

Combined Effects of Auxin Application and Beneficial Microorganisms on Rooting and Growth of *Ficus benjamina* L. Air-layers



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Abstract:

This study was conducted to define the best auxin application method with or without the inoculation with beneficial microorganism (*Bacillus subtilis* and arbuscular mycorrhizal fungi “AMF”) to improve rooting characteristics of *F. benjamina* air-layers. The treatments were arranged in a split-plot design. Seven combined treatments of auxin concentrations and application methods (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 assigned to the main plots. The sub-plots comprehended the application of *B. subtilis* and arbuscular mycorrhizal fungi in addition to the control. Data recorded on air-layers performance exhibited significant variation among different application methods and concentrations of IBA compared with the control with clear superiority of applying IBA at 3000 ppm by painting girdled zone in enhancing rooting%, number, length, fresh and dry weights of roots per rooted air-layer, as well as total contents of both carbohydrates and phenolics, and showed the shortest period required for root appearance. Similar trend was noticed regarding survival percentage, increment in plant height and number of leaves and shoots after detaching air-layers from mother plants. All root and growth characteristics showed significant increment when air-layers were treated with *B. subtilis* or AMF compared to the control with clear superiority of *B. subtilis*. The combined treatment of IBA at 3000 ppm by painting girdled zone and *B. subtilis* recorded the highest rooting (100%) and survival percentages (100%) and the best root and growth characteristics. This treatment could be recommended for propagation of *Ficus benjamina* by air layering.

Keywords: *Ficus benjamina*, IBA, *Bacillus subtilis*, arbuscular mycorrhizal fungi, air layering.

Introduction

Ficus benjamina belonging to family Moraceae, is a very popular ornamental species widely used as a pot-plant and in landscape gardening. As it has a high export potential, it can be cultivated commercially for exportation thereby earning foreign exchange (Gamlath *et al.*, 2010). So, it is necessary to find a suitable technique for propagation at large scale in

short time. It can be successfully propagated by air layering because it is simple and more rapid method for producing large plants with high quality.

Auxins such as indole-3-butyric acid (IBA) is known as root-promoting chemicals to accelerate root initiation and development, rooting percentage, number and quality of roots produced per cutting and the

uniformity of rooting (Wiessman-Ben and Tchoundjeu, 2000), which is attributed to the stability of IBA and stimulation of the rooting process (Hartmann *et al.*, 2014). In the same context, Nanda and Kochhar (1985) studied the application of root promoting substances during layering to get profuse rooting within a short time period and IBA has been found the most effective. In addition, a wide variety of auxin application methods have been reported (Blythe *et al.*, 2007). The most frequently used methods include the quick application with a concentrated solution (Blythe *et al.*, 2004 on *Ficus benjamina*), a talc powder application (Solanki *et al.*, 1986 on *Prosopis cineraria*, Hagen 1990 on *Prosopis chilensis* and Kumar, 2011 on guava), a lanolin paste (Suryanarayana and Rao, 1984 on *Punica granatum* and *Ficus carica*) or as a mixture with the rooting substrate in dilute concentrations (Wells, 1986 on *Mahonia aquifolium* and Gilani *et al.*, 2019 on guava).

Recently, root promotion on air-layered shoots has been demonstrated by the inoculation with beneficial microorganisms such as Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) (Rinallo *et al.*, 1999, Abdel-Rahman and El-Naggar, 2014 and Zenginbal and Demir, 2018). Previous studies have shown that bacteria in several genera (*Bacillus*, *Agrobacterium*, *Pseudomonas* and *Azospirillum*) can induce adventitious root formation (Rinallo *et al.*, 1999, Abdel-Rahman and El-Dsouky, 2010, Kumar, 2011, and Chawla and Mehta, 2015). The inoculation of rooting substrate with AMF during cutting

propagation has been reported to increase rooting in many woody plants (Scagel, 2001, Scagel, *et al.* 2003 and Fernades *et al.*, 2019). Adventitious root promotion is attributed to stimulate the synthesis of phytohormones such as auxins, gibberellins and cytokinins, as well as polyphenolic compounds by these beneficial microorganisms (Barea and Azcon-Aguilar, 1982, Mitchell *et al.*, 1986 and Goto, 1990).

However, studies comparing different auxin application methods and evaluating the relationship between beneficial microorganisms such as *Bacillus subtilis* and arbuscular mycorrhizal fungi (AMF) and air-layers treated with auxins are still scarce. Therefore, the present study aimed to improve rootability, root and vegetative characteristics as well as survival percentage of *Ficus benjamina* air-layers by using different application methods of auxins and inoculation with *B. subtilis* and AMF.

Material and Methods

The current investigation was conducted at the Floriculture Farm, Faculty of Agriculture, Assiut University, Egypt, during the 2016 and 2017 seasons to determine the effects of indole-3-butyric acid (IBA) and/or beneficial microorganisms (*Bacillus subtilis* and arbuscular mycorrhizal fungi “AMF”) on rooting and survival percentages as well as root and growth characteristics of *Ficus benjamina* air-layers.

Active strains of *Bacillus subtilis* and arbuscular mycorrhizal fungi “AMF” (*Glomus intraradices*) were obtained from the Unit of Biofertilizers, Fac. Agric., Ain Shams Univ., 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. benjamina* shoots.

Seven combined treatments of IBA concentrations and application methods; 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 used for treating air-layers.

