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NODUTATION, MYCORRHIZATION, GROWTH AND DROUGHT RESISTANCE OF *CASUARINA GLAUCA* SIEBER EX SPRENG UNDER TETRAPATITE SYMBIOTIC RELATIONSHIPS AMONG VESICULAR-ARBUSCULAR MYCORRHIZAL, ECTOMYCORRHIZAL FUNGI AND/OR *FRANKIA*

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ABSTRACT

Two experiments were carried out to study the tetrapartite symbiotic relationship of Casuarina glauca seedlings with vesicular- arbuscular mycorrhizal, ectomycorrhizal fungi and/or the endobiont Frankia in two seasons. The first experiment was conducted to study the effect of single, dual and triple inoculation with the symbiotic agents mentioned above on nodulation, mycorrhization characteristics, growth and dry matter of the seedlings using 8 treatments as follows:

1. *Single inoculation with the ectomycorrhizal fungus, namely, Coprinus sp ………(EM).*
2. *Single inoculation with vesicular- arbuscular mycorrhizal fungus, namely, Glomus mossae……. (VAM).*
3. *Single inoculation with Frankia…..(F).*
4. *Dual inoculation with EM and VAM……… (EM×VAM).*
5. *Dual inoculation with EM and F……… (EM×F).*
6. *Dual inoculation with VAM and F……… (VAM ×F).*
7. *Triple inoculation with EM,VAM and F ………. (EM×VAM×F).*
8. *Uninoculation (using sterilized inocula of the symbiotic agents used) ….. (Control).*

The obtained results showed:

- Nodule number (NN) and nodule dry matter (NDM) were enhanced due to the synergistic effect of VAM compared to EM. The triple inoculated plants showed NN and NDM lower than those of dual inoculated (EM×F and VAM×F) and single inoculated ones (F) for both seasons.

- The highest mycorrhization level (%) was detected in the roots of triple inoculated plants, i.e., Frankia displayed synergistic effects on mycorrhizal fungi, particularly with VAM.

- Shoot height (SH), height growth rate (HGR), stem diameter (SD), branchlet dry matter (BDM), root dry matter (RDM) and total dry matter (TDM) of EM×VAM-dual inoculated seedlings were higher than those of the other treatments in the most cases, except for those triple-inoculated ones, since there were no significant differences among them and EM×VAM dual inoculated ones. Stem dry matter (SDM) of VAM and inoculated plants was significantly higher than those of the other treatments for the first season, as well as, those triple-inoculated ones for the second season.

- Shoot/root ratio of F-inoculated plants was significantly higher than that of the other treatments in the first season, whilst in the second one; FM×F-dual inoculated and uninoculated ones displayed the highest ratio.

- The second experiment was done to study the effect of drought stress on the survival and life shoot ratio, expressed as height (LSR_H) and weight (LSR_W). However, for both seasons, the triple inoculated and EM×VAM-dual inoculated plants displayed the highest survival (%). In addition, the triple-inoculated plants showed the highest LSR_H and LSR_W for both seasons relative to those of all dual, single inoculated plants and control.

However, EM×VAM-dual inoculated plants and triple-inoculated ones displayed significantly higher growth and yield and drought resistance than those of single, dual inoculated and control plants.

1. INTRODUCTION

The complexity of the soil system is determined by the numerous and diverse interactions among its physical, chemical, and biological components, as modulated by the prevalent environmental conditions (Buscot, 2005).

The biological components play an important and sometimes critical roles in growth and survival of tree stands. The most important microorganisms that play a key role in forest and plantation establishment and survival are the symbiotic agents, amongst which are, N_2 fixing bacteria and mycorrhizal fungi.

Frankiae are soil-borne filamentous bacteria capable of forming root nodules and nitrogen fixation (non-legume symbiosis) that regarded as major contributors to nitrogen input in forests, wetlands, fields, and disturbed sites of temperate and tropical regions (Tate, 1995, Powlowski and Sirrenberg, 2003 and Ridgeway *et al.*, 2004). These associations involve roughly 200 species of angiosperms classified among six or seven orders and 24 genera (Baker and Mullin, 1992 and Ridgeway *et al.*, 2004). The contributions of fixed nitrogen to managed as well as unmanaged ecosystems by the actinorhizal symbioses are comparable to those of the more extensively studied *Rhizobium*-legume contributors. Actinorhizal plants are pioneer plants since they can extract up to 70% of their necessary nitrogen through N_2-fixation (Dommergues, 1996). Strains of genus *Frankia* are currently not assigned a species name, since much debate remains concerning the criteria on which taxonomy should be based (Roy *et al.*, 2007).

The mycorrhizal fungi are mainly divided into two groups, endo-mycorrhizae and and ectomycorrhizae (Balanchard and Tattar, 1981).The most important endomycorrhizal fungi is the vesicular-arbuscular mycorrhizal (VAM) ones; which have wide range

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hosts, including the most edaphic or higher plants herbs, weeds, shrubs and trees The fungi were formerly included in the order Glomales in the Zygomycota, but they have recently been moved to a new phylum Glomeromycota (Schueßler *et al.*, 2001).

The ectomycorrhizal fungi are rather tree partners and mainly belonging to Basidiomycota and Ascomycota. The community of these fungi in forest trees aids the plant to grow well in addition to increase its ability to absorb insoluble elements like phosphorus and enhanced its resistance to pathogenic diseases. The afforestation using some trees might be doomed to failure due to the absence of ectomycorrhizae (Daniel *et al.*, 1979).

There are some types of interactions among the soil symbionts under agro-ecosystems, notably, (i) the co-operation between plant growth promoting rhizobacteria (PGPR) and *Rhizobium* for improving N_2-fixation; (ii) microbial antagonism for the biocontrol of plant pathogens; and (iii) interactions between rhizosphere microbes and AM fungi to establish a functional mycorrhizosphere (Barea *et al* ., 2005). The beneficial effects of these bacteria in combination with VAM fungi have been reported by Domenech *et al* .(2004).

Large numbers of bacteria (including actinomycetes) and fungi can be associated with both VAM fungal structures (Budi *et al.*, 1999) and ectomycorrhizal structures (Frey-Klett *et al.*, 2005).

Kearney and Shantz (1911) and Shantz (1927) divided plants living in dry habitats into four groups: drought escaping "pseudoxerophytes" (Henkal, 1946), drought evading (via minimizing transpiration with no reduction in dry-weight increment), drought enduring "desiccation resistance" and drought resistant (which held water in leaves and stems in relative large quantities). Drought resistance in plants can occur via drought avoidance (maintenance of high internal water potential) or drought tolerance (survival of low internal water potential) (Ludlow, 1989). Relative drought resistance seems associated with the ability of seedlings (pines) to survive drought by maintaining favorable plant water potential (Barton and Teeri, 1993).

