Effect of Mycorrhizal Fungi and Rock Phosphate Application on The Growth of *Pinus roxburghii* Sarg. Seedlings Infected by *Rhizoctonia solani*

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ABSTRACT

This work was conducted to investigate the nature of tri- and tetra-partite systems among host plant, Ectomycorrhiza, VAM, (as symbiotic agents) and *Rhizoctonia solani* (as a pathogenic agent) alone or in combination and their effects on the survival (S) %, growth, biomass, dry weight of the seedlings of *Pinus roxburghii* Sarg. The research was conducted in two successive growing seasons, of 2013 and 2014. It was found that the infection % with EC+VAM was higher than the single inoculation with EC, owing to the synergistic effect of VAM, since the infection % was 40 and 20 %, respectively. The inoculated seedlings with EC+VAM had brought about the highest S% (68.75%). Uninoculated seedlings with *R. solani* displayed the tallest shoot height (SH) (15.74 cm). EC- inoculated seedlings and those dually inoculated ones, i.e., with EC+VAM had exhibited the highest SH (18.35 and 17.67cm, respectively). The inoculated seedlings of *P. roxburghii* with EC singly or dually with VAM which were fertilized with rock phosphate and uninoculated with *R. solani* displayed the highest shoot growth rate (SGR) (2.92 and 3.12 cm/ month, respectively). The inoculation of the seedlings with EC either singly or dually with VAM has brought about the highest root dry weight (RDW) (1.053 and 1.013g, respectively). The inoculation with ectomycorrhiza either singly or dually with VAM, which were fertilized with rock phosphate and uninoculated with *R. solani* displayed the highest shoot/root ratio as compared with the other treatments, since it was 3.481 and 3.559, respectively. Concerning, the significant interaction among rock phosphate, inoculation with *R. solani* and inoculation with symbiotic agents, the inoculated seedlings with ectomycorrhiza either only or with VAM, which were fertilized with rock phosphate and uninoculated with *R. solani* displayed the highest total dry weight (TDW) as compared with the other treatments, since it was 4.46 and 4.46 g, respectively. It has been concluded that the dual inoculation with EM and VAM combined with amendment of rock phosphate increased the resistance or mitigated the harmful effects off damping of the pathogenic- fungi and enhanced the survival as well as growth of *P. roxburghii* Sarg. seedlings.

Key words: Ectomycorrhiza, vesicular arbusculr mycorrhizal fungus, rock phosphate, *Pinus roxburghii* Sarg., *Rhizoctonia solani, Laccaria laccata*.

INTRODUCTION

Chir pine (*Pinus roxburghii* Sarg.) is an important soft- wood tree species used for the production of wood and resin, (Langenheim, 2003). Chir pine is widely planted for timber and manufacturing of pulp, (Tewari, 1994). Chir pine resin is principally used in paper, soap, cosmetics, paint, varnish, rubber and polish industries. Besides, these other uses include manufacture of linoleum, explosives, insecticides and disinfectants, as a flux in soldering, in brewing and in mineral beneficiation as a frothing agent. Pine oil from the woodis used in paints, varnishes, lacquers, pharmaceuticals, wetting agent in textiles, degreasing agent in leather manufacture, and as a synergist in insecticides. The essential 0ils of *Pinus roxburghii* has antibacterial and antioxidant activities,(Salem, *et al.*, 2014) .

Biological control of plant pathogens is currently accepted as a key practice in sustainable agriculture because it is based on the management of a natural resource, i.e. certain rhizosphere organisms, common components of ecosystems, known to develop antagonistic activities against harmful organisms (bacteria, fungi, nematodes etc.). Arbuscular mycorrhizal (AM) associations have been shown to reduce the damage caused by soil-borne plant pathogens. Although few AM isolates have been tested in this regard, some appear to be more effective than others. Furthermore, the degree of protection varies with the pathogen involved and can be modified by soil and other environmental conditions (Harrier and Watson, 2004; Whipps, 2004; Fritz *et al.*, 2006; Pozo and Azcón-Aguilar, 2007; Jung *et al.*, 2012)

Several studies have documented the protective role of mycorrhizae not only against fungal pathogens (Morin *et al*., 1999), but also against nematodes (Diedhiou *et al*., 2003, Koffi *et al.*, 2013 and Vos *et al.*, 2013) and insects (Halldorsson *et al*.,

2000). *Laccaria laccata* is an ubiquitous fungus which forms ectomycorrhizae with many tree species. The broad host range and the readiness of *L. laccata* for mycorrhizal formation make this fungus a candidate for artificial inoculation of tree seedlings aiming to provide biological control (Sinclair *et al*., 1982).