Selected mature shoots of *F. benjamina* were girdled by removing 2.0 cm ring of bark in the intermodal region, ca. 120 cm below the tip of shoot. The layering operation was done at the beginning of March for both seasons. Air-layers were treated with IBA and/or beneficial microorganisms. The girdling region was immediately covered with a rooting substrate (perlite and peat moss "1:1 v/v"). The rooting substrate was covered with transparent plastic bags and the both ends were secured firmly using gunny thread and then covered with aluminum foil. Air-layers in the control were treated with distilled water alone.

The experiment was laid out as a 7 x 3 factorial arranged in a split-plot design with three replicates. Seven combined treatments of IBA were assigned to the main plots. The sub-plots comprehended the inoculation with *B. subtilis* or AMF in addition to the control. Each sub-unit contained five air-layers. The air-layers were protected from any mechanical damage and loss of moisture.

All the air-layers were detached from mother plants two and half

months after air layering, then dipped in water to remove adhering rooting substrate. Data were recorded on rooting percentage, number of days to emerge visual roots, number of roots, root length, fresh and dry weights of roots per rooted air-layer. After detaching the rooted layers from mother plants, they were planted in polyethylene bags filled with clay soil and were kept under plastic house conditions. Six months later, the increment in shoot length and number of leaves and shoots parameters per rooted layer as well as survival percentage were recorded.

At the time of detaching the air-layers from mother plants, the basal 2.5-3.0 cm portion of root zone was used for determining total carbohydrates, phenols and endogenous phytohormones. Some samples were oven-dried at 70° C for 48 h and ground to a fine powder then placed for chemical procedures. Total carbohydrates content was determined colorimetrically 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 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

Results in Table 1 indicated that applying IBA by painting girdled zone was superior to the other application methods, followed by using IBA in the talc paste while application of IBA into the rooting substrate

recorded lower results compared to the other methods. Besides, exogenous application of IBA at different concentrations significantly increased the rooting percentage of layered shoots compared to the control. The highest rooting percentages (91.1%) were obtained from treated air-layers with 3000 ppm IBA by painting girdled zone. Similar results were obtained by Puri and Nagpal (1988) on *Carissa carandas* and *Dalbergia sissoo*, Dessalegn and Reddy (2003) on *Simmondsia chinensis*, Suresh *et al.* (2016) on *Syzigium jambos*, Chauhan (2009) and Reddy *et al.* (2014) in *Ficus carica*, Udhavrao (2017) on *Punica granatum* and Gilani *et al.* (2019) on *Psidium guajava*. These previous studies indicated that the increase in rooting percentage might be attributed to the vital role of higher IBA concentration in mobilization and utilization of carbohydrates and nitrogen fraction with the presence of co-factor at girdled zone which may have helped in better root initiation. Hence, IBA at higher concentration resulted in better rooting in *F. benjamina* air-layers.

Table 1. Percentage of rooted layers in *Ficus benjamina* 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	66.67	60.00	60.00	53.33	66.67	66.67	62.22
in the substrate	50	66.67	80.00	73.33	73.33	66.67	80.00	73.33	73.33
	100	73.33	80.00	80.00	77.78	66.67	86.67	80.00	77.78
by painting	1500	73.33	86.67	86.67	82.22	73.33	93.33	86.67	84.44
	3000	80.00	100.00	100.00	93.33	80.00	100.00	86.67	88.89
Talc paste	1500	73.33	86.67	80.00	80.00	73.33	86.67	80.00	80.00
	3000	80.00	93.33	93.33	88.89	73.33	93.33	86.67	84.44
Mean		71.43	84.76	81.90		69.52	86.67	80.00	
LSD at 0.05									
IBA Treatments		1.98				3.18			
Microorganisms		1.38				1.78			
Interaction		3.65				4.71			

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

On the other hand, inoculation with *B. subtilis* and AMF significantly increased rooting percentage comparing with the untreated layers (control). *Bacillus subtilis* treatment was more effective on stimulating adventitious root formation (85.8%) than AMF treatment (81.0%) as average of two seasons, respectively. These results agree with those obtained by Rinallo *et al.* (1999), Abdel-Rahman and El-Dsouky (2010) and Kumar (2011). Also, Chawla and Mehta (2015) stated that PGPR increased adventitious roots formation. PGPR encouraged beneficial effect on plant growth and accelerate the availability of nutrients and assimilate and well as the production of phytohormones. It has been reported that *B. subtilis* produces IAA (Goto, 1990 and Rajan and Radhakrishna, 2013).

The interaction effect between IBA application methods and beneficial microorganisms was significant during both seasons. Rooting percentage reached 100% in air-layers treated with both IBA at 3000 ppm by painting girdled zone and *B. subtilis* in both seasons. When AMF com-

bined with the same IBA treatment, rooting percentage reached 93.35% as average of both seasons. These results are in accordance with those obtained by Rinallo *et al.* (1999), Chawla (2011), Zenginbal and Demir (2018) and Fernades *et al.* (2019). The higher rooting percentage of *F. benjamina* air-layers may be due to synergistic effect of IBA in combination with *B. subtilis* or AMF (Rinallo, 1999, Scagel, *et al.*, 2003, Kumar, 2011 and Abdel-Rahman and El-Naggar, 2014).