The role of mycorrhizal fungi in increasing drought tolerance of their partners hosts were studied by some workers. Also, synergistically both of *Frankia* and mycorrhizal fungi are improving drought resistance (Sempavalan, 1995).

This work aimed to monitor and evaluate the complex inrelationships among tree species (*Casuarina glauca*), the ectomycorrhizal and endomycorrhizal, vesicular arbuscular fungi (VAM) and the N_2 Fixing bacterium *Frankia* , i. e. under tetrapartite interrelationship. The study aimed to assess the synergistic or antagonistic impact of each symbiotic agent on each other, its effects on the plant growth, and survival under drought stress hazard as well.

2. MATERIALS AND METHODS

This work was carried out in 2 seasons (2005- 2006 and 2006- 2007) in Experimental Station of Forestry and Wood Tech. Dept., Fac. of Agric., Alex. Univ., Alexandria, Egypt in two experiments as follows:

2.1. First Experiment:

Effect of single, dual and triple inoculation on mycorrhization, nodulation and growth and biomass yield of *Casuarina glouca* trnsplants.

2.1.1. Preparation of transplants

Seeds of certified *Casuarina glauca* sieber Ex Spreng trees, grown in Desert of Sadat City, were sown in a sterilized soil (3: 1 sand and clay (v/v); contained 140.50, 1.08 and 97. 14 mg nitrogen, phosphorus and potassium/ kg of soil; respectively). Resultant transplants were transplanted in polyethylene bags whichcontained 2 kg (for each bag) of the same soil mixture that previously sterilized with 10.00% formaldehyde. Before transplanting, plants were watered with 0.1% of the fungicide, vitvax and 1.0 g/kg soil of tetracycline to kill , if any, pathogenic or symbiotic agents in rhizosphere that may infect root system.

2.1.2. Preparation of inocula

2.1.2.1. *Frankia* inocula

Preparation of inocula was done 3 days before the inoculation. From pure culture of *Frankia* colony designated CGAA, 5 discs of filamentous hyphae were picked up via cork borers of 0.5 cm diam., incorporated with 100.00 g of sterilized peat and farm yard residue with 200 ml of sterilized water using warn blender that gave rise a slurry textured inocula. Such inocula were kept in sterilized jars at room temperature (27° ± 3° C).

2.1.2.2. VAM inocula

Soil inocula of *Glomus mossae* were used. Such inocula contained root debris and apparently viable clamydospores (ca 150 spores $g^{-1}$of soil).

2.1.2.3. Ectomycorrizae inocula

Basidiospores were collected from the head of the fruit body of the fungus then germinated in Melin-Norkas agar media described by Marx (1969) and kept in incubators at 27°C for two weeks then homogenized in a warn blender to slurry textured inocula and kept in refrigerator at 5°C until use.

2,1.2.3. Inoculation

Transplants of *Casuarina glauca* aged 6 months were artificially inoculated with *Frankia* inocula by addition of about 20 g based on the dry weight of inocula in rhizosphere at a depth of 30 cm of the soil surface. As for VAM, 1.0 g/plant of inocula were added in holes of 5.0 cm depth then covered with the soil and watered with a tap water to reach field capacity every 2 days. Ten plants were inoculated with each inoculum.

The first inoculation was done on Mars 25, 2006. Control plants were inoculated, but the with previously sterilized inocula of the symbiotic agents used. The inoculation dates are set out in Table (1).

Shoot height of all plants was recorded after inoculation to calculate the growth rate (cm/ month).

2.1.3: Experimental design

The experimental design used in this study was complete randomized arrangements (CRD) with 8 treatments and 5 replicates (5 plants for each treatment) according to Steel and Torrie (1980).

2.1.4. Examination of root and sampling

2.1.4.1. Examination of root system

The inoculated and uninoculated plants were examined one month after the inoculation to check the incidence, if any, of *Frankia* prenodule and VAM fungus. Samples of feeder roots were taken from the plants, washed free from debris with a tap water then softened, cleaned, stained with 0.1 % trypan blue according to the method described by Phillips and Hayman (1970). Infection level of roots with VAM was determined by measuring the length of actual colonized feeder root segment with arbuscules, vesicules, extramatical hyphae,mantle and/ or Hartig's net relative to total length of the root in samples.

Ten feeder roots of one-cm- length were examined in each plant then the average of infection was assessed using the following equation:

$$\mathrm{IL} = \frac{\mathrm{FL}}{\mathrm{TL}} \times 100$$

Where; IL is an infection level (%), FL is a length of feeder root colonized with VAM or ectomycorrihzal structures and TL is the total length of the feeder root examined,

2.1.4.2. Determination of growth, biomass parameters

Shoot height of the plants was assessed just after inoculation as well as at the end of the experiment to compute the growth rate (cm/ month), stem diameter (cm) and dry matter of branchlets, stem and roots (g) that were previously removed carefully from the soil and oven dried at 100°C to constant weight. Number, diameter (cm) and dry weight of nodules (g) were determined in inoculated plants with *Frankia*.

2.2. Experiment 2

As described in 2.1. using the same experimental design, 15 inoculated transplants with the same symbiotic agents mentioned above were subjected to four 8- day drought cycles, by prevention irrigation after 5 months of the artificial inoculation.

Drought cycles were terminated by rewatering when soil moisture potential reached -1.2 (±0.2) megapascal. Control (15 plants of the same treatment) were watered every third day.

2.2.1. Survival (%) and life-shoot ratios determinations.

Forty days after starting drought cycles, seedling were carefully extracted from the soil then, their roots were washed free from soil debris. The survival (S%) was determined. Life-shoot ratio, based on height of alive shoot (LSR_H) was calculated by the following equation:

$$LSR_H\% = \frac{\text{Height of alive shoot of the transplant}}{\text{Total height of the same transplant}}$$

Life-shoot ratio, based on oven dry weight (LSR_W) was calculated by the following equation:

$$LSR_W\% = \frac{\text{Oven-dry weight of alive shoot of the transplant}}{\text{Total oven dry weight of the same transplant}}$$

3. RESULTS AND DISCUSSIONS

3.1. First Experiment:

3.1.1 Nodulation: Examination of inoculated roots revealed berries-like nodules in all artificially inoculated plants, yet with different levels (Fig.1). However, statistical analysis of variance revealed that there were significant differences between single-, dual- and triple- inoculated plants in number and dry matter of nodules for both seasons. However, the dual inoculation with *Frankia* and vesicular-arbuscular mycorrhizae (F x VAM) induced significantly higher nodule number than those obtained in single, dual inoculation with ectomycorrhizal (EM) inoculum and triple inoculation using F x VAM x EM inocula. On the other hand, nodule dry matter of E×VAM- treated plants was significantly higher than that detected in the other single, dual and triple inoculated plants.

