth of air-layers after six months from detachment were observed in air-layers treated with 3000 ppm IBA by painting girdled zone in combination with *B. subtilis* followed by the same IBA treatment combined with AMF.

**Keywords:** *Ficus elastica* var. *decora*, IBA, beneficial microorganisms, air layering

### Introduction

*F. elastica* var. *decora*, belonging family Moraceae, is one of the best known figs cultivated throughout the world. This species was formerly important as a source of an inferior natural rubber. Several cultivars are grown including cv. 'Decora' (*F. decora* Hort.) with leaves dark glossy green, ivory midribs, and red beneath (Baily, 1963).

Rubber fig is propagated through sexual and asexual methods. In sexual method, seed is employed only to produce new varieties by hybridization and for rootstock purpose. In asexual method, it can be successfully propagated by cuttings, air lay-

ering, grafting and budding. Although multiplication through cutting is the common method, the problem lies in the very low or undesirable percentage of success. On the other hand, air layering is a well-known method for vegetative propagation of economically important woody plants. Comparing to other vegetative propagation methods, air layering is reliable and easy means of propagation, especially in species, which are difficult-to-root on cuttings. The retention of desirable characteristics, the creation of uniform rootstock and the ability to mass production of identical plants quickly and efficiently are all advantages of asexual propagation which

all can be fulfilled through air layering (Paul and Aditi, 2009). The success of air layering as a practice in propagation is probably due, in part, to the effect of endogenous auxins accumulating at the base of the girdled shoots (Cameron and Thompson, 1969). Propagation by air layering has been reported for several ornamental *Ficus* species such as *F. benjamina*, *F. triangularis*, *F. microcarpa* and *F. carica* as stated by Gamlath *et al.* (2010) and Reddy *et al.* (2014).

Transplanting rooted layers and stem cuttings with good root system may give better adaptation in the field and thus ultimately resulting in good performance, survival and growth. The importance of long root system is that it should allow the uptake of nutrients outside the initial exhaustion zone (Clark *et al.*, 2000). To promote root formation and improved roots and vegetative growth characteristics, IBA is one of the most effective and widely used for this purpose, exhibiting low toxicity, low mobility, and high chemical stability (Hartmann *et al.*, 2011).

There are many application methods for auxins to ensure high successful rate propagation through air laying, including direct application of auxin at the girdled zone, talc paste or application of auxins into the rooting substrate with dilute concentrations.

Singh *et al.*, (2007) recorded the best rooting and growth results due to the treatment of air-layers with the highest concentration of IBA (6000 ppm) directly at the girdled zone. In an experiment conducted by Porlingis *et al.* (1999) on air layering of olive,

they evaluated several factors including IBA incorporated in the paste. They found that the lanolin-Tween 20 paste (3:1 w/w) compared with lanolin alone, in the presence of IBA, accelerated rooting and finally increased rooting percentage and root number and weight. Another method is by application of auxin in dilute concentrations into the rooting substrate (Wells, 1986 on *Mahonia aquifolium* and Gilani *et al.*, 2018 on guava).

In addition to using the synthetic auxins, natural biostimulants such as plant growth promoting rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) play an essential role in improving rooting performance particularly for the development of lateral roots which are the preferred infection sites for the fungi (Ludwig-Muller and Guther, 2007, Abdel-Rahman and El-Dsouky, 2010 and Rajan and Radhakrishna, 2013). The mechanisms involved in plant growth promotion by these beneficial microorganisms involve producing substances such as phytohormones and polyphenolic compounds (Barea and Azon-Aguilar, 1982, Mitchell *et al.*, 1986, Goto, 1990 and Scigel *et al.*, 2003).

Therefore, the current study aimed to define the effect of auxin application methods with or without beneficial microorganisms (*B. subtilis* or AMF) on rooting and survival percentage, as well as root and growth characteristics of *Ficus elastica* var. *decora* propagated by air layering.

## Materials and Methods

This study was carried out at the Floriculture Farm, Faculty of Agriculture, Assiut University, Egypt, during the 2016 and 2017 seasons to clarify the effects of different application methods of indole-3-butyric acid (IBA) and/or *B. subtilis* and AMF on rooting and survival percentages as well as root and growth characters of *F. elastica* var. *decora* propagating by air layering.

Active strains of *B. subtilis* and AMF (*Glomus intraradices*) were obtained from the Unit of Biofertilizers, Fac. Agric., Ain Shams Univ., Shobra El-Kheima, Egypt. Bacterial suspension prepared of *B. subtilis* ( $10^8$  CFU/ml) and AMF inoculum (2500 spores/l) were supplied to rooting substrate at a rate of 10 ml/layer for each of them after girdling process of *F. elastica* var. *decora* shoots.

Air layering of *F. elastica* var. *decora* was carried out by removing 2.0 cm ring of bark in the intermodal region from mature shoots (ca. 120 cm long). The layering process was done at the beginning of March for both seasons. Air-layers were treated with IBA alone or in combination with *B. subtilis* or AMF. The rooting substrate used was a mixture of perlite and peat moss (1:1 in volume). The layers were covered with transparent plastic bags and the both ends were secured firmly and then covered with aluminum foil. The un-treated air-layers (control) were treated with distilled water alone.

