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## Welcome to Review Of Research

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## NODULATION AND GROWTH PARAMETERS OF CASUARINA GROWN IN SANDY AND CALCAREOUS SOILS AS INFLUENCED BY LOCALLY ISOLATED FRANKIA STRAINS ISOLATED FROM ECOLOGICALLY DIFFERENT AREAS IN EGYPT

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### Abstract:-

Sixteen *Frankia* pure isolates were isolated from three ecologically different Egyptian governorates i.e., five isolates from Kafr El-Sheikh, five isolates from New Valley and six isolates from Ismailia. According to their physiological properties, it was found that the isolated *Frankia* were identified as fifteen different *Frankia* strains belonging to the species of *Frankia casuarina*. In sandy soil, no nodules were formed in the control seedlings. The inoculated seedlings produced nodules dry weight ranged from 0.41 to 1.93 g/seedling with increases over the Br reference strain reached 13 to 15%. Inoculation of *Casuarina* seedlings with the local *Frankia* strains increased roots length and roots dry weight. The increases over the Br reference strain ranged from 3 to 130% and 0.4 to 139% consecutively. The isolated *Frankia* strains positively affect *Casuarina* stems length and number of branches in comparison with the Br reference strain. No significant differences were found regarding *Casuarina* shoots dry weight. For nodules dry weight, no nodules were formed in the control treatment. The isolated *Frankia* induced nodules dry weight ranged from 0.55 to 2.17 g/seedling, while the reference strain (Br) produced dry weight of nodules reached 1.20 g/seedling. For each of roots length and stems length, the isolated *Frankia* strains produced increases over the Br reference strain ranged from 2 to 104% and from 1 to 439% respectively. The dry weight of shoots of *Casuarina* seedlings grown in calcareous soil also positively affected by inoculation with the local *Frankia* strains in comparison with the Br reference strain, the percentage of increases ranged from 15 to 349. As for nitrogen content of roots and shoots of *Casuarina* seedlings grown in sandy soil, most of the locally isolated *Frankia* strains significantly overcame the Br reference strain. The increases in roots nitrogen content ranged from 17 to 137%, while for shoots they ranged from 10 to 123%. N-uptake in sandy soil was significantly affected by the majority of local *Frankia* strains; the increases reached 4% as a minimum and 354% as a maximum over the Br reference strain. In calcareous soil, the increases in nitrogen content of roots over the Br reference strain ranged from 21 to 225%. For shoots, these increases ranged between 14 and 131% over the Br reference strain. The isolated *Frankia* strains resulted in increases in N-uptake over the Br reference strain ranged between 20 and 395%. The majority of local *Frankia* strains were highly active where they acted well in both types of tested soils where the variations were not significant. The same trend was recorded with shoots nitrogen content due to the application of *Frankia* strains.

**Keywords:** *Frankia* sp., *Casuarina glauca*, nodulation, growth parameters, nitrogen uptake, sandy and calcareous soils.

Title: "NODULATION AND GROWTH PARAMETERS OF CASUARINA GROWN IN SANDY AND CALCAREOUS SOILS AS INFLUENCED BY LOCALLY ISOLATED FRANKIA STRAINS ISOLATED FROM ECOLOGICALLY DIFFERENT AREAS IN EGYPT",  
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**INTRODUCTION**

*Casuarina* is the most extensively grown trees in Egypt where they represent over 80 % of the number of cultivated trees (National Research Council, 1984). Interest in planting *Casuarina* in desert areas of Egypt and other arid lands as windbreaks and in woodlots has increased in the last two decades (El-Lakany et al. 1990). *Casuarinas* are planted as windbreaks, to stabilize sand, in agro-forestry systems and for amenity and ornamental purposes (Zhong Chonglu, 1990). The ability of *Casuarina* species to fix atmospheric nitrogen with association of soil actinomycetes, genus *Frankia*, is one attribute which makes these trees grown better and potentially important for fuel-wood production, agro- forestry and reclamation of infertile soils in tropical and subtropical zones. Recently, *Casuarina* trees have proved to increasing annually the soil nitrogen content by BNF, around its rhizosphere in higher rates in comparison with those of legumes-Rhizobium symbioses, as it reached about 300 Kg N/h/year. Nodulation of *Casuarina* roots by their specific genus *Frankia* under field conditions is irregular due to many factors, such as the absence of effective strains of *Frankia*, low phosphorus content, dryness and salt stress. In Egypt, since the soil is alkaline and poor in nutrients, as well as, the absence of infective *Frankia*, could be proposed as the main factors for the lack of nodule formation or irregular nodulation on *Casuarina* roots. So, the work within hand is concerning with investigating the impact of different *Frankia casuarina* strains that isolated from ecologically different Egyptian locations in comparison with the foreign Br reference strain (ORS 020608) on nodulation and growth of *Casuarina glauca* grown in sandy and calcareous soils.