However, triple inoculated plants and dual (EM×F-treated plants) displayed higher nodule dry matter than that of F-treated plants for both seasons. (Tables 2 and 3). Oliveira *et al.* (2005) obtained similar results, since he found that the dual inoculation of *Alnus glutinosa* with VAM fungus, *Glomus intraradices* and *Frankia* resulted in greater numbers and larger root nodules than when inoculated with *Frankia* spp. alone. The mycorrhizal roots, however, stimulate and increased the populations of bacteria (including Actinomyceteous one), since the VAM mycelium releases energy-rich organic compounds. However, an increased growth and activity of microbial saprophytes can be expected to occur in the mycorrhizosphere (Andrade *et al.*, 1997 and Budi *et al.*, 1999), thus the synergistic effect would take place. Similar synergistic effects were obtained by Rajendran and Devaraj (2003) expressed in nodular biomass, since they found that the combined inoculation of *Frankia* + Phosphobacteria + VAM fungus had better nodule development than with a single inoculation of *Frankia*. They found also that the nodulation was significantly low even after inoculation with effective *Frankia* and they attributed the poor nodulation to the P stress or deficient and the presence of VAM fungi is necessary to facilitate its absorption by the host, since such element is required for nodulation and N_2 fixation. The positive or synergistic effects of VAM on nodulation of the N_2 *Rhizobium* was obtained by Gabor *et al.* (1987).

3.1.2. Mycorrhization: The examination of all rootlets of all inoculated plants either with VAM, EM, singularly, dually or triple revealed, however, the formation of VAM structures (Fig. 2a and 2b) an ectomycorrhizal ones as well (Fig. 3a and 3b) after three months of artificial inoculation) that the infection level (IL) of plants inoculated with the all symbionts has brought about the highest IL for both seasons (Tables 2 and 3). On the other hand, the infection by VAM or EM were enhanced by the dual inoculation with *Frankia* inoculum. The infection level with VAM was lower than that obtained in the plants inoculated only with EM inoculum for both seasons. Under tripartite system, Sempavalan *et al.* (1995) stated that there were a high degree of co-ordination between nodulation, mycorrhizal colonization and *Casuarina equisetifolia* seedling growth in mineral deficient conditions, thus there is a co-operative, but not a competitive interaction between *Frankia* and *Glomus* for nodulation and mycorrhizal colonization of *Casuarina* spp. The mechanisms by which the isolates of symbiotic bacteria activity in VAM plants can be summarized as follows: (i) improved rooting, and VAM formation and functioning of mycorrhizosphere (ii) effects on the receptivity of the root; (iii) effects on the root-fungus recognition; (iv) effects on the fungal growth; (v) modification of the chemistry of the rhizospheric soil; and (vi) effects on the germination of the fungal propagules. On the other hand, other reports stated that the presence of VAM fungi is known to enhance nodulation and N fixation by legumes (Amora- Lazecano *et al.*, 1998; Johansson *et al.*, 2004 and Barea, 2005). Moreover, VAM fungi and nitrogen fixing bacteria often act synergistically on infection rate, mineral nutrition and plant growth.

3.1.3. Growth parameters

The plants that were inoculated only with *Frankia* and dually with VAM and EM inocula displayed the highest shoot height for both seasons. In the second season, the triple inoculated plants displayed no significant differences with those of *Frankia* and EM□VAM- inoculated ones. However all single-, dual- and triple- inoculated plants showed higher shoot growth significantly than those of the control one.

As for height growth rate (HGR), in the first season, plants inoculated with EM □ VAM- inocula displayed the highest rate; whilst in the second one, the same treatment and those inoculated with *Frankia* showed the highest level. Generally, all seedlings single, dual or triple inoculated with *Frankia*, EM and/ or VAM displayed significantly higher HGR than that of the control.

Stem diameter (SD) of dual inoculated transplants with EM □ VAM was significantly thicker than those of the all other treatments in the first season; yet in the second one, the same plants as well as those triple inoculated with all symbiotic agents used displayed the highest SD relative to the other treatments. Furthermore, the dual inoculated transplants with EM and VAM displayed significantly higher SD than that of single inoculated ones.

As for branchlet dry matter (BDM), EM □ VAM- inoculated plants showed the highest level in the first season; while in the second one, the same plants and those triple- inoclated ones (with EM □ VAM □ *Frankia*) displayed the highest level (Tables 2 and 3).

Stem dry matter (SDM), i.e, without branchlets, of both of VAM and F □ VAM- inoculated plants was significantly higher than those obtained in all other treatments in the first season; while in the second one, the same plants as well as those triple-inoculated ones with all symbiotic agents used showed the highest level.

As found in case of BDM, for both seasons, however, EM × VAM- and triple-inoculated plants displayed the highest root dry matter (RDM) relative to the other treatments. In addition, single, dual and/or triple inoculation with symbiotic-agent inocula brought about RDM significantly higher than that of non-inoculated ones (Tables 2 and 3).

Shoot/ root ratio, however, was erratic for both seasons, since in the first season, it was found that the F-inoculated plants showed the highest value, followed by EM×F- dual- inoculated ones. Meanwhile, control plants displayed the highest value in the second season. However, the lowest shoot/root ratio was detected in triple' and EM×VAM-dual inoculated plants in the first and second seasons, respectively. From these findings, it is concluded that symbiotic agents, particularly EM×VAM enhanced the root growth (expressed as dry weight) and in turn brought about the reduction of shoot/root ratio (Table 2 and 3). The effect on root may be attributed to the direct effects of mycorrhizal fungi, since they contributed in an increase in RDM with their hyphae and indirectly by enhancing minerals and water uptake and so that the growth of the root.

Total dry matter (TDM) of EM×VAM- dual and triple (EM×VAM×F) inoculated plants was higher than that of the other treatments in the first and second season, respectively (Tables 2 and 3).