The experiment was arranged in split-plot design, with three replicates. Application methods of auxin (control, 50 and 100 ppm IBA in rooting substrate, 1500 and 3000 ppm

IBA by painting girdled zone, 1500 and 3000 ppm IBA in talc paste) were represented in the main plots, whereas, the beneficial microorganisms (control, *B. subtilis* and AMF) were represented in the sub-plots. Each experimental unit included 5 air-layers. The air-layers were kept under constant observation to prevent from any mechanical damage and loss of moisture.

After 10 weeks from layering, the air-layers were separated from mother plants. The roots of the separated air-layers were washed in water to remove adhering rooting substrate. Data were recorded on number of days for appearance of roots, rooting percentage, number of roots, root length, fresh and dry weights of roots per rooted air-layer. Plant height and number of leaves and shoots as well as survival percentage were recorded after 6 months from transplanting the layers in polyethylene bags filled with clay soil and were kept under plastic house conditions.

For determining total carbohydrates and phenols, as well as endogenous phytohormones and anatomy structure, the basal 2.5-3.0 cm portion of rooted shoots was used. Some samples were oven-dried at 70°C for 48 hours and ground to a fine powder and stored until used for determination of total carbohydrates and phenols. Total carbohydrates content was colorimetrically determined using anthrone sulphuric acid method described by Fales (1951). The total phenolic content was estimated colorimetrically by Folin-ciocalteau reagent (FCR) method (Maliauskas *et al.*, 2004).

Endogenous phytohormones in tissues of layered shoots bases were analyzed using GC-MS (7890A-5975B) Gas Chromatograph Mass equipped with a flame ionization detector for separation of phytohormones constituents. The chromatograph apparatus was fitted with capillary column DB-5ms. The oven temperature was set at 40 ° c for 2 min., then temperature program ramp increase with a rate of 10° c/min to 150° c for 3 min., then it was increased at the rate of 10° c/min to 220° c for 6 min and then at 15° c/min to 280° c for 15 min and finally the a post run was at 260° c for 2 min. Flow rates of gases was 0.5 mL/min for 10.9 min then 1 mL/min for 30 min. Detector and injector temperatures were 300° C and 260° C, respectively. Hence, the total run time was 48 min. The obtained chromatogram and report of GC Mass analysis for each sample were analyzed to calculate the percentage of main components of phytohormones (indole acetic acid and its derivatives, zeatin and gibberellic acid).

Data obtained during the two seasons of the study were statistically analyzed using Statistix 8.1 analytical software and the means were compared using a least significant difference (L.S.D.) test based on Gomez and Gomez (1984).

## Results and Discussion

### Rooting percentage

Data presented in Table 1 show significant effects of different application methods of IBA on rooting percentage of *F. elastica* var. *decora* air-layers during both seasons. The best application method of IBA was the direct application by painting girdled zone which showed superiority over the other methods. The effect of IBA concentration was also obvious on rooting percentage. Treating air-layers with 3000 ppm IBA by painting girdled zone resulted in the highest rooting percentage (93.3 and 86.7%) in both seasons, respectively. These results are in agreement with the findings of Ray *et al.* (2001), Chawla (2011), Reddy *et al.* (2014) and Baghel *et al.* (2016). The stimulatory effect of IBA in increasing rooting percentage may be due to the important roles of auxin including accumulation of metabolites at the site of application of auxin, stimulation of cell division, cell elongation, root formation, enhanced hydrolysis of carbohydrates, synthesis of RNA, enzymes, new protein and cell-wall components and stem elongation (Lwin *et al.*, 2012 and Kasem and Abd El-Baset, 2014).

**Table 1. Percentage of rooted layers in *Ficus elastica* var. *decora* as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

IBA application methods “ppm”		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		53.33	60.00	60.00	57.78	46.67	60.00	60.00	55.56
In the substrate	50	66.67	80.00	80.00	75.56	66.67	73.33	80.00	73.33
	100	73.33	86.67	80.00	80.00	66.67	80.00	73.33	73.33
By painting	1500	73.33	100.00	93.33	88.89	66.67	80.00	80.00	75.56
	3000	80.00	100.00	100.00	93.33	73.33	100.00	86.67	86.67
Talc paste	1500	73.33	86.67	80.00	80.00	66.67	80.00	73.33	73.33
	3000	80.00	100.00	86.67	88.89	73.33	86.67	80.00	80.00
Mean		71.43	87.62	82.86		65.71	80.00	76.19	
<b>LSD at 0.05</b>									
IBA Treatments		1.94				3.58			
Microorganisms		1.05				2.2			
Interaction		2.79				5.82			

Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*

The results also highlight the importance of the treatment with beneficial microorganisms to improve rooting percentage. Air-layers treated with *B. subtilis* had significantly higher rooting percentage (83.8% as average mean of both seasons) than the layers treated with AMF and the control. This is consistent with the previously reported by Goto (1990), Rinallo *et al.* (1999) and Rajan and Radhakrishna (2013). Also, McAfee *et al.* (1993) showed that rooting of *Pinus* and *Larix* cuttings was higher when they were inoculated with *Agrobacterium rhizogenes*. Esitken *et al.* (2003) and Ercisli *et al.* (2004) tested plant growth promoting rhizobacteria (PGPR) for rooting in rose hip and sour cherry cuttings and found that PGPR were effective to obtain high rooting percentages. Root induction by beneficial microorganisms is the accepted result of phytohormones, such as IAA production, inhibition of ethylene synthesis and mineralization of nutrients (Goto, 1990, Steenhoudt and Vanderleyden, 2000 and Erturk *et al.*, 2010).