**MATERIALS AND METHODS**

***Casuarina* seedlings**

Seeds of *Casuarina glauca* were kindly obtained from Desert Development Center (DDC) of American University in Cairo, Egypt. *Casuarina* seeds were surface sterilized by immersing seeds for 2 minutes in concentrated H<sub>2</sub>SO<sub>4</sub>, then washed with sterile tap water until the wash reached pH 7 (Selim and Schwencke, 1995). The sterile *Casuarina* seeds were germinated in sterile growth medium composed of washed sand and peat moss (1:1) per volume for two weeks under room temperature.

***Frankia* strains**

Sixteen isolates of *Frankia* were isolated from three ecologically different locations i.e., five isolates from Kafr El-Sheikh governorate, five isolates from New Valley governorate and six isolates from Ismailia governorate in addition to a reference *Frankia* strain [(Br) ORS 020608] that was isolated from *Casuarina equisetifolia* grown in Brazil, that kindly supported by Department of Plant Ecology, Faculty of Science, Chile University, Chile (Muller *et al.*, 1991).

**Experimental Soils**

Representative composite samples of virgin sandy and calcareous soils were collected from El-Nobarria city and Mariout Research Station (MRC) respectively. The collected soil samples were air dried, ground to pass through 2 mm sieve and kept at room temperature in plastic bags. The microbiological, physical and chemical properties of these soils were presented in Tables (2&3).

**Table 1: The microbiological analysis of the experimental sandy and calcareous soils.**

Microorganism Soil	Total counts of various microbial groups ( CFU/g dry soil)				
	Total	Fungi	Actinomycetes	N <sub>2</sub> - fixing microorganisms	Spore-forming bacilli
Sandy	3.4 x10 <sup>6</sup>	2.7 x10 <sup>4</sup>	1.1 x10 <sup>5</sup>	5.3 x10 <sup>5</sup>	1.6x10 <sup>5</sup>
Calcareous	1.0 x10 <sup>7</sup>	6.1 x10 <sup>4</sup>	2.7 x10 <sup>5</sup>	1.3 x10 <sup>5</sup>	1.7x10 <sup>4</sup>

**Table 2: Mechanical and chemical properties of the experimental sandy and calcareous soils.**

parameter soil	Mechanical analysis				W.H.C. (%)	Chemical analysis										O.M. (%)
	Sand (%)	Silt (%)	Clay (%)	Texture class		pH (2.5:1)	EC (dS/m)	CaCO <sub>3</sub> (%)	Cations (meq/L)				Anions (meq/L)			
									Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>		
Sandy	92.5	5.0	2.5	Sandy	26	7.7	1.7	1.4	4.8	3.1	8.7	0.4	0.3	11.3	5.4	0.51
Calcareous	5.3	30.7	64	Clay loam	40	8.34	0.51	30.2	1.4	1.0	2.2	0.5	0.1	3.1	1.9	0.79

W.H.C.: Water holding capacity; O.M.: Organic matter.

**Effect of inoculation with *Frankia* strains on *Casuarina glauca* grown in sandy and calcareous soils.**

The different isolated *Frankia* strains and the reference one as well were tested for their ability to nodulate their host plant, as well as their efficiency to fix atmospheric N symbiotically. A green-house experiment was carried out at the National Research Centre (NRC) using two different types of soils. These soils were collected from Mariout Research Station (MRC) and El-Nobaria city. The soils were sterilized by autoclaving for two hours at 121oC during three successive days. These soils were packed in sterile plastic pots of 25 cm diameter at the rate of 3 kg/pot. Pregerminated seeds (under sterile condition) of *Casuarina glauca* were transplanted at the rate of three seedlings/pot. The pots were inoculated with 25 ml of 10 days-old of the respective *Frankia* strain then covered with a thin layer of the same sterile soil. Irrigation was done with N-free Evan’s nutrient solution recommended by Huss-Danell (1978) at intervals according plant needs. Two control treatments were used in this study; the first was cultivated without inoculation with any of *Frankia* strains, while the second control was inoculated with the reference *Frankia* strain (Br). The greenhouse experiment continued for ten months, and then *Casuarina* seedlings were collected from all treatments as well as the control and subjected to the following estimations: Dry weight of nodules (g/seedling), shoots dry weight (g/seedling), plant nitrogen content (%) and plant nitrogen uptake (mg/seedling).