However, dual or triple inoculation of mycorrhizal fungi (EM×VAM or EM×VAM×F) produced significantly higher TDM of plants than that of single-inoculated ones, either with EM or VAM only. It worth pointing also, that the TDM of the EM × VAM or EM×VAM×F infected plants was as much as two folds that of the control. However, the positive effects of such symbiotic agents inoculated in TDM may be directly attributable to the significant contribution of the stem.

The positive effects of dual (tripartite) or triple (tetrapartite) inoculation of most growth and yield characteristics, expressed as growth and yield were previously obtained by some workers, notably Chatapraul *et al.* (1989) and Markham (2005) on *Alnuus* plants that triple and dual inoculated with *Frankia*, the VAM fungus *Glomus fasciculatus* and the ectomycorrhizal fungus, *Paxillus involutus* gave the highest growth records.

3.2. Second Experiment

3.2.1 Drought Effects

The plants subjected to drought stress responded differently owing to the presence of the symbiotic agents, expressed as survival, life shoot ratio on the height basis (LSR_H) and on dry weight basis (LSR_W). Survival of EM×VAM-dual and triple- inoculated plants with inocula of the three symbiotic agents used was higher than those of single inoculated ones and control (Tables 4 and 5) in the first season. In the second season, EM×F and triple-inoculated plants showed the highest survival value (86.66%). It has been noticed also, that the all infected plants, either single-, dual- or triple- inoculated ones, showed survival levels significantly higher than that of the control one, but dual- and triple- inoculated ones regarded superior relative to the single inoculated ones.

As for LSR_H for both seasons, triple-inoculated plants displayed the highest level. On the other hand, LSR_W values of EM-, EM ×F- and triple inoculated plants were significantly higher than those of the other treatments.

It is concluded from these findings that EM is of the most positive symbiotic agent in enhancing LSR_W followed by *Frankia*. It is obviously noticed also that all inoculated plants (single-, dual- or triple- inoculated ones) displayed LSR_W significantly higher than that of the control (Tables 4 and 5).

In this concern, Gabor *et al.* (1987) found that transpiration and leaf conductance were significantly greater in stressed VAM than in non-VAM plants during the drought stress. Similarly, Aliasgharzad *et al.* (2006) suggested that plant water status is effectively improved by this plant-microbe association (the N_2 fixing bacterium, *Bradyrhizobium japonicum* and VAM fungus). Also, under tripartite interrelationship, the synergistic impacts of VAM on nodulation and the positive role in enhancing plant drought tolerance was monitored by Rutz-Lozano *et al.* (2001), since he stated that the most remarkable observations was the substantial reduction in oxidative damage to lipids and proteins in nodules of mycorrhizal plants subjected to drought as compared to the nodules of non-mycorrhizal plants.

The positive impacts of symbionts in tetrapartite interrelationship studied may be ascribed to the positive role in enhancing growth rate and their key role in N_2 fixation and other elements. In this connection, Radin (1990) pointed out that leaf N concentration, as well as K element (Mansfield *et al.*, 1990) can affect stomatal behaviour and in turn enhancing water status and minimizing water losses under drought stress. Also, the availability of nitrogen in the soil plays a critical role in survival under stress conditions, since its shortage may lead to drastically reduction or impeding of proline synthesis and other amino acids involved in drought tolerance, since it has been demonstrated that the drought stress stimulates an accumulation of proline in plant tissues which results in a 10-100-fold increase in the amount of proline in leaves (Aspinall and Paleg, 1981) and a 4-5-fold increase in the content of proline in legume root nodules (Kohl *et al.*, 1991). However, it is recommended to inoculate transplants of *C.glauca* and other tree species with the symbiotic agents (mycorrhizae and nitrogen fixing bacteria,.e.g., *Rhizobium* and *Frankia*) in nursery before transplanting in afforestation and/ or reforestation programs, in arid or semiarid zones.

4. CONCLUSIONS

- Nodulation and mycorrhization under tetrapartite system, to certain extent, are of the mutual synergistic interrelationship.
- Vesicular-arbuscular mycorrhizal fungus is more matual symbiotic agent with *Frankia* than ectomycorrhizal one, yet both of mycorrhizal types throve better than if they separately established.
- Nodulation characteristics under tripartite (FxVAM) were better than tetrapartite (EMxVAMxF). It may be ascribed to slight antagonistic effects that might owing to increasing antibiotics of EM fungus, rather than VAM one or accumulative effects of both.
- Most growth and biomass characteristics, amongst which; shoot height, height growth rate, stem diameter, branchlet and total dry matter of *C. glauca* were improved with EMxVAM, yet in the second season, both of EMxVAM and EMxVAMxF displayed the highest total dry matter.
- The most drought resistance characteristics, notably, survival and life-shoot ratio were enhanced under tetrapartite relationship.
- It is recommended, however that more research are needed using symbiotic agents from Egyptian soil and also to inoculate seeding with more than one convenient symbiotic agent either in afforestation or reforestation programs.