The interaction between IBA application methods and beneficial microorganisms exerted significant differences in both seasons. The combined treatment of IBA at 3000 ppm by painting girdled zone and *B. subtilis* resulted in the highest rooting percentage (100%) in both seasons, followed by the same IBA treatment + AMF (Table 1). These results are in accordance with Rinallo *et al.* (1999), Scagel, *et al.* (2003), Yasser (2015) and Zenginbal and Demir (2018). They revealed that IBA-bacteria or IBA-mycorrhizal fungi combined treatments showed greater capacity than IBA or beneficial microorganisms alone treatments in enhancing rooting percentage.

#### **Root and vegetative growth characteristics**

It is evident from the data in Tables 2-6 and Figs. 1 & 2 that the treatment of *F. elastica* var. *decora* air-layers with IBA alone or in combination with beneficial microorganisms significantly increased all root and vegetative growth characteristics compared to the control.

Concerning IBA effect, treating air-layers with IBA at 3000 ppm by painting girdled zone induced significantly faster root appearance and gave higher number and length of roots as well as heavier fresh and dry weights of roots. Also, the same treatment gave the same trend in increasing vegetative growth characteristics (plant height, number of shoots and leaves per rooted layer) after 6 months from transplanting compared to the control and the other treatments in both seasons. These results are in agreement with those obtained by Singh and Singh (1996), Dharshan (2008), Rymbai and Reddy (2010) and Reddy *et al.* (2014). Gilani *et al.* (2018) reported that application of IBA at 150 ppm into the rooting substrate increased root and shoot characteristics of guava air-layers. Also, Reddy *et al.* (2014) indicated that *Ficus carica* air-layers treated with IBA at 3000 ppm reduced number of days required for root appearance, highest number of roots, maximum root length, maximum fresh and dry weights of roots, maximum survival percentage of air-layers and number of new leaves planting. Better root and shoot promoting activity of IBA could be attributed to its property of slow movement and its relatively slow destruction by auxin degrading enzyme system (Chovatia and Singh, 2000, Dharshan, 2008 and Kumar, 2011). This also may be due to the fact that early root initiation might have provided enough time for higher rate of cell division and cell elongation which ultimately might have promoted higher length of roots. Cumulative root length had a positive relation with the number of roots per

rooted layer. Therefore, IBA affect the root length either through increasing the number of roots per rooted layer or by increasing cell division and enlargement at each root (Desalegn and Reddy, 2003). IBA most probably enhanced the translocation of carbohydrate and other endogenous plant substances and nutrients to the rooting zone for root initiation and development of the layered shoots (Yeboah *et al.*, 2014).

As for the effect of beneficial microorganisms, inoculation of air-layers with *B. subtilis* or AMF resulted in early root appearance and better root and shoot characteristics comparing to the control, where *B. subtilis* was superior to AMF. These results are in accordance with those revealed by Rinallo *et al.* (1999), Abdel-Rahman and El-Naggar (2014) and Yasser (2015). The increase in root production observed with the mycorrhizal treatments may be related to the IBA treatment, since this phytohormone can play a specific and direct role in establishing symbioses between fungi and plant roots, as well as stimulating the fungus to lateral root formation in the host (Etemadi *et al.*, 2014). Apart from the application of synthetic auxin, natural plant auxin play essential role as signals during the establishment of AMF symbiosis particularly for the development of lateral roots which are the preferred infection sites for the fungi (Ludwig-Muller and Guther, 2007). On the other hand, the addition of PGPR enhance the availability and uptake of plant nutrients, the production of growth promoting substances and the suppression of deleterious bacteria which might have encouraged the

rooted layers to put on better root and vegetative growth and hence an increase in plant height (Chawla, 2011).

The interaction effects between IBA and beneficial microorganism showed that the earliest root appearance and the best root and vegetative growth characteristics were obtained from combined IBA at 3000 ppm by painting girdled zone with *B. subtilis*, followed by the same IBA treatment

+ AMF. These results are consistent with the findings of Rinallo *et al.* (1999), Scagel *et al.* (2003), El-Nashar (2008), Abdel-Rahman and El-Naggar (2014). Yasser (2015) and Abdul Hakim *et al.* (2018) showed that combination of auxins with beneficial microorganisms significantly increased root and shoot characteristics of *Punica granatum* cuttings compared with the control.