Root characteristics

The results presented in Tables 2, 3, 4, 5 and 6 revealed that all IBA treatments considerably induced early root appearance and improved root characteristics of air-layers. Among IBA treatments, IBA at 3000 ppm by painting girdled zone resulted in significant decrease in number of days required for roots appearance and was significantly superior over rest of the concentrations. This treatment also increased each of number and length of roots as well as fresh and dry weights of roots. These results are in agreement with those obtained by Suryanarayana and Rao (1984), Des-

salegn and Reddy (2003), Chawla (2011), Reddy *et al.* (2014) and Gilani *et al.* (2019). They observed that treating air-layers with IBA accelerate root appearance and improve root characteristics. They added that the response of air-layers to increasing IBA concentrations might be due to the activity of auxin and the accumulation of rooting co-factors at cambial region above the ringed portion. Also, enhancement of auxin concentration in the cell that may be adequate for initiating root primordia early by increasing cell division that further speed up callus formation. Nanda (1975) ascribed the promotive effect of IBA to better utilization of reserved carbohydrates in the hardwood with the application of exogenous auxin by converting starch into simple sugars, which is required for the production of new cells and for increased respiratory activity in regenerating tissues at the time of root initiation. The increase in fresh and dry weights of roots may be due to exogenous application of auxin which generally stimulate the movement of natural auxin and other materials downward from the leaves and shoot tips and accumulate at the girdling zone resulted in root formation with higher fresh weight as reported by Maurya *et al.* (2012) and Baghel *et al.* (2016).

Regarding the effect of beneficial microorganisms, early root appearance and better root characteristics were obtained by inoculation of air-layers with *B. subtilis* or AMF comparing to the control with a remarked superiority to *B. subtilis* over AMF. These results are in accordance with those revealed by Rinallo *et al.*

(1999), Abdel-Rahman and El-Naggar (2014), Chawla and Mehta (2015) and Zenginbal and Demir (2018). Promotion of adventitious roots formation is attributed to the production of phytohormones such as auxins, gibberellins and cytokinins as well as polyphenolic compounds by these beneficial microorganisms (Barea and Azcon-Aguilar, 1982, Mitchell *et al.*, 1986, Goto, 1990 and Erturk *et al.*, 2010). It is well documented that AMF stimulates the biomass of the root system (Sarkar *et al.*, 2015). This observation appeared that the increase in fresh and dry weights of roots of layered shoots in *F. benjamina* plants associated with AMF was due to the symbiosis between the roots of the host plants and the mycorrhizal fungi that resulted in an increase in nutrients and water uptake, thus favoring biomass production.

The combined effects of IBA application methods and beneficial microorganisms were significant and showed that early root appearance was obtained under 3000 ppm IBA by painting girdled zone combined with *B. subtilis* treatment followed by 3000 ppm IBA by painting girdled zone + AMF which were remarkably superior to other treatments. It has previously been demonstrated that the combined treatment of the beneficial microorganisms and IBA showed greater capacity than IBA or microorganisms alone in accelerating and enhancing rooting in several plant species (Scagel *et al.*, 2003, Abdel-Rahman and El-Naggar, 2014, Abdul Hakim *et al.*, 2018 and Fernades *et al.*, 2019). They attributed this effect to the synergistic influence since IBA is known for promoting emergence of

roots when used at optimum concentration in combination with beneficial microorganisms. Fuscon (2014) has lately cleared the interactive role of auxin and arbuscular mycorrhizal fungi and their host during the pre-colonization phase as being important

in the process of lateral root formation. In addition, this also enhances changes in the release of carbohydrates to the roots and modulation of phytohormones concentration resulting in improving root system (Schott *et al.*, 2013).

Table 2. Number of days to emerge visual roots in *Ficus benjamina* 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)		48.7	40.3	42.0	43.7	47.3	40.7	41.7	43.2
in the substrate	50	39.3	36.0	37.7	37.7	39.3	35.7	36.0	37.0
	100	37.7	36.7	37.3	37.2	37.0	34.3	35.0	35.4
by painting	1500	37.7	36.3	36.7	36.9	34.7	34.0	35.0	34.6
	3000	36.0	29.3	32.7	32.7	34.0	29.7	33.7	32.1
Talc paste	1500	37.0	35.3	35.7	36.0	33.7	35.7	35.3	34.9
	3000	35.0	32.3	33.7	33.7	34.7	32.3	33.7	33.6
Mean		38.8	35.2	36.5		37.2	34.6	35.6	
LSD at 0.05									
IBA Treatments		0.7				0.9			
Microorganisms		1.0				0.7			
Interaction		2.6				1.8			

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

Table 3. Root number per air-layer of *Ficus benjamina* 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)		6.67	15.33	13.33	11.78	7.00	17.67	15.67	13.44
In the substrate	50	20.83	25.67	24.50	23.67	19.83	32.50	27.83	26.72
	100	23.17	29.17	27.37	26.57	21.17	38.33	36.67	32.06
By painting	1500	28.33	36.33	33.67	32.78	27.00	43.00	37.50	35.83
	3000	30.83	40.33	36.33	35.83	38.50	49.00	47.67	45.06
Talc paste	1500	28.33	33.50	30.83	30.89	29.50	40.00	38.33	35.94
	3000	28.33	37.00	35.67	33.67	31.00	43.00	40.00	38.00
Mean		23.79	31.05	28.81		24.86	37.64	34.81	
LSD at 0.05									
IBA Treatments		1.36				1.36			
Microorganisms		0.68				1.04			
Interaction		1.80				2.75			