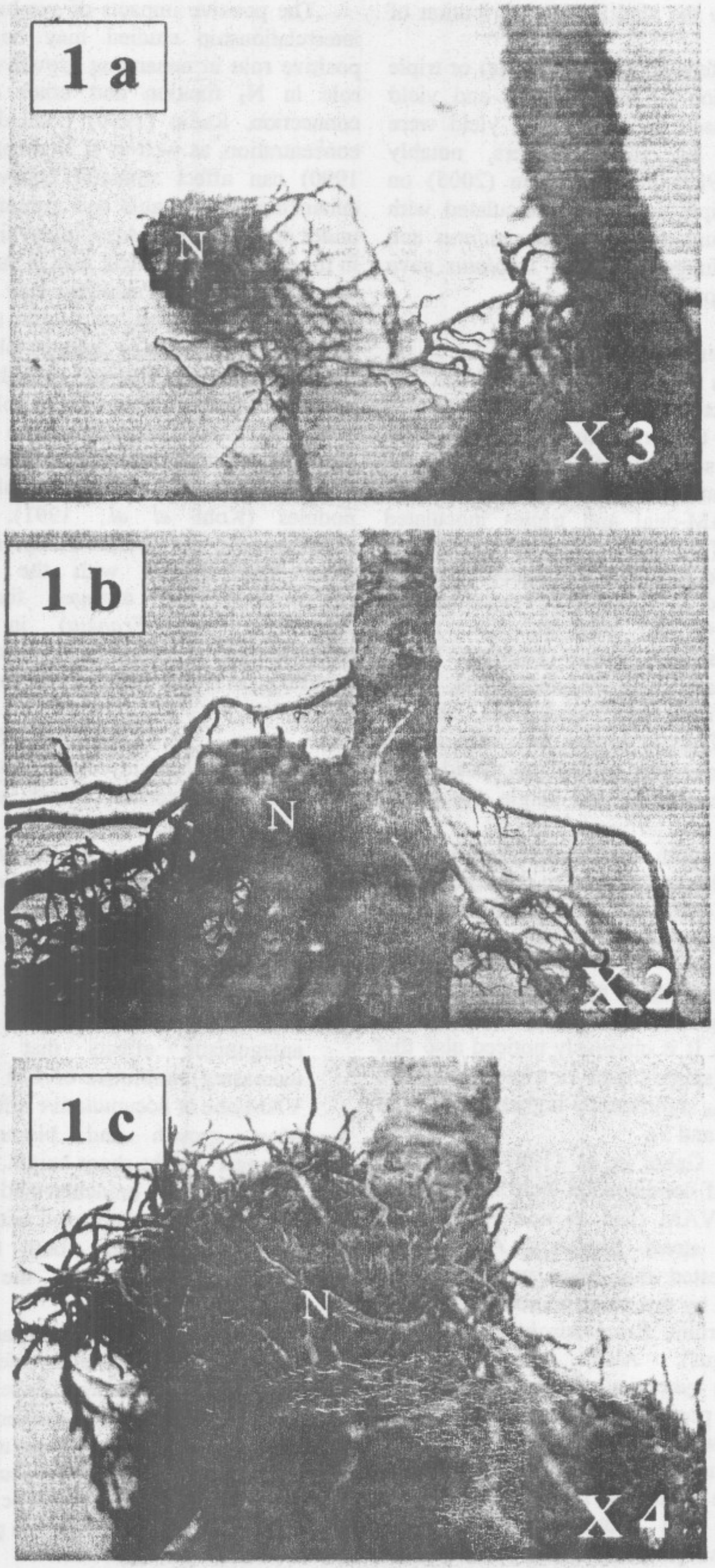
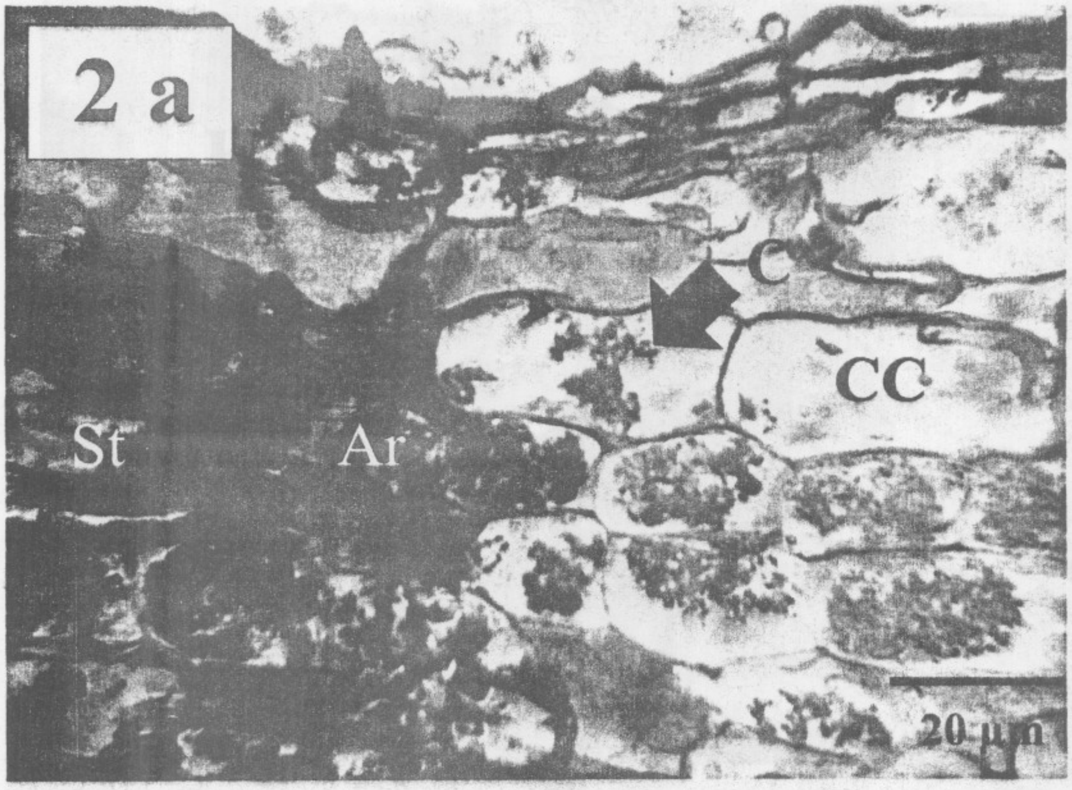


Fig. (1): Berries-like nodules (N) of *Casuarina glauca* roots inoculated only with *Frankia* inocula (a), dually with vesicular arbuscular mycorrhizal (b) and with ectomycorrihzal fungal inocula (c).