**Table 2. Number of days to emerge visual roots in *Ficus elastica* var. *decora* air-layers as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

IBA application methods "ppm"		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		51.7	44.0	45.7	47.1	53.0	42.7	43.7	46.4
In the substrate	50	43.3	39.7	40.7	41.2	41.7	38.0	39.7	39.8
	100	41.0	37.0	39.3	39.1	39.7	36.0	37.3	37.7
By painting	1500	38.3	35.0	36.3	36.6	36.3	34.7	34.3	35.1
	3000	36.3	31.0	33.7	33.7	35.0	30.3	33.3	32.9
Talc paste	1500	38.7	36.0	37.3	37.3	37.3	34.7	35.7	35.9
	3000	37.0	33.0	34.3	34.8	35.3	32.7	34.0	34.0
Mean		40.9	36.5	38.2		39.8	35.6	36.9	
<b>LSD at 0.05</b>									
IBA Treatments		1.3				1.1			
Microorganisms		0.6				0.5			
Interaction		1.6				1.4			

Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*

**Table 3. Root number per air-layer of *Ficus elastica* var. *decora* as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

IBA application methods "ppm"		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		7.68	13.67	11.50	10.95	10.67	12.33	12.00	11.67
In the substrate	50	9.73	19.44	18.33	15.84	12.50	15.50	14.67	14.22
	100	9.89	20.77	19.44	16.70	14.00	19.50	19.33	17.61
By painting	1500	15.67	24.67	23.67	21.33	14.33	22.57	19.50	18.80
	3000	23.13	26.17	27.67	25.65	17.50	26.67	24.67	22.94
Talc paste	1500	16.20	21.47	19.77	19.14	14.83	19.83	18.00	17.56
	3000	16.00	24.43	23.33	21.26	15.17	22.00	19.00	18.72
Mean		14.04	21.52	20.53		14.14	19.77	18.17	
<b>LSD at 0.05</b>									
IBA Treatments		1.67				1.50			
Microorganisms		0.82				0.71			
Interaction		2.18				1.87			

Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*

**Table 4. Root length (cm) per air-layer of *Ficus elastica* var. *decora* as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

IBA application methods "ppm"		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		8.90	11.83	10.87	10.54	10.17	13.33	13.00	12.17
In the substrate	50	11.83	16.11	14.33	14.09	13.83	16.67	15.17	15.22
	100	12.33	17.44	14.67	14.81	14.50	18.00	14.50	15.67
By painting	1500	14.78	18.33	17.33	16.81	15.67	19.50	17.33	17.50
	3000	16.17	21.00	18.67	18.61	17.67	22.17	21.67	20.50
Talc paste	1500	12.62	16.50	15.87	15.00	14.83	16.50	17.17	16.17
	3000	14.53	17.23	17.17	16.31	16.33	18.17	18.67	17.72
Mean		13.02	16.92	15.56		14.71	17.76	16.79	
<b>LSD at 0.05</b>									
IBA Treatments		0.43				0.47			
Microorganisms		0.33				0.36			
Interaction		0.87				0.96			

Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*

**Table 5. Root fresh weight (g/air-layer) of *Ficus elastica* var. *decora* as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

IBA application methods "ppm"		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		3.93	7.90	6.83	6.22	4.49	7.25	6.33	6.02
In the substrate	50	8.33	14.93	14.67	12.64	7.60	14.10	12.67	11.46
	100	8.40	17.60	16.00	14.00	9.52	16.91	16.05	14.16
By painting	1500	9.30	22.00	19.00	16.77	10.66	19.42	17.63	15.90
	3000	15.93	23.00	22.33	20.42	13.50	23.81	19.67	18.99
Talc paste	1500	9.20	18.70	16.90	14.93	9.89	17.86	17.14	14.96
	3000	11.17	21.10	20.00	17.42	12.30	19.74	18.07	16.70
Mean		9.47	17.89	16.53		9.71	17.01	15.36	
<b>LSD at 0.05</b>									
IBA Treatments		0.77				1.27			
Microorganisms		0.74				0.85			
Interaction		1.95				2.26			

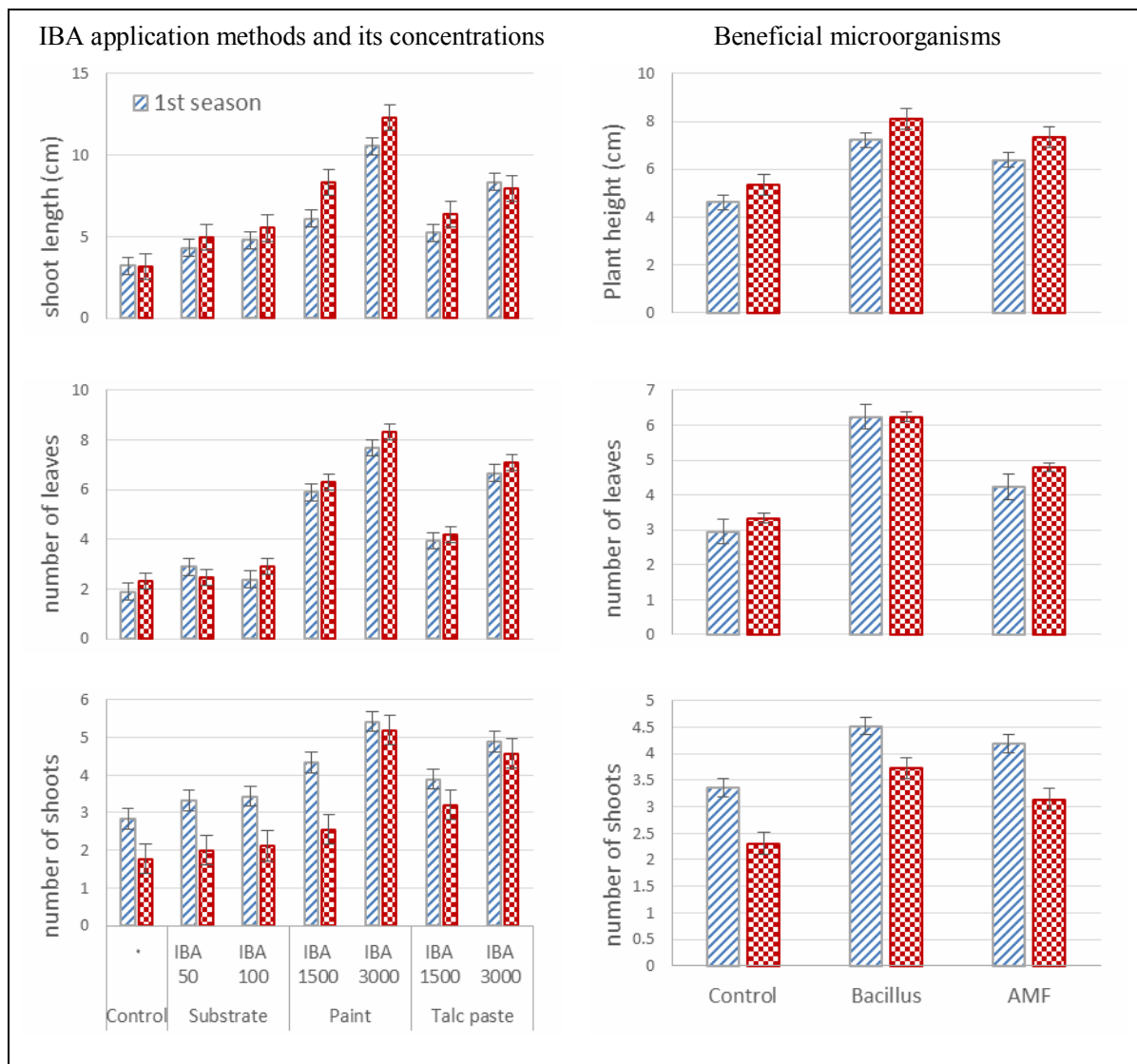
Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*