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

Table 4. Root length (cm) per air-layer of *Ficus benjamina* 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)		6.83	13.17	11.33	10.44	12.17	19.67	18.33	16.72
In the substrate	50	10.60	14.17	13.33	12.70	16.33	22.67	21.67	20.22
	100	12.67	13.17	14.33	13.39	19.00	24.50	16.00	19.83
By painting	1500	14.00	17.00	15.17	15.39	22.17	27.33	25.67	25.06
	3000	16.67	20.00	19.00	18.56	24.50	29.00	28.83	27.44
Talc paste	1500	13.50	16.00	14.67	14.72	22.00	18.83	23.50	21.44
	3000	14.33	16.83	16.17	15.78	23.00	20.33	26.33	23.22
Mean		12.66	15.76	14.86		19.88	23.19	22.90	
LSD at 0.05									
IBA Treatments		0.65				2.27			
Microorganisms		0.54				1.35			
Interaction		1.43				3.58			

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

Table 5. Root fresh weight (g/air-layer) of *Ficus benjamina* 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)		5.60	8.90	8.37	7.62	7.34	11.68	10.67	9.90
In the substrate	50	10.20	13.87	12.27	12.11	10.77	14.91	12.95	12.88
	100	11.07	15.43	13.90	13.47	13.11	20.17	16.56	16.61
By painting	1500	14.27	21.17	18.67	18.03	18.84	23.87	22.33	21.68
	3000	18.10	32.63	30.27	27.00	24.30	33.37	29.18	28.95
Talc paste	1500	12.37	20.73	17.03	16.71	17.37	20.65	19.52	19.18
	3000	14.70	25.23	22.23	20.72	19.26	24.56	23.30	22.37
Mean		12.33	19.71	17.53		15.86	21.32	19.22	
LSD at 0.05									
IBA Treatments		0.84				0.53			
Microorganisms		0.69				0.48			
Interaction		1.83				1.27			

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

Table 6. Root dry weight (g/air-layer) of *Ficus benjamina* 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)		1.60	3.07	2.93	2.54	2.19	3.35	3.31	2.95
In the substrate	50	3.21	3.93	3.60	3.58	3.59	4.24	4.03	3.95
	100	3.23	4.41	4.08	3.91	4.21	5.92	4.94	5.02
By painting	1500	4.12	5.94	5.00	5.02	5.74	6.59	6.09	6.14
	3000	4.95	8.19	7.50	6.88	6.92	9.76	8.83	8.50
Talc paste	1500	3.48	5.21	4.48	4.39	4.68	5.80	5.71	5.40
	3000	3.98	6.16	5.84	5.33	5.18	6.63	6.16	5.99
Mean		3.51	5.27	4.78		4.64	6.04	5.58	
LSD at 0.05									
IBA Treatments		0.66				0.48			
Microorganisms		0.23				0.33			
Interaction		0.62				N.S.			

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

Vegetative growth characteristics

Data presented in Figures 1 and 2 show that treatment of *F. benjamina* air-layers with IBA and/or beneficial microorganisms significantly increased plant height and number of shoots and leaves per rooted layer at 6 months after transplanting compared to the control in both seasons. However, application of 3000 ppm IBA by painting girdled zone combined with *B. subtilis* was the most effective on increasing vegetative growth characteristics of rooted layers, followed by 3000 ppm IBA by painting girdled zone + AMF treatment comparing with the individual treatments of IBA or beneficial microorganisms. Since these separated layers are having better root system, they could absorb sufficient water and other nutrients, which could be excellent in all studied characters. These results are in agreement with the finding of Khalid *et al.* (2004), Chawla (2011) and Kumar (2011). In addition, Abdel-Rahman and El-Dsouky (2010) and Abdel-Rahman and El-Naggar (2014) found that combined IBA-bacteria or IBA-AMF treatments were more effective in increasing root and vegetative growth than control or IBA and beneficial microorganisms alone.

The increment in vegetative growth characteristics as a results of IBA treatments may be due to the role of IBA on cell division and/or cell elongation (Noggle and Fritz, 1989). Besides, the stimulatory action of auxin in softening the cell wall, increasing its plasticity leading to cell enlargement of the protoplast by water uptake and consequently stimulating the growth (Tagwa and Bonner, 1957). The enhancing effect of *B. subtilis* on the vegetative growth characters obtained in the present investigation was also found by Karakurt *et al.* (2009) and Erturk *et al.* (2010).

The PGPR may increase the level of root hormone by production of IAA, cytokinin other plant hormones. Thus, the amount of IAA and cytokinin may appear to be directly correlated with plant growth. Many plant-associated bacteria have the ability to produce plant growth regulators especially IAA which further play an important role in plant growth promotion (Patten and Glick, 2002 and Khalid *et al.*, 2004) and hence may have increased growth emergence after transplanting.