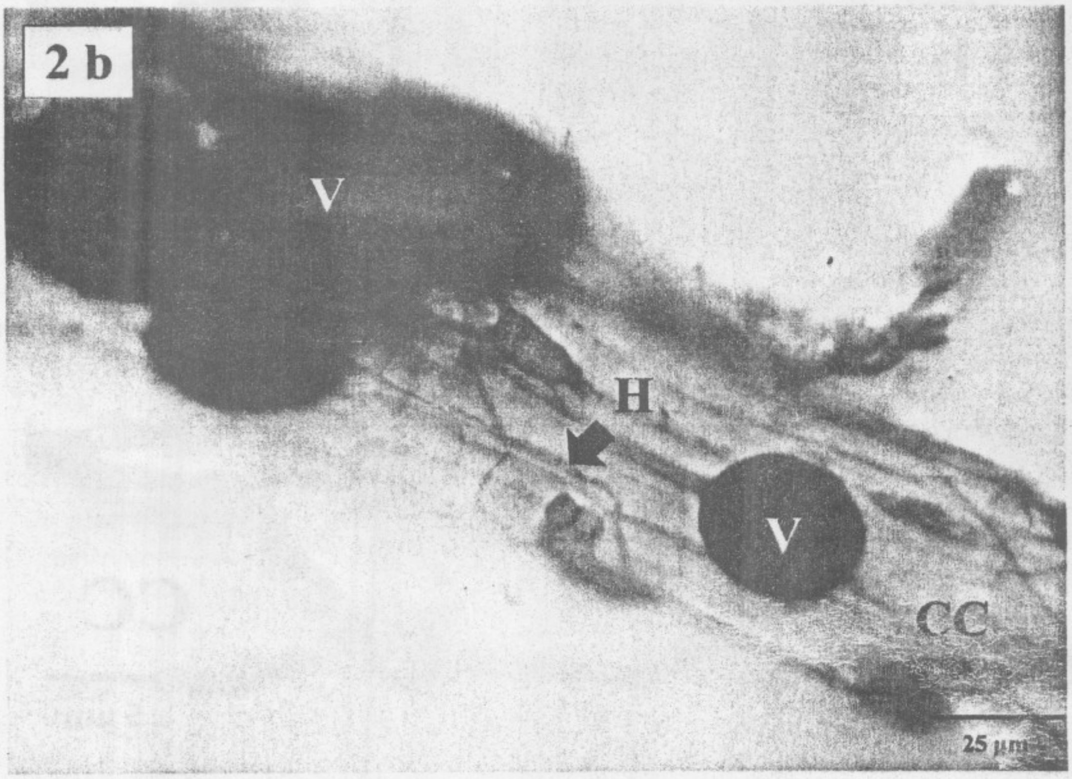
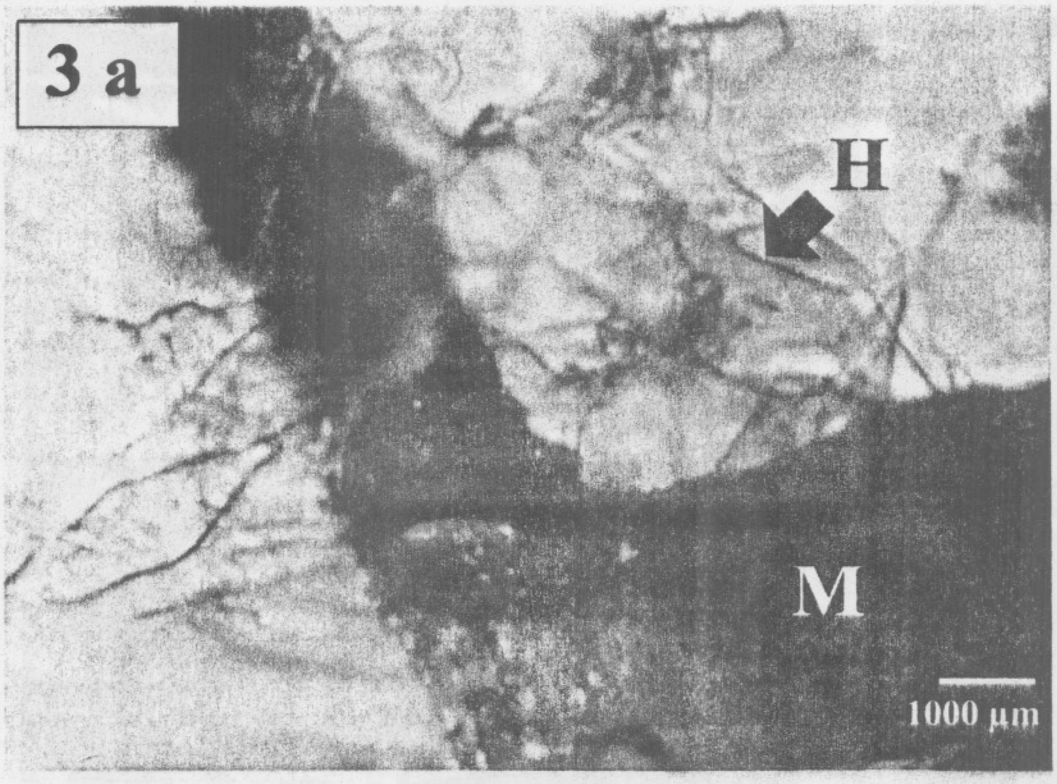


Fig.(2a): Infected root of *Casuarina glauca* with vesicular-arbuscular fungus, displayed the arbscul. (Ar), clump (C), and intramatrical hyphae (H) colonized cortex cells of the root, St is the stele (free from any infection). Fig. (2b): Infected root of *Casuarina glauca* with vesicular-arbuscular mycorrhizal fungus. displayed the vesicles (V), hyphae (H), colonized cortex cells (CC) of feeder root



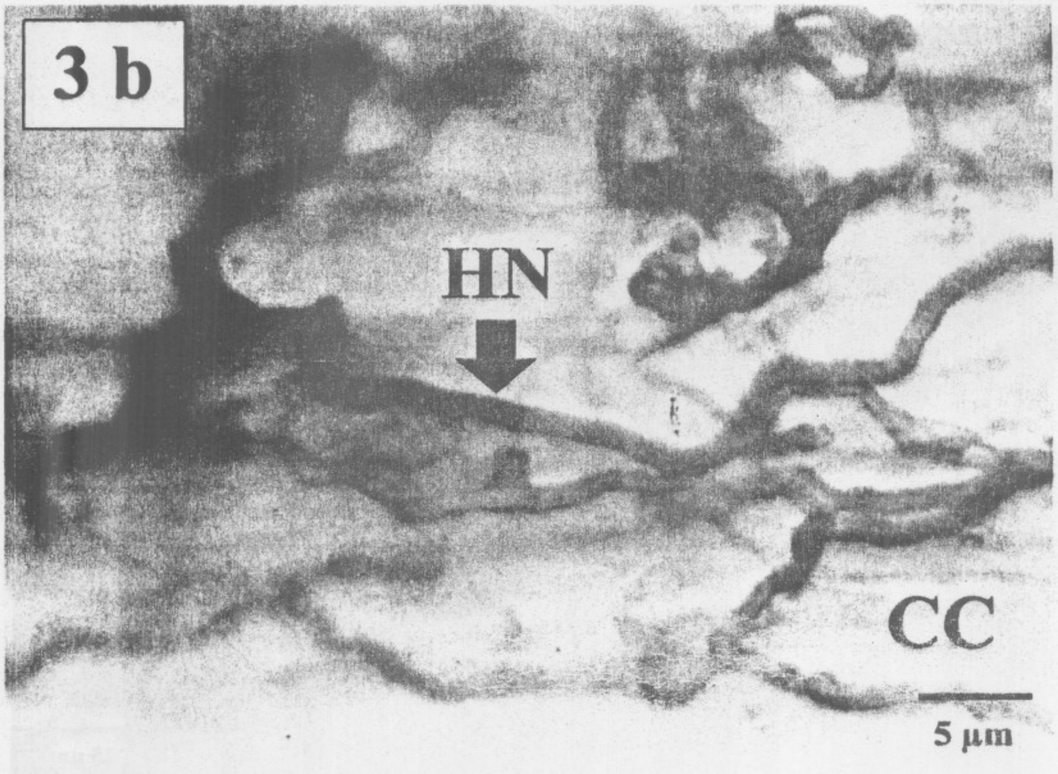


Fig. (3a): **Infected root of *Casuarina glauca* with ectomycorrhizal fungus, displayed the mantle (M)(as pseudparechymatous sheath) and extrametrical hyphae (H).**