**Table 6. Root dry weight (g/air-layer) of *Ficus elastica* var. *decora* as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

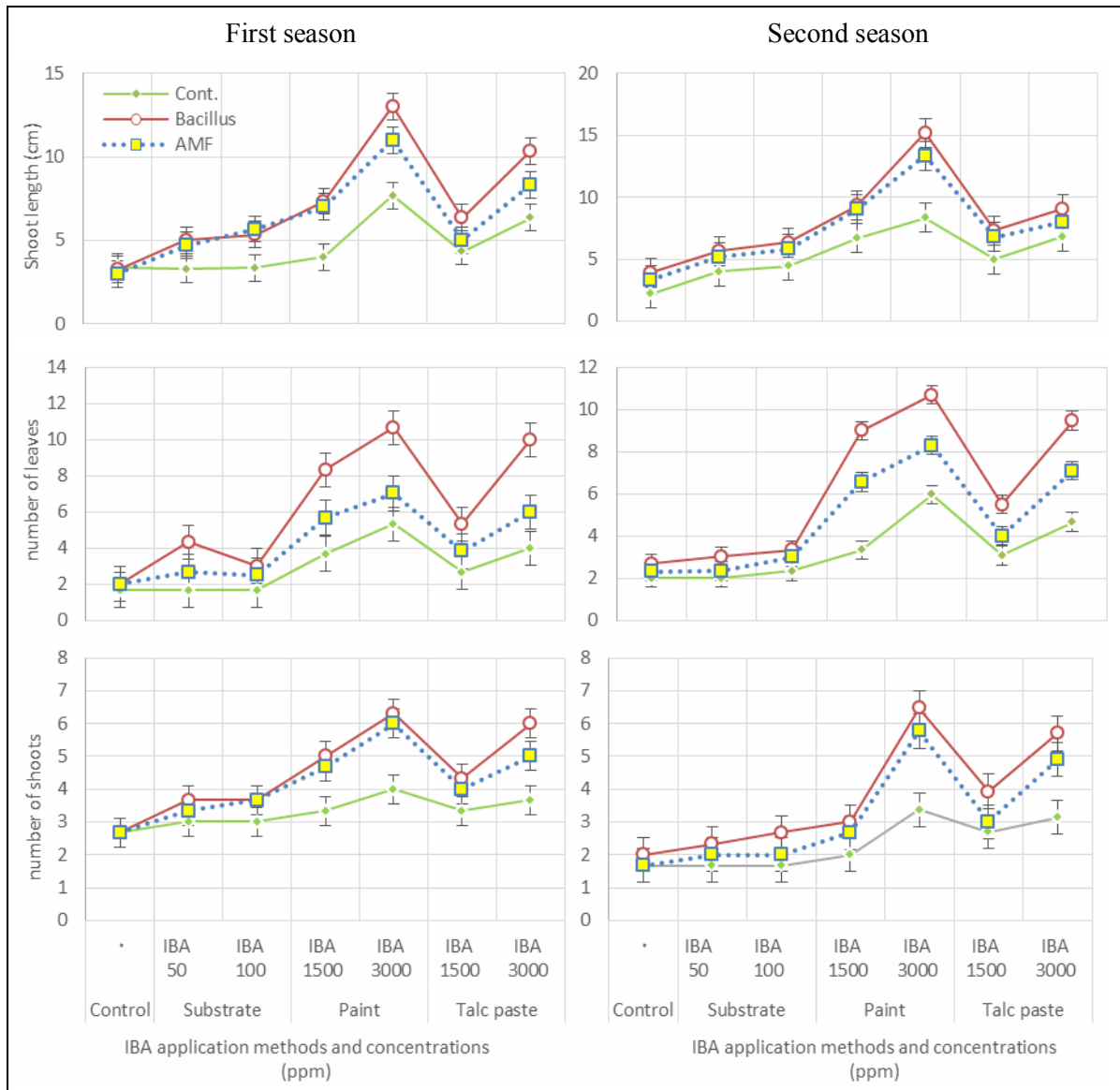
IBA application methods "ppm"		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		1.63	3.29	3.03	2.65	1.89	3.19	3.12	2.73
In the substrate	50	3.37	4.32	4.32	4.01	3.34	4.29	4.16	3.93
	100	3.67	4.69	4.37	4.24	3.40	4.64	4.41	4.15
By painting	1500	3.70	5.58	4.90	4.73	3.89	5.17	4.46	4.51
	3000	4.68	5.66	5.39	5.24	4.05	5.91	5.18	5.05
Talc paste	1500	3.58	4.63	4.03	4.08	3.55	4.90	4.41	4.29
	3000	3.84	4.92	4.92	4.56	4.00	5.43	5.00	4.81
Mean		3.50	4.73	4.42		3.45	4.79	4.39	
<b>LSD at 0.05</b>									
IBA Treatments		0.42				0.41			
Microorganisms		0.31				0.26			
Interaction		N.S.				N.S.			

Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*





**Fig. 1.** Effect of IBA at different concentrations and application methods and beneficial microorganisms on vegetative growth characteristics of *F. elastica* var. *decora* during both seasons. Vertical bars above mean denote LSD values ( $p \leq 0.05$ ).



**Fig. 2.** Interaction effects between IBA at different concentrations and application methods and beneficial microorganisms on vegetative growth characteristics of *F. elastica* var. *decora* during both seasons. Vertical bars above mean denote LSD values ( $p \leq 0.05$ ).

### Survival percentage

Data presented in Table 7 show that the best treatments which improved root and shoot characteristics, also enhanced survival percentage of successfully rooted air-layers. Generally, treating air-layers with IBA at 3000 ppm by painting girdled zone combined with *Bacillus* or AMF produced the best survival percentage (100%) in both seasons comparing to ca. 50% in untreated layers (control) as an average of both seasons (Table

7). These results are in agreement with the finding of Kumar (2011), Chawla (2011) and Khalid *et al.* (2004). Improvement in root and vegetative growth characteristics by the combined treatment of 3000 ppm IBA with the beneficial microorganisms could explain the percentage of survival after transplanting derived by the same treatment (Kumar, 2011 and Yasser, 2015). In addition, beneficial microorganisms including bacteria and mycorrhizal fungi has a

great role in promoting better uptake of water and nutrients by the plant and hence improving survival per-

centage (Kumar and Syamal, 2005 and Cakmakci *et al*, 2006).

**Table 7. Survival percentage of new produced plants after 6 months from transplanting in *Ficus elastica* var. *decora* as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

IBA application methods "ppm"		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		50.03	50.00	50.00	50.01	50.03	50.00	50.00	50.01
In the substrate	50	61.10	77.77	72.23	70.37	61.10	77.77	72.23	70.37
	100	66.67	88.90	88.90	81.49	77.77	88.90	88.90	85.19
By painting	1500	72.23	88.90	77.80	79.64	72.23	77.80	77.80	75.94
	3000	77.80	100.00	92.00	89.93	77.80	100.00	91.67	89.82
Talc paste	1500	66.67	77.80	77.80	74.09	66.67	77.80	77.80	74.09
	3000	77.80	91.67	88.90	86.12	77.80	91.67	88.90	86.12
Mean		67.47	82.15	78.23		69.06	80.56	78.19	
<b>LSD at 0.05</b>									
IBA Treatments		1.13				4.46			
Microorganisms		0.94				2.32			
Interaction		2.48				6.13			

Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*

### Total carbohydrates and phenols content

The results presented in Tables 8 and 9 show that the combination of IBA with beneficial microorganisms significantly increased content of total carbohydrates and phenols in layered shoots tissues compared to untreated layers. The highest contents of total carbohydrates and phenols were obtained by treating air-layers with 3000 ppm IBA by painting girdled zone + *B. subtilis*, followed by the same IBA treatment + AMF. The increment in polyphenolic compounds produced by IBA plus *B. subtilis* or AMF has a direct role in decreasing auxin oxidation and accordingly improve rootability as well as root and shoot characteristics (Mitchell *et al.*, 1986 and Scagel and Linderman, 1998). Also, this might be due to that the monophenol can increase root growth as the enzyme polyphenol oxidase can induce second OH group in to the ring of a monophenol

(Goodwin, 1976) and convert it into diphenol, this in turn induces better rooting (Kunal and Syamal, 2005). Phenolic compounds are shown to interact with proteins leading to altered metabolism form roots in air-layers (Kefeli and Kutacek, 1976).