The experimental design was complete randomized block design. Each treatment was represented by three replicates and each replicate contained three seedlings. The obtained results were subjected to analysis of variance (ANOVA) according to the procedure outlined by Snedecor and Cochran (1982). Means of various treatments were compared using multiple range test (Duncan, 1955) at 5% level of probability.

**RESULTS AND DISCUSSION**

**Isolation and identification of *Frankia* strains:**

A new sterilization method for *Casuarina* root nodules was adopted (Gomaa et al. (2008). physiological properties i.e., use efficiency of sodium propionate, sodium pyruvate, sodium acetate, sucrose, glucose and mannitol as different carbon sources; antibiotics growth sensitivity at 15, 30 and 60 µg ml<sup>-1</sup> medium of Rifampicin, Kanamycin, Chloramphenicol, Spectinomycin, Tetracycline, Ampicilin, Erythromycin, Neomycin sulfate, Streptomycin sulfate and Vancomycin hydrochloride; salinity tolerance 2.0, 4.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0 and 10.5 % of sodium chloride; plasmids number and RAPD PCR , it was found that the various obtained *Frankia* isolates were identified as sixteen different *Frankia* strains all belonging to *Frankia casuarina* (Gomaa et al., 2008 and Sabir et al., 2009).

**The influence of inoculation with various *Frankia* strains on certain growth parameters of *Casuarina glauca* grown in sterile sandy soil:**

For nodules dry weight (g/seedling), Table (3) indicates that no nodules were formed in the uninoculated control treatment under sterile condition. With regard to the inoculated seedlings, Table (3) also demonstrates that the nodules dry weight of various treated seedlings ranged from 0.41 to 1.93 g/seedling being the highest with *Frankia* strain K 01 isolated from Kafr El-Sheikh and the lowest with *Frankia* strains N 05 and I 05 isolated from New Valley and Ismailia respectively. In comparison with the reference strain (Br), the only two *Frankia* strains of K 01 and I 04 isolated from Kafr El-Sheikh and Ismailia consecutively that recorded significant increase in nodules dry weight. Moreover, insignificant increases were found with *Frankia* strains K 05, I 02, I 03 and I 06 either against the reference strain or the other isolated strains.

Concerning the root length of *Casuarina* seedling as affected by inoculation with the various *Frankia* strains, Table (3) indicates that irrespective of the treatment, the root lengths ranged from 30.33 cm for the control to 111.0 cm for the seedlings inoculated with *Frankia* strain I 02 isolated from Ismailia. In comparison with the reference strain (Br), the local *Frankia* strains number K 01, K 03, N 04, I 02 and I 03 gave significant augments in the root length. The other tested *Frankia* strains induced results similar to the reference strain (Br) without significant differences. For the root dry weight parameter of *Casuarina* seedling, Table (3), also demonstrates that it ranged from 20.33 (g/plant) for K 01 isolate of Kafr El-Sheikh to 1.03 (g/plant) for the control (un-inoculated). The local *Frankia* strains K 01, K 02, K 03, N 02, N 03 and N 05 gave significant augments compared to the reference strain (Br). Moreover, insignificant variations were found among the remainder tested strains when compared to the Br strain. As to the stem length of *Casuarina* seedlings, Table (3) shows that the stem lengths ranged from 66.67 cm for the control treatment to 101.0 cm due to the application of K 01 *Frankia* strain of Kafr El-Sheikh. Further, comparable results to the reference *Frankia* strain without significant differences were recorded due to the inoculation with all local *Frankia* strains. It is worthy to mention that the local *Frankia* strains (except the strains K 02, K 04, K 05 and I 04) significant by surpassed the control treatment.