Fig. (3a): **Infected root of *Casuarina glauca* with ectomycorrhizal fungus, displayed the intercellular hyphae in Hartig's net (HN) arrangement.**

Table (1): Dates of artificial inoculation with ectomycorrhizal fungus (EM), vesicular– arbuscular mycorrhizal one (VAM), *Frankia* (F) inocula and their sterilized inocula (em, vam and f; respectively).

Treatment	First inoculation Mars 25, 2006 and 2007	Second inoculation Mars 30, 2006 and 2007	Third inoculation April 04, 2006 and 2007
EM	EM	vam	f
VAM	VAM	em	f
F	F	vam	em
EM VAM	EM	VAM	f
EM	EM	F	vam
EM VAM F	EM	VAM	F
control	em	vam	f

Table (2): Nodulation, mycorrhization, growth and biomass characteristics of *Casuarina glauca* transplants that were single, dual and or triple inoculated with ectomycorrhizal- (EM), vesicular arbuscular mycorrhiza- fungi (VAM) and/ or *Frankia* (F) in the first season.

	EM	VAM	F	EM × VAM	EM × F	F × VAM	EM × VAM× F	Cont.
Nodule number	-	-	1.50^{b}	-	1.50^{b}	2.00^{a}	1.00^{c}	
Nodule dry matter/plant (g)	-	-	0.84^{c}	-	1.04^{b}	1.60^{a}	1.10^{b}	-
Mycorrhization level (%)	30^{d}	24^{e}	-	38^{b}	30^{c}	42^{b}	50^{a}	-
Shoot height (cm)	67.13^{c}	75.12^{b}	88.83^{a}	88.20^{a}	66.2^{c}	75.14^{b}	82.75^{ab}	57.43^{d}
Height growth rate (cm/ month)	4.25^{e}	5.62^{b}	5.13^{cd}	6.03^{a}	4.98^{d}	5.22^{c}	5.00^{d}	2.84^{f}
Stem diameter (mm)	3.43^{d}	4.08^{c}	3.92^{b}	5.90^{a}	4.70^{b}	4.12^{c}	4.93^{b}	2.94^{e}
Branchlet dry matter (g)	1.52^{c}	1.88^{bc}	1.82^{bc}	2.72^{a}	1.63^{c}	1.92^{b}	2.00^{b}	1.30^{cd}
Stem dry matter (g)	1.61^{b}	1.80^{ab}	1.60^{b}	1.52^{bc}	1.12^{d}	1.84^{a}	1.49^{c}	0.93^{d}
Root dry matter (g)	1.46^{c}	1.68^{b}	1.20^{d}	2.03^{a}	1.06^{de}	1.82^{b}	2.20^{a}	0.95^{e}
Shoot/ root ratio	2.14^{d}	2.19^{c}	2.85^{a}	2.08^{d}	2.59^{b}	2.06^{d}	1.58^{e}	2.34^{c}
Total dry matter (g)	4.59^{d}	5.16^{c}	4.62^{d}	6.20^{a}	3.81^{e}	5.58^{e}	5.69^{b}	3.18^{e}

Within each row, means of the same postscript letter are not significantly different at 0.05 of probability level.

Table (3): Nodulation, mycorrhization, growth and biomass characteristics of *Casuarina glauca* transplants that were single, dual and or triple inoculated with ectomycorrhizal- (EM), vesicular arbuscular mycorrhiza- fungi (VAM) and/ or *Frankia* (F) in the second season.

	EM	**VAM**	**F**	**EM × VAM**	**EM × F**	**F × VAM**	**EM × VAM × F**	**Cont.**
Nodule number	-	-	1.50^{b}	-	1.30^{b}	2.25^{a}	1.00^{c}	-
Nodule dry matter/plant (g)	-	-	0.92^{c}	-	0.80^{d}	1.70^{a}	1.20^{b}	-
Mycorrhization level (%)	34^{b}	20^{c}	-	42^{b}	23^{c}	40^{b}	52^{a}	-
Shoot height (cm)	64.33^{c}	77.75^{b}	82.25^{a}	86.14^{a}	75.08^{b}	74.5^{b}	76.75^{b}	55.66^{d}
Height growth rate (cm/ month)	4.08^{c}	5.42^{b}	5.79^{a}	5.98^{a}	5.05^{c}	5.03^{c}	4.79^{d}	2.21^{e}
Stem diameter (mm)	3.75c	3.87^{d}	3.87^{d}	4.83^{a}	4.40^{b}	4.25^{bc}	4.75^{a}	2.80^{e}
Branchlet dry matter (g)	1.30^{de}	2.00^{b}	1.20^{ef}	2.21^{a}	1.43^{d}	1.85^{c}	2.13^{ab}	1.16^{ef}
Stem dry matter (g)	1.50^{c}	1.76^{a}	1.42^{d}	1.56^{b}	1.05^{e}	1.71^{a}	1.69^{ab}	0.75^{f}
Root dry matter (g)	1.35^{c}	1.75^{b}	1.10^{d}	1.92^{a}	1.00^{d}	1.75^{b}	2.00^{a}	0.74^{f}
Shoot/ root ratio	2.07^{cd}	2.14^{c}	2.38^{b}	1.96^{d}	2.48^{ab}	2.03^{cd}	2.41^{b}	2.58^{b}
Total dry matter (g)	4.15^{d}	5.51^{b}	3.72^{e}	5.69^{a}	3.48^{f}	5.31^{c}	5.82^{a}	2.65^{g}

Within each row, means of the same postscript letter are not significantly different at 0.05 of probability level.

Table (4): Survival (%), life-shoot ratio based on alive height (H) and alive oven dry weight (w) in the first season.