As for carbohydrates, it has been suggested that auxin-carbohydrates are important in regulating root formation (Hansen *et al.*, 1978, Abdel-Rahman and El-Dosouky, 2010 and Chawla, 2011). Arslonov (1979) reported that the exogenous application of auxins resulted in the utilization of the stored food substances for quicker root formation. It is assumed that some substances in addition to auxins as carbohydrates are produced in leaves and transported to the rooting zone which enhance the rooting process (Raviv and Reuveni, 1984). Carbohydrates may function solely as a source of metabolic fuel which is necessary to provide the energy

needed for root formation. Carbohydrates may also influence the auxin status thereby influencing adventitious root formation. In this regard, it has been suggested that auxin synthesis or transport is linked to the supply of photosynthate since conditions which reduced photosynthesis also reduced endogenous auxin levels (Vardar, 1968). In addition, the increase in carbohydrates content in bases of layered shoots in *F. elastica* var. *decora* as a result of the inoculation with *B. subtilis* or AMF may be due to the production of IAA by beneficial microorganisms, which

stimulated adventitious root formation, and resulted in better absorption of water and nutrients from the soil as well as increasing of vegetative growth. Reuveni and Raviv (1981) found a positive correlation among carbohydrates content, growth promoters and the rooting response. Abdel Rahman and El-Dsouky (2010) indicated that application of IBA in combination with *B. subtilis* was more effective treatment in increasing carbohydrates content in cutting bases when compared to the control, or IBA and/or *B. subtilis* alone.

**Table 8. Total carbohydrates (%) in tissue of girdled zone in *Ficus elastica* var. *decora* as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

IBA application methods "ppm"		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		15.93	19.17	18.14	17.75	18.13	19.33	18.51	18.66
In the substrate	50	19.01	22.06	20.79	20.62	18.97	20.86	19.57	19.80
	100	19.86	23.03	20.00	20.96	19.34	22.40	20.70	20.81
By painting	1500	21.40	22.94	23.17	22.50	20.00	23.03	23.73	22.26
	3000	19.86	23.85	25.21	22.97	20.47	25.20	25.73	23.80
Talc paste	1500	19.43	21.92	21.14	20.83	19.33	21.79	21.33	20.82
	3000	22.31	23.40	23.47	23.06	21.83	23.40	23.70	22.98
Mean		19.69	22.34	21.70		19.73	22.29	21.90	
<b>LSD at 0.05</b>									
IBA Treatments		0.30				1.02			
Microorganisms		0.36				0.65			
Interaction		0.95				1.73			

Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*

**Table 9. Total phenolics (mg GAE/ g DW) in tissue of girdled zone in *Ficus elastica* var. *decora* as affected by IBA treatments and beneficial microorganisms during the 2016 and 2017 seasons.**

IBA application methods "ppm"		Beneficial microorganisms							
		First season (2016)				Second season (2017)			
		Cont.	<i>Bacillus</i>	AMF	Mean	Cont.	<i>Bacillus</i>	AMF	Mean
Control (non-treated)		12.70	15.73	10.80	13.08	13.47	17.00	10.80	13.76
in the substrate	50	13.10	16.83	16.60	15.51	14.70	18.13	17.00	16.61
	100	15.70	19.10	14.78	16.53	16.90	20.83	15.55	17.76
by painting	1500	17.70	20.90	15.00	17.87	18.30	22.20	15.63	18.71
	3000	20.30	20.97	23.17	21.48	19.40	21.57	24.17	21.71
Talc paste	1500	17.80	20.10	18.53	18.81	17.33	22.33	21.00	20.22
	3000	19.97	23.53	17.20	20.23	17.73	25.07	17.80	20.20
Mean		16.75	19.60	16.58		16.83	21.02	17.42	
<b>LSD at 0.05</b>									
IBA Treatments		0.59				0.87			
Microorganisms		0.36				0.43			
Interaction		0.94				1.13			

Cont. = control, AMF = Arbuscular Mycorrhizal Fungi, *Bacillus* = *Bacillus subtilis*

### Promoters and inhibitors

GC MS analysis (Fig. 3) of two samples extracted from rooting zone of the air-layers for the control and best treatment for improving rooting and survival percentages, as well as root and shoot characteristics (IBA at 3000 ppm by painting girdled zone + *B. subtilis*) showed the presence of only 2 constituents (indole acetic acid and its derivatives at 20.9% and zeatin at 0.28%) in control and 3 ones (indole acetic acid and its derivatives at 22.9%, zeatin at 0.06% and longiborneol as flavonol at 1.65% as a percentage of total organic compounds per sample). The present results showed that there was a positive relationship between the best rooting and survival percentages, as well as root and vegetative growth characteristics and the high concentration of

indole acetic acid and its derivatives obtained by the best treatment of 3000 ppm IBA by painting girdled zone plus *B. subtilis* comparing to the control. The promotive effects of IBA + *B. subtilis* combined treatment in adventitious root formation could be attributed to the increase in IAA production by *B. subtilis* (Goto, 1990). On the other hand, Maldiney *et al.* (1986) found that increasing cytokinins appear to have inhibitory effects on adventitious root formation and they reported a change in their levels during root initiation. As for gibberellic acid, some investigators reported inhibitory effects of GA<sub>3</sub> on adventitious root formation and number of roots (Kato, 1958, Bostrack and Struckmeyer, 1967 and Mauriat *et al.*, 2014).