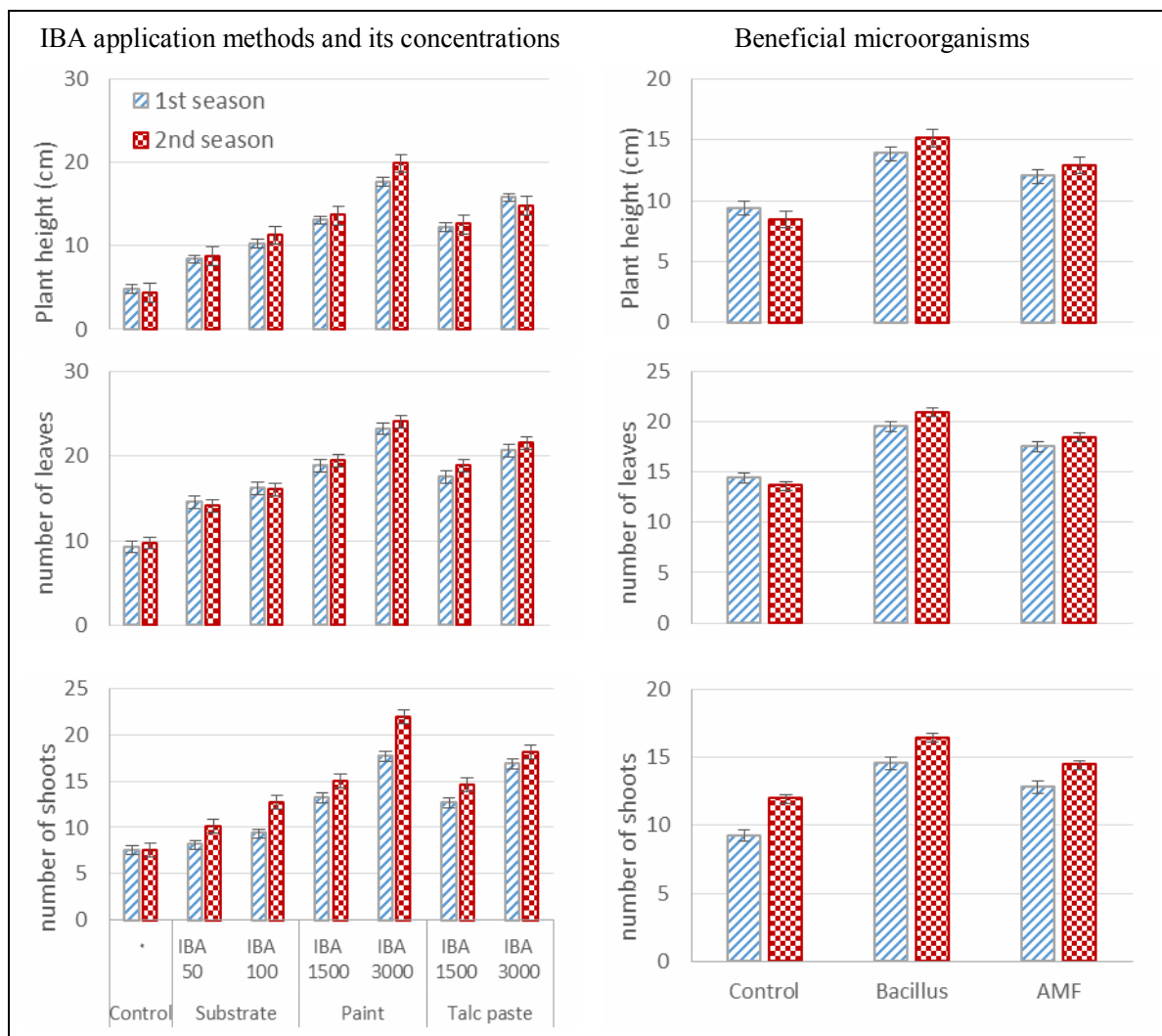


Fig. 1. Effect of IBA at different concentrations and application methods, and beneficial microorganisms on vegetative growth characteristics of *F. benjamina* during both seasons. Vertical bars above mean denote LSD values ($p \leq 0.05$).