**Table 3: Effect of inoculation with different strains of *Frankia* on *Casuarina glauca* seedlings grown in sterile sandy soil.**

Parameter Treatment	Nodules D.W. (g/seedling)	Root length (cm)	Root D.W. (g/seedling)	Stem length (cm)	Branches (no/seedling)	Shoots D.W. (g/seedling)
Cont.	0.00 d	30.3 f	1.03 c	66.7 f	32 bc	3.67 b
Br	0.77 c	48.3 ef	8.50 bc	90.7 abcde	78 a	11.93 ab
K 01	1.93 a	91.7 ab	20.33 a	101.0 a	17 bc	20.00 a
K 02	0.58 cd	55.0 ef	15.17 ab	79.3 cdef	7 c	10.57 ab
K 03	0.66 c	90.7 abc	15.67 ab	93.7 abcd	27 bc	19.17 a
K 04	0.75 c	65.7 bcde	9.667 bc	79.7 cdef	21 bc	8.10 ab
K 05	1.04 bc	58.3 e	10.53 bc	77.0 def	10 c	13.20 ab
N 01	0.51 cd	49.7 ef	6.87 bc	94.7 abc	77 a	13.00 ab
N 02	0.68 c	64.0 cde	14.13 ab	95.7 abc	29 bc	17.00 a
N 03	0.60 cd	55.0 ef	11.03 ab	92.0 abcd	76 a	14.00 ab
N 04	0.56 cd	89.7 abcd	8.53 bc	96.7 ab	82 a	11.17 ab
N 05	0.41 cd	55.0 ef	14.03ab	95.3 abc	84 a	17.83 a
I 01	0.57 cd	63.3 de	7.60 bc	88.0 abcde	43 b	17.33 a
I 02	0.87 bc	111.0 a	10.30 bc	84.7abcde	24 bc	17.97 a
I 03	0.97 bc	91.7 ab	9.67 bc	85.0 abcde	45 b	17.67 a
I 04	1.47 ab	41.7 ef	7.83 bc	75.0 ef	20 bc	11.73 ab
I 05	0.41 cd	68.0 bcde	8.17 bc	85.7 abcde	25 bc	21.00 a
I 06	0.93 bc	51.3 ef	6.40 bc	80.0 bcde	28 bc	11.80 ab

Br: The reference strain; K 01-K 05: *Frankia* strains of Kafr El-Sheikh; N 01-N 05: *Frankia* strains



of New Valley; I 01-I 06: *Frankia* strains of Ismailia governorate; D.W., dry weight.

As for the number of branches per plant, it ranged from 7 to 84 being the highest with *Frankia* isolate N 05 and the lowest with the isolate K 02. Comparable results to the Br reference strain were obtained due to the inoculation with local *Frankia* strains N 01, N 03, N 04 and N 05 (Table 3). Except the previously stated *Frankia* strains, the remainder ones gave matchable results to the control treatment without significant variations. The obtained results recorded in Table (3) show that all treatments inoculated with local *Frankia* strains gave insignificant increases compared to the reference strain (Br). On the other hand, all treatments inoculated with *Frankia* strains gave significant increases in comparison with the control (un-inoculated). The strain K 01 of Kafr El-Sheikh gave the highest value of shoots dry weight (20 g/plant) when compared with the other strains.

**The influence of inoculation with various *Frankia* strains on certain growth parameters of *Casuarina glauca* grown in sterile calcareous soil:**

Table (4) shows the impact of diverse *Frankia* strains and the reference strain as well on nodulation and certain growth parameters of *Casuarina glauca* seedlings grown in calcareous soil. Concerning nodules dry weight, no nodules were recorded for the control treatment. The *Frankia* strains induced nodules dry weight ranged from 0.55 to 2.17 g/seedling; meanwhile the reference strain produced nodules dry weight reached 1.20 g/seedling. In comparison with the reference strain (Br), the isolated *Frankia* strains produced comparable nodules dry weight; where the variations were not significant, except the result of I04 strain that significantly overmatched the Br strain. Regarding root lengths of *Casuarina* seedlings, Table (4) also indicates that they ranged from 30.67 cm in the control treatment to 94.33 cm due to the inoculation with strain I 04. The *Frankia* strains I 01, I 04 and I 05 induced root lengths significantly overcame those produced by either the control or the Br reference strain, while the other tested *Frankia* strains produced insignificant variations in root lengths in comparison with the Br reference strain. As for roots dry weights, they ranged from 2.93 to 22.57 g/seedling being the highest due to inoculation with *Frankia* strain I 04.