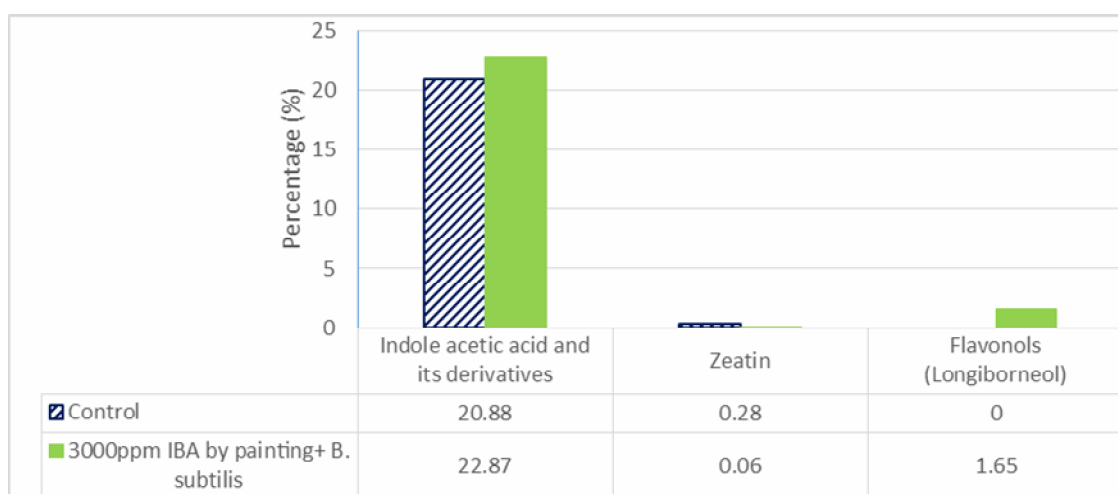


Fig. 3. GC-MS analysis of two samples extracted from root zone of *Ficus elastica* var. *decora* layers treated with IBA at 3000 ppm by painting girdled zone + *B. subtilis* and the control.

### Conclusions

From the above mentioned results, it appears that all treatments tested significantly increased rooting and survival percentages, as well as root and shoot characteristics compared to the control. The best results were obtained from treatment of air-

layers with 3000 ppm IBA by painting girdled zone combined with *B. subtilis*, followed by the same IBA treatment + AMF. Hence, this treatment is recommended for improving the propagation of *F. elastica* var. *decora* through air layering.

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## تحسين صفات التجذير في التراقيد الهوائية لنبات فيكس ديكورا باستخدام إندول حمض البيوتيريك في وجود بكتيريا باسيلس ساتلس وفطر الميكوريزا

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قسم الزينة وتنسيق الحدائق - كلية الزراعة - جامعة أسيوط

### المخلص

أجريت التجربة الحالية بمزرعة أبحاث الزينة بكلية الزراعة - جامعة أسيوط خلال موسمي ٢٠١٦، ٢٠١٧ بهدف مقارنة الطرق المختلفة للإضافة وتركيزات إندول حمض البيوتيريك والتي اشتملت على: مخلوط الأكسين بيئة التجذير (بتركيز ٥٠، ١٠٠ جزء في المليون)، دهان منطقة التحليق (بتركيز ١٥٠٠، ٣٠٠٠ جزء في المليون) و مخلوط بعجينة التلك (بتركيز ١٥٠٠، ٣٠٠٠ جزء في المليون). وذلك مع تلقيح بيئة التجذير بالكائنات الحية الدقيقة المفيدة (بكتيريا باسيلس ساتلس أو فطر الميكوريزا. هذا بالإضافة إلى معاملتي المقارنة للأكسين والكائنات الحية الدقيقة. صممت التجربة بنظام القطع المنشقة مع ثلاث مكررات، حيث اشتملت القطع الرئيسية على معاملات إندول حمض البيوتيريك، بينما اشتملت القطع المنشقة على الكائنات الحية الدقيقة. وأظهرت النتائج المتحصل عليها أن دهان منطقة التحليق بإندول حمض البيوتيريك بتركيز ٣٠٠٠ جزء في المليون نتج عنه حدوث زيادة معنوية في نسبة التجذير، سرعة التجذير، أفضل صفات جذرية وخضرية (عدد، طول، الوزن الطازج والجاف للجذور لكل ترقيدة هوائية مجذرة، أيضا نسبة البقاء، ارتفاع النبات، عدد الأوراق، وعدد فروع لكل نبات بعد فصل التراقيد الهوائية من النباتات الأم)، كما أدت هذه المعاملة إلى الحصول على محتوى أعلى من الكربوهيدرات، الفينولات الكلية، إندول حمض الخليك ومشتقاته مقارنة بالمعاملات الأخرى. تفوقت التراقيد الهوائية الملقحة ببكتيريا باسيلس ساتلس على تلك المعاملة بفطر الميكوريزا. كما تم الحصول على أفضل النتائج المتعلقة بنسبة التجذير، صفات التجذير، المحتوى الكلى للكربوهيدرات والفينولات، نسبة البقاء وزيادة نمو التراقيد الهوائية نتيجة المعاملة المشتركة للتراقيد الهوائية بدهان منطقة التحليق بإندول حمض البيوتيريك بتركيز ٣٠٠٠ جزء في المليون بالاشتراك مع بكتيريا باسيلس ساتلس، يليها المعاملة بنفس معاملة إندول حمض البيوتيريك في وجود فطر الميكوريزا.