	EM	VAM	F	EM × VAM	EM × F	F × VAM	EM× VAM × F	Cont.
Survival (%)	70.00^{b}	55.33^{c}	77.33^{b}	80.00^{a}	73.33^{b}	73.33^{b}	80.00^{a}	20.00^{d}
Life shoot ratio (H)	00.60^{b}	00.50^{c}	00.52^{c}	00.47^{c}	00.62^{b}	00.50^{c}	00.86^{a}	0.33^{d}
Life shoot ratio (W)	00.90^{ab}	00.78^{c}	00.82^{bc}	00.86^{bc}	00.90^{ab}	00.80^{c}	00.94^{a}	0.60^{d}

Within each row, means of the same postscript letter are not significantly different at 0.05 of probability level.

Table (5): Survival (%), life-shoot ratio based on alive height (H) and alive oven dry weight (w) in the second season.

	EM	VAM	F	EM × VAM	EM × F	F × VAM	EM × VAM × F	Cont.
Survival %	73.33^{b}	46.66^{c}	73.33^{b}	73.33^{b}	86.66^{ab}	73.33^{b}	86.66^{a}	26.66^{d}
Life shoot ratio (H)	00.68^{b}	00.47^{c}	00.61^{b}	00.45^{c}	00.60^{b}	00.47^{c}	00.88^{a}	00.33^{d}
Shoot life ratio (W)	00.92^{ab}	00.72^{c}	00.86^{ab}	00.80^{b}	00.90^{a}	00.83^{b}	00.95^{a}	00.70^{c}

Within each row, means of the same postscript letter are not significantly different at 0.05 of probability level.

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الملخص العربى

تكوين العقد المثبتة للنيتروجين، تكوين الميكوريزا، نمو ومقاومة جفاف نباتات الكازوارينا البيضاء فى ظل نظام التكافل الرباعى مع الميكوريزا الداخلية والخارجية والفرانكيا

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تم إجراء تجربتين لدراسة علاقة التكافل الرباعية بين شتلات الكازوارينا البيضاء، والميكوريزا الداخلية والميكوريزا الخارجية وبكتيريا الفرانكيا الداخلية وذلك فى موسمين. التجربة الأولى تمت لدراسة تأثير التلقيح الفردى، المزدوج أو/ والثلاثى (بجميع التوليفات) باستخدام المتكافلات المشار إليها أعلاه وذلك على نمو ومقدار المادة الجافة وصفات العقد وتكوين الميكوريزا وذلك باستخدام ٨ معاملات كالآتى:

١- العدوى الفردية باستخدام فطر الـــ *coprinus sp.* المكون للميكوريزا الخارجية.... (EM).
٢- العدوى الفردية باستخدام فطر الـــ *Glomus mossae* المكون للميكوريزا الداخلية.... (VAM).
٣- العدوى الفردية باستخدام لقاح بكتيريا الفرانكيا *Frankia*... (F).
٤- العدوى المزدوجة باستخدام فطرى الميكوريزا الخارجية والداخلية .... (EM× VAM).
٥- العدوى المزدوجة باستخدا ملقاح فطر الميكوريزا الخارجية والفرانكيا... (EM × F).
٦- العدوى المزدوجة باستخدام لقاح فطر الميكوريزا الداخلية والفرانكيا... (VAM × F).
٧- العدوى الثلاثية باستخدام لقاحات فطرى الميكوريزا الخارجية والداخلية وبكتيريا الفرانكيا... (EM × VAM × F).
٨- عدم العدوى (باستخدام لقاحات تم تعقيمها للمتكافلات الثلاثة كمعاملة مقارنه).

وقد أمكن التوصل للنتائج التالية:

- قد لوحظ أن عدد العقد الجذرية للفرانكيا وكذلك وزنها الجاف قد زاد نتيجة التأثير المتضافر لفطر الميكوريزا الداخلية مقارنةً بفطر الميكوريزا الخارجية، وقد وجد أن النباتات الملقحة ثلاثياً قد كونت عدداً أقل من العقد وأقل وزن جاف من تلك الملقحة ثنائياً (EM × F و VAM × F) والملقحة فردياً (F) وذلك فى تجارب الموسمين.
- وجد أن أعلى نسبة إصابة بالميكوريزا كانت فى جذور النباتات المعداه ثلاثياً، أى أن الفرانكيا كان لها أثر تضافرى (إيجابى) على تكوين الميكوريزا وزادت من نسبة الإصابة بها خصوصاً الميكوريزا الداخلية.
- بالنسبة لصفات النمو والمادة الجافة فى نباتات الكازوارينا البيضاء، فقد وجد أن طول النباتات، معدل النمو الطولى، قطر الساق، الوزن الجاف للفريعات الوزن الجاف للجذور والوزن الكلى الجاف فى النباتات المعداه ثنائياً بفطرى الميكوريزا (EM × VAM) فضلاً عن تلك المعداه ثلاثياً باللقاحات الثلاثة فى كثير من الأحيان كانت أعلى من مثيلها فى المعاملات الأخرى وذلك فى موسمى التجربة. بالنسبة للوزن الجاف للساق، وجد أن النباتات المعداه بفطري الميكوريزا وكذلك المعداه فقط بـــ VAM قد أعطت أعلى مستوى فى الموسم الأول، وفى الموسم الثانى انضمت إليهم المعاملة باللقاحات الثلاثة.

بالنسبة لنسبة الفرع/ الجذر، وجد أن النباتات الملقحة بالفرانكيا كانت أعلى معنوياً من بقية المعاملات في الموسم الأول، أما فى الموسم الثانى فإن النباتات المعداه ثنائياً (FM × F) وغير المعداه (كنترول) قد أظهرت أعلى مستوى.

وقد أجريت التجربة الثانية لدراسة تأثير الجفاف على حيوية ونسبة الفرع الحى معبراً عنها بالطول (LSR_H) ومعبراً عنها بالوزن الجاف (LSR_W). وعموماً، من واقع نتائج الموسمين وجد أن التلقيح الثلاثى وكذلك المزدوج (EM × VAM) قد أدى إلى زيادة نسبة حيوية النباتات عند تعرضها للجفاف وكذلك زادت نسب كل من LSR_H وLSR_W مقارنةً ببقية المعاملات.

تتضح من النتائج أن أعلى مستويات نمو لنباتات الكازوارينا كانت بفضل اللقاح المزدوج بفطرى الميكوريزا وإن كان لإضافة لقاح الفرانكيا أثر محدود فى هذه الصفات إلا أنه كان معنوياً فى زيادة مقاومة النباتات للجفاف.