Each of N 04 and I 04 *Frankia* strains produced significant differences in root dry weight when compared to the Br reference strain, the other tested *Frankia* strains produced root dry weights were comparable to that obtained with the Br reference strain. As to stem lengths of *Casuarina* seedlings grown in calcareous soils (Table 4) inoculated with *Frankia* resulted in significant increases over the control treatment. The stem lengths, in general, varied from 65.0 to 100.7 cm where the highest value was recorded owing to the inoculation with I 04 *Frankia* strain. Further, *Frankia* strains of K 05, N 04, I 02, I 05 and I 05 also induced significant augmentations in stem lengths in comparison with either the control or the Br reference strain. The remainder *Frankia* strains produced stem lengths similar to that obtained due to the inoculation with the Br reference strain without significant variations. Concerning branches numbers per seedling, it was found that, except three *Frankia* strains (K 01, N 03 and I 02), the rest isolated *Frankia* induced branches number similar to those obtained due to the Br reference strain with no significant differences. Irrespective of the diverse treatments, *Casuarina* seedlings had branches number ranged between 7 and 38.

**Table 4: Effect of inoculation with different strains of *Frankia* on *Casuarina glauca* seedlings grown in sterile calcareous soil.**

Parameter Treatment	Nodules D.W. (g/seedling)	Root length(cm)	Root D.W. (g/seedling)	Stem length(cm)	Branches (no/seedling)	Shoot D.W. (g/seedling)
Cont.	0.00 e	30.67 f	2.93 d	65.00 g	36.66 ab	5.01 e
Br	1.20 bcd	46.33 def	4.19 cd	74.67 efg	38.67 a	5.78 e
K 01	1.47 abc	57.00 cde	3.83 cd	76.67 efg	16.33 bcd	10.00 bcde
K 02	0.55 de	42.33 ef	4.23 cd	84.33 bcdef	30.00 ab	9.13 bcde
K 03	1.90 ab	55.33 cde	6.17 bcd	85.00abcdef	25.00 abcd	13.77 bcd
K 04	1.90 ab	63.67 cd	8.67 bcd	98.33 ab	19.67 abcd	15.83 b
K 05	0.55 de	46.00 def	3.57 cd	73.33 fg	17.67 abcd	5.70 e
N 01	1.30 abcd	43.00 ef	3.63 cd	77.33 efg	35.00 ab	6.63 de
N 02	1.20 bcd	49.33 def	15.77 abc	80.00 defg	18.33 abcd	14.60 bc
N 03	0.85 cde	47.33 def	10.30 abcd	88.67abcdef	7.67 cd	11.43 bcde
N 04	0.56 de	45.00 def	17.44 ab	98.67 ab	18.33 abcd	14.73 bc
N 05	0.73 de	45.33 def	3.83 cd	80.33 cdefg	37.67 ab	7.57 cde
I 01	1.82 cde	84.00 ab	12.00 abcd	87.33abcdef	22.33 abcd	15.60 b
I 02	2.07 ab	56.33 def	8.30 bcd	94.67 abcd	6.67 d	15.50 b
I 03	1.60 ab	60.67 def	7.50 bcd	90.00 abcde	32.67 ab	14.80 bc
I 04	2.17 a	94.33 a	22.57 a	100.7 a	29.33 abc	25.93 a
I 05	1.93 abcd	73.67 bc	12.83 abcd	96.00 abc	28.33 abcd	16.47 b
I 06	1.20 ab	63.67 cd	7.47 bcd	93.67 abcd	32.33 ab	13.87 bcd

Br: The reference strain; K 01-K 05: *Frankia* strains of Kafr El-Sheikh; N 01-N 05: *Frankia* strains of New Valley; I 01-I 06: *Frankia* strains of Ismailia governorate; D.W, dry weight.

Concerning the dry weight of shoots, Table (4) indicates that it ranged from 5.0 g/seedling in the control treatment to 25.93 g/seedling owing to the inoculated with I 04 *Frankia* isolate. The majority of isolated *Frankia* strains induced significant augmentations in comparison with the Br reference strain (K 03, K 04, N 02, N 04, I 01, I 02, I 03, I 04, I 05 and I 06), while the other tested *Frankia* strains produced insignificant variations in shoot dry weights when compared to the Br reference strain.