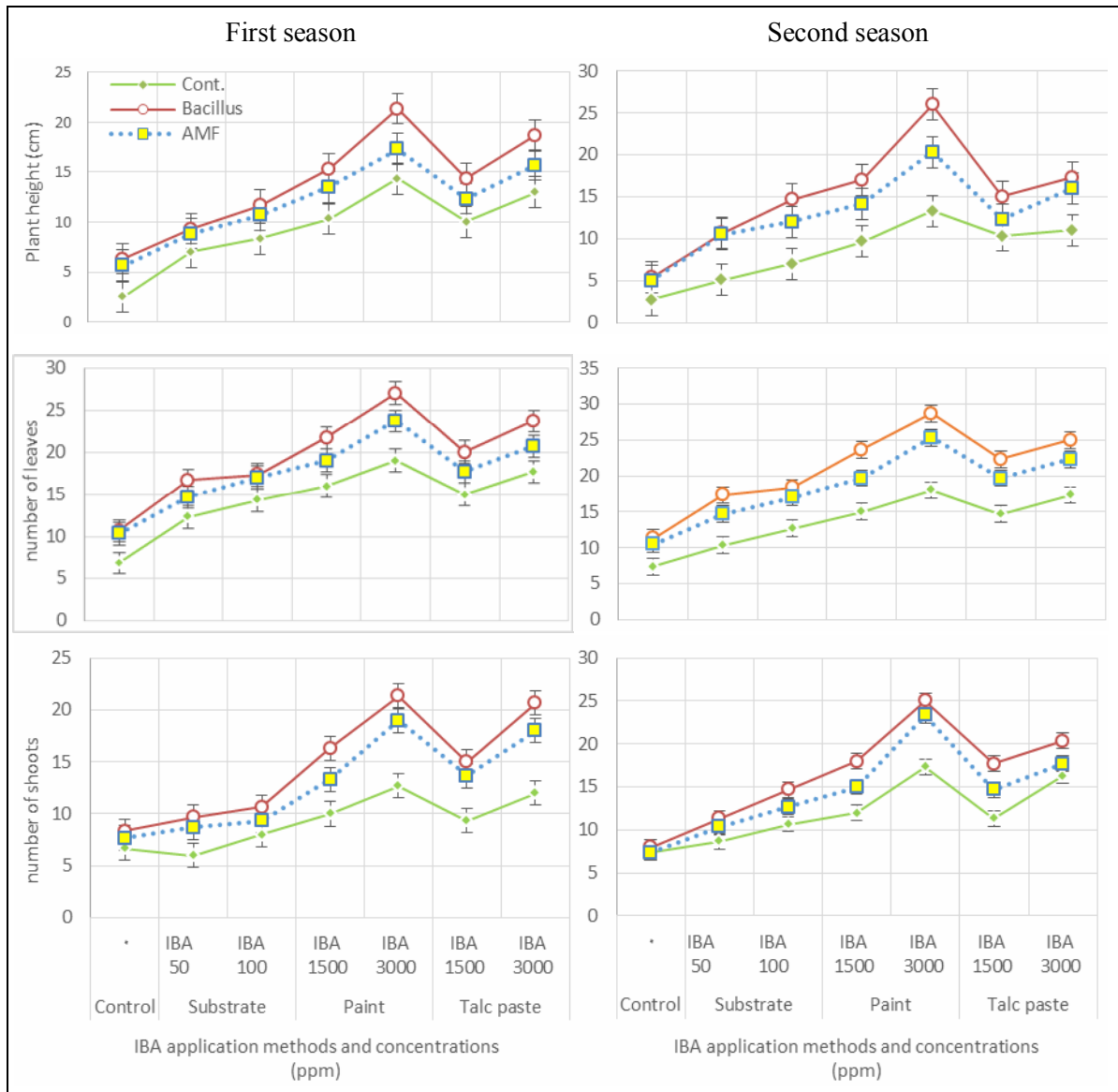


Fig. 2. Interaction effects between IBA at different concentrations and application methods and beneficial microorganisms on vegetative growth characteristics of *F. benjamina* during both seasons. Vertical bars above mean denote LSD values ($p \leq 0.05$).

Survival percentage

Survival percentage of successfully rooted air-layers is almost important characteristic which has to be carefully traced when propagating plants by air-layering. This is logic since attaining successful rooting with extra root characteristic has no value without successfully survive these air-layers. Accordingly, data on survival percentage of *F. benjamina* air-layers were represented in Table

7. It could be inferred that treating air-layers of *F. benjamina* with IBA at 3000 ppm by painting girdled zone combined with *Bacillus* or AMF which produced the best rooting characteristics seem to influence the survival percentage as well. These combined treatments gave the maximum survival percentage (100%) in both seasons comparing to untreated layers (53.7%) as an average of two seasons. These results are in concur-

rence with the findings of Singh *et al.* (2007), Kumar (2011) and Baghel *et al.* (2016). The higher percentage of survival after transplanting the rooted air-layers can be attributed to the possession of better root characteristics like higher number and length of roots (Kumar, 2011 and Reddy *et al.*, 2014). In addition, it might be due to the rooting co-factors and their balance with nutritive substances and auxin (Rymbai and Reddy, 2010 and Baghel *et al.*, 2016).

Beneficial microorganisms including bacteria and mycorrhizae

fungi encourage plant growth and accelerate the availability of nutrients and assimilates, as well as the production of substances promoting plant growth. This effect happens through the biological nitrogen fixation, increasing inorganic phosphate solubilization of organic phosphorus compounds, as well as water and nutrient uptake (Esitken *et al.*, 2003, Das *et al.*, 2006, Aslantas *et al.*, 2007 and Chawla, 2011) and hence improving survival percentage.

Table 7. Survival percentage of new produced plants after 6 months from transplanting in *Ficus benjamina* 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	55.57	50.00	51.87	50.00	61.10	55.57	55.56
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	61.10	88.90	88.90	79.63
By painting	1500	72.23	80.57	77.80	76.87	72.23	88.90	77.80	79.64
	3000	77.80	100.00	91.67	89.82	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	81.75	78.19		66.67	83.73	78.98	
LSD at 0.05									
IBA Treatments		4.40				4.27			
Microorganisms		2.44				2.69			
Interaction		6.47				7.11			

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

Total carbohydrates and phenols content

The obtained results (Tables 8 and 9) show that total carbohydrates and phenols contents were higher in the air-layers treated with IBA and/or beneficial microorganisms compared to untreated layers at the end of the rooting period. The air-layers treated with 3000 ppm IBA by painting girdled zone + *B. subtilis* contained higher levels of total carbohydrates and phenols which led to maximum

rooting, followed by the combined treatment of 3000 ppm IBA + AMF. These results are in agreement with those obtained by Hess (1964), Yeboah *et al.* (2014) and Suresh *et al.* (2016). They stated that higher levels of total carbohydrates and phenols in plant tissues could stimulate adventitious root formation and improve root development. The present study indicate a positive relationship between rootability of *F. benjamina* air-layers treated with IBA combined with *B.*

subtilis or AMF and carbohydrates and phenols content in basal parts of the layered shoots. These results could be attributed to certain evidences supported by the effective role of carbohydrates and phenols as well as production of IAA using *B. subtilis* and AMF in the presence of IBA to exert an effect on the rootability of air-layers.

As for phenols, several studies (Hess, 1962, Karunakara, 1997 and Kumar, 2011) showed that the phenolic compounds act as auxin co-factors in root promotion. The auxins such as applied IBA and IAA produced by beneficial microorganisms bound the phenol molecules and translocate them to the girdled zone to prevent infection of the developed roots, as well as serve as protection against the oxidation of the auxins by IAA-oxidase (Pandey and Pathak, 1981 and Hartmann *et al.*, 2014).

On the other hand, carbohydrates have most often been considered to be the principal source of energy and carbon during rooting proc-

ess (Haissig, 1974). Carbohydrates concentration in plant tissues may be influenced by IBA treatment and beneficial microorganisms inoculation, which can enhance mobilization of carbohydrate in leaves and upper stem and increase transport to the rooting zone (Haissig, 1982, Woodward and Bartel, 2005 and Abdel-Rahman and El-Naggar, 2014). Several investigators (Abdel-Rahman and El-Dsouky, 2010, Kumar, 2011 and Kasem and Abd El-Baset, 2014) confirmed the importance of carbohydrates accumulation in plant tissues to improve number of roots and shoots. Arslonov (1979) observed breakdown of carbohydrates during the initial stages of root growth in lemon cuttings, and also noted a rise in catalase and peroxidase activities which accompanied by the breakdown of carbohydrates. The difference in the magnitude of decline of starch content between the treated and untreated layers showed that the exogenous application of auxins might have enhanced the hydrolysis of starch.

Table 8. Total carbohydrates (%) in tissue of girdled zone in *Ficus benjamina* 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)	12.67	19.23	18.34	16.75	16.16	19.30	17.39	17.62	
In the substrate	50	18.17	21.93	18.79	19.63	18.26	21.53	20.64	20.14
	100	18.32	19.01	18.59	18.64	20.56	22.34	20.10	21.00
By painting	1500	19.77	21.37	19.19	20.11	20.80	22.74	23.10	22.21
	3000	19.58	23.40	19.38	20.79	20.24	24.93	24.53	23.23
Talc paste	1500	20.20	21.38	20.52	20.70	20.03	21.73	21.78	21.18
	3000	19.92	22.57	22.37	21.62	22.12	23.02	23.40	22.84
Mean	18.38	21.27	19.60		19.74	22.23	21.56		
LSD at 0.05									
IBA Treatments	0.92				0.74				
Microorganisms	0.63				0.51				
Interaction	1.67				1.35				

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

Table 9. Total phenolics (mg GAE/ g DW) in tissue of girdled zone in *Ficus benjamina* 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)		8.93	11.47	10.80	10.40	9.43	13.27	10.80	11.17
in the substrate	50	9.54	12.67	11.67	11.29	11.53	14.97	14.63	13.71
	100	9.40	13.50	12.88	11.93	14.63	16.67	14.28	15.19
by painting	1500	10.33	15.19	13.37	12.96	14.67	17.07	14.30	15.34
	3000	14.30	19.10	17.57	16.99	16.13	19.20	19.90	18.41
Talc paste	1500	11.11	14.57	14.37	13.35	14.87	16.90	17.23	16.33
	3000	14.10	17.87	16.03	16.00	15.30	17.77	16.33	16.47
Mean		11.10	14.91	13.81		13.80	16.55	15.35	
LSD at 0.05									
IBA Treatments		0.45				1.04			
Microorganisms		0.29				0.68			
Interaction		0.78				1.79			

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

Promoters and inhibitors

Data presented in Fig. 3 for the GC MS analysis of the samples extracted from basal portion root zone (2.5-3.0 cm) of the rooted layers for the control and the layers treated with IBA at 3000 ppm by painting girdled zone + *B. subtilis* which induced the highest effect upon rooting and survival percentages as well as root and vegetative growth characteristics, exhibited the presence of 3 phytohormones components in control and only 2 in the best treatment. The phytohormones components in the control sample were indole acetic acid and its derivatives at 8.54%, zeatin at 0.28% and GA₃ at 0.13%. Meanwhile, the treatment of IBA at 3000 ppm by painting girdled zone + *B. subtilis* sample had indole acetic acid and its derivatives at 17.4% and zeatin at 0.26% as a percentage of total organic compounds per sample. The obtained results showed that the best treatment increased the concentration of IAA and its derivatives comparing to the control, which is strongly correlated with the im-

provement in rooting and survival percentages as well as root and vegetative growth characteristics. This effect may be due to the role of combined treatment of IBA and *B. subtilis* in increasing the level of IAA at the girdled zone of *F. benjamina* air-layers. Thus, *B. subtilis* has the ability to produce plant growth regulators especially IAA which further play an important role in plant growth and root formation (Goto, 1990 and Rajan and Radhakrishna, 2013). Suresh *et al.* (2016), Ghosh *et al.* (2017) and Gilani *et al.* (2019) also reported that the response of air-layers to IBA with increasing concentration might be due to the activity of auxin and the accumulation of rooting co-factors at cambial region above the girdled zone, as well as enhancement of auxin concentration in the cell that may be adequate for initiating root primordia early by increasing cell division that further speed up callus formation. In addition, cytokinins and gibberellic acid have inhibitory effects on adventitious root formation as suggested by Pawlicki and We-

lander (1992) and Brian *et al.* (1960) where gibberellic acid probably inhibited the outgrowth of root primordia. This could be related to a partial inhibition of endogenous starch synthesis in the plastids, as described by

Coleman and Greyson (1979). Also, Haissig (1972) concluded that GA₃ hindered the development of root primordia via an inhibition of mitotic activity within the initiating root primordia.