Effect of *Frankia* strains on nitrogen content of roots and shoots and nitrogen uptake of *Casuarina* seedlings grown in sterile sandy and calcareous soils:

Nitrogen content and nitrogen uptake of *Casuarina* seedling grown in sandy and calcareous soils are presented in Table (5). In sandy soil, the nitrogen content of *Casuarina* seedling roots reached 0.45% in the control treatment and 3.55% in consequence of inoculation with K 01 *Frankia* strains. Both *Frankia* strains K 01 and K 05 induced significant increases in nitrogen content of roots in comparison with the Br reference strain. Moreover, comparable nitrogen content values to the Br reference strain without significant variations were obtained due to the *Frankia* strains of Kafr El-Sheikh (K 02, K 03 and K 04) and the New Valley (N 01-N 05) in addition to one strain (I 01) from Ismailia.



**Table 5: Effect of different *Frankia* strains on nitrogen content of roots and shoots and nitrogen uptake of *Casuarina* seedlings grown in sandy and calcareous soils.**

Parameter Treatment	Sandy soil			Calcareous soil		
	N-content (%)		N-uptake (mg/seedling)	N-content (%)		N-uptake (mg/seedling)
	Roots	Shoots		Roots	Shoots	
Cont.	0.45 e	0.40 d	39.98 p	0.20 fig	0.37 d	45.26 p
Br	1.50 bcde	1.50 abcd	612.90 j	1.14 defg	1.45 bcd	258.22 n
K 01	3.55 a	3.35 a	2782.77 a	2.80 ab	2.23 abc	695.65 i
K 02	0.76 bcde	1.40 bcd	555.98 k	1.50 bcdefg	0.90 cd	320.64 l
K 03	1.63 bcd	1.40 b	1055.65 e	0.15 g	0.32 d	93.72 o
K 04	1.90 de	1.70 abcd	639.61 i	2.05 bcde	0.30 d	575.75 j
K 05	2.45 ab	2.10 ab	1079.72 d	1.65 bcdef	1.95 abcd	333.72 k
N 01	2.05 abc	2.00 ab	804.61 g	1.38 cdefg	1.65 abcd	310.88 m
N 02	2.45 ab	1.65abcd	1276.33 c	1.42 bcdefg	1.00 cd	734.95 g
N 03	1.75 bcd	1.78 abc	883.60 f	2.37 abcd	0.90 cd	710.57 h
N 04	1.05 bcde	1.78 abc	557.60 k	2.45 abcd	2.55 abc	1383.3 c
N 05	1.80 bcd	2.30 ab	1306.26 b	2.90 ab	2.15 abc	575.70 j
I 01	0.85 cde	0.64 cd	371.46 n	2.70 abc	1.05 cd	1035.00 e
I 02	0.79 cde	0.85 bcd	463.63 nm	2.30 abcd	1.75 abcd	963.90 f
I 03	1.40 bcde	1.10 abcd	683.43 h	3.55 abcd	3.35 ab	1538.70 b
I 04	0.55 e	1.25 abcd	352.13 o	3.70 a	2.55 abc	3031.25 a
I 05	0.85 cde	0.95 abcd	525.01 l	2.25 abcd	1.55 bcd	1113.40 d
I 06	1.20 bcde	0.85 bcd	373.10 n	1.90 bcde	0.80 cd	576.18 j

Br: The reference strain, K 01-K 05: *Frankia* strains of Kafr El-Sheikh, N 01-N 05: *Frankia* strains of New Valley, I 01-I 06: *Frankia* strains of Ismailia governorate.

For shoots nitrogen content it ranged from 0.4% in the control to 3.35% due to inoculation with *Frankia* isolate K 01, while the Br reference strain induced a value reached 1.50%. The *Frankia* strains number K 01, N 04, N 05, I 01, I 02, I 03 and I 04 produced significant variations when compared to the Br reference strain, meanwhile the other strains produced results similar to that obtained owing to the Br reference strain without significant differences. Concerning the nitrogen uptake, it ranged between 39.98 mg/seedling in the control and 2782.77 mg/seedling due to K 01 *Frankia* strain. Significant increases in comparison with the Br reference strain were recorded due to the *Frankia* strains number K 01, K 05, N 02, N 05 and I 03. The remnant *Frankia* strains resulted in nitrogen uptake values were matchable to that obtained with the Br reference strain without significant variations. In calcareous soil, the nitrogen content of *Casuarina* seedlings roots reached 0.20% in the control treatment while the highest value was 3.70% and recorded with I 04 *Frankia* strain. The significant variations were recorded in consequence of the inoculation with *Frankia* strains number K 01, N 05, I 01 and I 04 against the Br reference strain. Further, the other tested *Frankia* strains induced nitrogen content values were similar to that obtained with the Br reference strain with insignificant differences. With regard to the nitrogen content of shoots in the calcareous soil, it ranged from 0.37% in the control treatment to 3.35% owing to the isolated *Frankia* number I 03. The significant increases over the Br reference strain were induced due to the inoculation with *Frankia* isolate number I 03 while the other isolated *Frankia* produced nitrogen content similar to the Br reference strain. Regarding the nitrogen uptake, *Casuarina* seedlings recorded values ranged from 45.26 mg/seedling in the control to 3031.25 mg/seedling due to inoculation with I 04 *Frankia*

isolate. Except *Frankia* strain number K 03, the other isolated *Frankia* did increase nitrogen uptake by *Casuarina* seedlings as these increases were significant when compared to the Br reference strain.

**Comparison among performance of diverse *Frankia* strains in sandy and calcareous soils:**

The performance of diverse *Frankia* strains in both calcareous and sandy soils was presented in Table (6). As to nitrogen content of *Casuarina* seedling roots, noticeable increases in the calcareous soil were recorded due to the inoculation with various *Frankia* strains of Ismailia governorate (I 01-I 05) where the increases were significant, except the isolate number I 06, in comparison with the data obtained from the sandy soil. Furthermore, insignificant increases were found in the calcareous soil owing to the strains number K 02, K 04, N 03, N 04 and N 05 when compared to their effects in the sandy soil. Contrarily, in the sandy soil it was found that the *Frankia* strains number K 01, K 03, K 05, N 01 and N 02 in addition to the Br reference strain gave insignificant increases in roots nitrogen content grown in sandy soil in comparison with those grown in calcareous soil. For shoots nitrogen content, Table (6) also indicates that *Frankia* isolate number I 03 induced the highest shoot nitrogen content in *Casuarina* seedlings grown in calcareous soil. Moreover, the difference was significant when compared to the same treatment in the sandy soil. On the other side, significant variation was observed due to the inoculation with *Frankia* isolate number K 04 in the sandy soil in comparison with its effect in the calcareous soil. Insignificant variations between calcareous and sandy soils for shoots nitrogen content of *Casuarina* seedlings were found due to the inoculation with the other *Frankia* strains. In regard to N-uptake, it was found that, it ranged from 39.98 to 3031.25 mg/seedling. The *Frankia* strains of Ismailia governorate (I 01-I 05) in addition to one strain from New Valley (N 04) recorded the highest values of N-uptake in calcareous soil where they significantly overcame their effects in sandy soil. Contrarily, the *Frankia* strains of Kafr El-Sheikh (K 01-K 05) and New Valley (N 01, N 02, N 03 and N 05) significantly affect N-uptake in sandy soil in comparison with their effects in calcareous soil. It is worthy to mention that the Br reference strain act well in sandy soil in comparison with its effect in calcareous soil where the difference was significant.

Inoculation of *Casuarina glauca* grown in sandy soil with various tested *Frankia* strains increased each of nodules dry weight, root lengths, roots dry weight, stems length, branches number and shoots dry weight. The increases over the Br reference strains ranged from 13 to 151%, 3 to 130%, 0.4 to 139%, 1 to 11%, 5 to 8% and from 9 to 76% consecutively for the aforementioned studied parameters. These increases could be attributed to the nitrogen fixing capacity of *Frankia* strains that work efficiently in low fertile soil (Masuka and Makoni, 1995).

**Table 6: Comparison among performance of diverse *Frankia* strains in sandy and calcareous soils against Br Reference strain.**