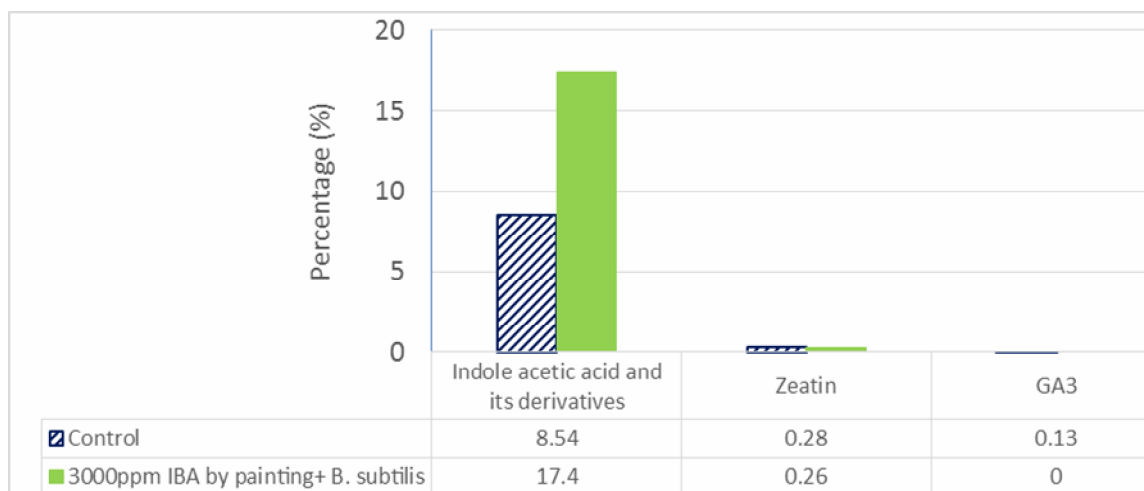


Fig 3. Gas chromatography–mass spectrometry (GC-MS) analysis of two samples extracted from root zone of *Ficus benjamina* layers treated with IBA at 3000 ppm by painting girdled zone + *B. subtilis* and the control.

Conclusions

From results obtained, it could be concluded that the better rooting and survival percentages of *Ficus benjamina* air-layers were observed under the treatment of 3000 ppm IBA by painting girdled zone combined with *B. subtilis*, followed by 3000 ppm IBA by painting girdled zone + AMF for all root and growth characteristics before and after detachment of air-layers. Hence, this treatment is recommended for obtaining rooted layers from *F. benjamina* with superior root and vegetative growth characteristics in a short period of time as well as a high survival rate.

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تأثيرات المشتركة لإضافة الأكسين والكائنات الحية الدقيقة المفيدة على تجذير ونمو الترافيد الهوائية لنبات فيكس بنجامينا

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قسم الزينة وتنسيق الحدائق - كلية الزراعة - جامعة أسيوط

الملخص

أجريت هذه الدراسة بمزرعة أبحاث الزينة بكلية الزراعة - جامعة أسيوط خلال موسمى ٢٠١٧، ٢٠١٦ بهدف تحديد الطريقة المثلى والتركيز المناسب لإضافة إندول حمض البيوتيريك بالطرق التالية:

- ١- مخلوط ببيئة التجذير (بتركيز ٥٠، ١٠٠ جزء فى المليون).
 - ٢- دهان منطقة التحليق (بتركيز ١٥٠٠، ٣٠٠٠ جزء فى المليون).
 - ٣- مخلوط بعجينة التلك (بتركيز ١٥٠٠، ٣٠٠٠ جزء فى المليون).
- وذلك مع تلقيح بيئة التجذير بالكائنات الحية الدقيقة المفيدة (بكتيريا باسيلس ساتلس أو فطر الميكوريزا. هذا بالإضافة إلى معاملتى المقارنة للأكسين والكائنات الحية الدقيقة.
- صممت التجربة بنظام القطع المنشقة، حيث تم توزيع معاملات إندول حمض البيوتيريك فى القطع الرئيسية والكائنات الحية الدقيقة فى القطع المنشقة. وقد أوضحت النتائج ما يأتى:
- أظهر التحليل الإحصائى اختلافات جوهرية بين طرق إضافة وتركيزات إندول حمض البيوتيريك مقارنة بالكنترول.
 - أظهرت معاملة دهان منطقة التحليق بإندول حمض البيوتيريك بتركيز ٣٠٠٠ جزء فى المليون تقوفاً "ملحوظاً" فى زيادة نسبة تجذير الترافيد الهوائية، الصفات الجذرية (سرعة التجذير، عدد الجذور، طول الجذر، الوزن الطازج والجاف للمجموع الجذرى بكل ترقية).
 - اتضح وجود ارتباط وثيق بين القدرة العالية على التجذير وزيادة محتوى الأنسجة من الكربوهيدرات، الفينولات والهرمونات النباتية المنشطة للتجذير.
 - وجدت علاقة موجبة بين تنشيط التجذير، مواصفات النمو الخضري ونسبة البقاء للمعاملات التى تفوقت، حيث أنها أظهرت زيادة واضحة فى ارتفاع النبات، عدد الفروع والأوراق فى النباتات الجديدة.
 - نتج عن تلقيح بيئة التجذير ببكتيريا باسيلس ساتلس أو الميكوريزا تأثيراً منشطاً ملحوظاً فى نسبة نجاح الترافيد الهوائية، كما أظهرت زيادة ملموسة فى المواصفات الجذرية والخضرية مقارنة بالكنترول، وقد تفوقت البكتيريا عن الميكوريزا فى هذا الصدد.
 - أظهر التأثير المتبادل بين المعاملات أن دهان منطقة التجذير بإندول حمض البيوتيريك (٣٠٠٠ جزء فى المليون) مع التلقيح ببكتيريا باسيلس ساتلس تقوفاً فى زيادة نسبة التجذير (١٠٠%)، كما حسنت مواصفات النمو الجذرية والخضرية للنباتات الناتجة. ولذا يمكن النصح بتطبيق هذه المعاملة عند إكثار نبات فيكس بنجامينا بالترقيد الهوائى بهدف الإنتاج التجارى.