Parameter Treatment	Roots N -content (%)		Shoots N -content (%)		N-uptake (mg/seedling)	
	Sandy soil	Calcareous soil	Sandy soil	Calcareous soil	Sandy soil	Calcareous soil
Cont.	0.45 kl	0.20 l	0.40 hijk	0.37 ijk	39.98 w	45.26 w
Br	1.50 defghijk	1.14 fghijkl	1.50defghijk	1.45cdefghijk	612.90 n	258.22 u
K 01	3.55 ab	2.80 abc	3.35 a	2.23 abcde	2782.77b	695.65 m
K 02	0.76 ijkl	1.50 defghijk	1.40cdefghijk	0.90 efgghijk	555.98 op	320.64 st
K 03	1.63cdefghijk	0.15 l	1.40cdefghijk	0.32 jk	1055.65gh	93.72 v
K 04	1.90 cdefghi	2.05 cdefgh	1.70 cdefghij	0.30 k	639.61 n	575.75 o
K 05	2.45 abcde	1.65cdefghijk	2.10 abcdef	1.95 bcdefg	1079.72 g	333.72 st
N 01	2.05 cdefgh	1.38 efgghijkl	2.00 abcdefg	1.65cdefghijk	804.61 k	310.88 t
N 02	2.45 abcde	1.42 efgghijkl	1.65cdefghijk	1.00 defghijk	1276.33 e	734.95 l
N 03	1.75 cdefghij	2.37 bcdef	1.78 bcdefgh	0.90 efgghijk	883.60 j	710.57 lm
N 04	1.05 ghijkl	2.45 abcde	1.78 bcdefgh	1.85 bcdefg	557.60 o	1383.31d
N 05	1.80 cdefghij	2.90 abc	2.30 abcd	2.15 abcdef	1306.26 e	575.70 o
I 01	0.85 hijkl	2.70 abcd	0.64 ghijk	1.05 defghijk	371.46 r	1035.00 h
I 02	0.79 hijkl	2.30 bcdefg	0.85 fghijk	1.75 bcdefghi	463.63 q	963.90 i
I 03	1.40 efgghijkl	3.55 ab	1.10 defghijk	3.35 a	683.43 m	1538.70 e
I 04	0.55 jkl	3.70 a	1.25cdefghijk	2.55 abc	352.13 rs	3031.25 a
I 05	0.85 hijkl	2.25 cdefg	0.95 defghijk	1.55cdefghijk	525.01 p	1113.40 f
I 06	1.20 efgghijkl	1.90 cdefghi	0.85 efgghijk	0.80 fghijk	373.10 r	576.18 o

Br. The reference strain, K01-K05: *Frankia* strains of Kafr El-Sheikh, N01-N5: *Frankia* strains of New Valley, I01-I06: *Frankia* strains of Ismailia governorate.

Furthermore, inoculation of *Casuarina* seedlings grown in calcareous soil with locally isolated *Frankia* strains augmented each of nodules dry weight, roots length, stems length and shoots dry weight. The percentage of increases ranged between 8 and 81, 2 and 104, 1 and 439 and 3 and 35 and 15 and 349 respectively over the Br reference strain. Miguel *et al.* (1978) and Wheeler *et al.* (1984) confirmed the previously mentioned results, they attributed the promotion effects of inoculation with *Frankia* sp. to the nitrogen fixation in addition to some phytohormones that produced by some *Frankia* strains. Moreover, Bulloch (1994) stated that the inoculated *Casuarina* trees were significantly larger in the stem diameter and height. 10% and 123%. Regarding the N-uptake in sandy soil, it was found that the majority of isolated *Frankia* strains increased N-uptake by also, the isolated *Frankia* strains resulted in augmentation of N-uptake over the Br reference strain, these increases ranged from 20 to 1073%. These observations are in harmony with those obtained by Zhang *et al.* (1990) who stated that different *Casuarina* genotypes inoculated with pure *Frankia* strains showed significant nitrogen fixation and nitrogen uptake per plant. Performance of various tested *Frankia* strains in both calcareous and sandy soils was taken into consideration using nitrogen content and nitrogen uptake of *Casuarina* seedlings as measuring parameters. Concerning nitrogen content of *Casuarina* roots; the majority of *Frankia* strains i.e., K 01, K 02, K 03, K 04, K 05, N 01, N 02, N 03 and N 05 acted well in both types of soils where the variations were not significant. This observation could be ascribed to the stress of isolation areas from which these strains were isolated (E.C., 12.9 and 10.6 dS/m; CaCO<sub>3</sub>, 2.9 and 3.5% and Na<sup>+</sup>, 67.3 and 57.4 meq/l). The same trend was found with shoots nitrogen content with additional strains of *Frankia* (N 04, I 01, I 02, I 04, I 05 and I 06). The *Frankia* strains isolated from Kafr El-Sheikh and New valley, except N 04 strain, acted well for N-uptake in sandy soil where the differences were significant when compared with N-uptake in calcareous soil. Contrarily, the *Frankia* strain N 04 and those isolated from Ismailia affected significantly N-uptake in calcareous soil in comparison with their effects in sandy soil. Khalil (1999) found that *Casuarina* was significantly enhanced in calcareous soil than sandy soil; she attributed this effect to the amount of minerals in the soil.

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