Ectomycorrhizal (ECM) fungal associations are prevalent among forest tree species particularly in the families of Betulaceae, Fagaceae, Myrtaceae, Pinaceae, Salicaceae etc. Ectomycorrhizae are not only necessary for conifer survival and health but have been shown to inhibit development of some soil borne pathogens (Sinclair *et al.* 1975 and Mohan *et al.*, 2015).

Arbuscular mycorrhizae are characterized by the formation of unique structures, arbuscules and vesicles by fungi of the phylum Glomeromycota. AM fungi help plants to capture nutrients such as phosphate, sulfur, nitrogen and micronutrients from the soil (Smith *et al.*, 2010a). It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonization of land by plants and in the evolution of the vascular plants. (Brundrett, 2002).

This work was aimed to investigate the nature of tri- and tetra-partite systems among host plant, VAM, ectomycorrhiza and *Rhizoctonia solani* alone or in combinations and their effects on the, survival, growth, biomass and phosphate content of the seedlings of *Pinus roxburghii Sarg.*

MATERIALS AND METHODS

This study was conducted at the nursery of Forestry and Wood Technology Dept., at the Experimental Station, Faculty of Agriculture, University of Alexandria, Abies region, Alexandria. The study aimed at investigating the nature of tetrapartite system, implying host plant, ectomycorrhiza, VAM and pathogen either alone or in combination and their effect on the growth, biomass and phosphate content of the *Pinus roxburghii* Sarg. seedlings. The experiments were carried out during two successive growing seasons of 2013 and 2014.

1. The plant

Pinus roxburghii Sarg. which considered one of the common conifer trees grown in Egypt. The seeds of *P. roxburghii* Sarg.were collected from 28 years old trees grown at the Fac. Agric. Univ., El-Shatby, Alexandria.

2. Soil:

The soil used in this study was a mixure of sand, peat and perlite at the ratio of 1:1:1, by volume. After homogenization of the mixure, 5 g of humic acid/kg media were added and 3g rock phosphate / kg medium was added to 50% of total seedlings. Rock phosphate used in this investigation contained 15.4% P.

The medium was sterilized using 5% formaldehyde solution and left for 10 days for aeration

3. Seed sowing:

Surface sterilized seeds with 10% NaHCl were sown per pot.

4. Symbiotic agents:

4.1. Ectotrophic (EC)

Inocula of *Laccaria laccata* fungus were obtained from Tour the Burns Lab. in Canada and were used in the study.

4.1.1. Preperation of Ectomycorrhizal inocula

Active colonies (hyphal mat) of the fungus *Laccaria laccata* growing on Modified-Melin-Norkrans (MMN) medium, (Marx, 1969) were extracted from petri dishes, milled with sterilized water in a warm blender, to obtain slury- texured inocula, then mixed with sterilized peat perfectly, kept in refrigerator until use. One hole (1cm*5cm) was made in the soil of each pot and filled with one tea spoon about 0.5g of the inocula (Duponnosis *et al*., 2006).

4.2. Vesicular arbuscular mycorrhizal fungus (VAM)

Inocula (medium and root debris) of *Glomus fasciculatum* fungus. which were obtained from Experimental Station of Philipps University, Botany Dept., Marburg/ Lahn, Germany were used.

4.3. EC+VAM

A combination of the two microorganisms mentioned above, was also applied to the seedlings, opposite each other.

5. Active pathogenic isolate of the fungus (*Rhizoctonia solani* J.G. Kühn):

Inocula (Mycellium) of *Rhizoctonia solani* which causes damping off disease and is considered as one of the soil borne fungi.

6. Treatment with Rhizolex 50WP

The fungicide Rhizolex (O,O-dimethyl-O(2,6-dichloro-4-methyl-phenyl)-phosphorothioate) , was added as a treatment (1g/kg soil) .

7. Survival (%)

Four weeks after inoculation with the *Rhizoctonia solani*, the number of survived plants which showed no pre-nor post-emergance damping off symptoms were counted, and the survival (%) was calculated using the following equation:

$$S = \frac{NLP}{TNP} \times 100$$

Where:

S: Survival (%)

NLP: Number of living plants

TNP: Total number of the plants

8. Growth parameters

Growth parameters, i.e.; height (cm), dry weight of the seedlings (g) and shoot growth rate (cm/ month) were asessed. By the end of the experiment, seedlings were lifted carefully from

the pots, then the roots were washed gently with tap water. Each seedling was divided into root and shoot (needles and stem) and their fresh weights were determined then oven-dried at 70°C for 48 hours to a constant weight to determine the dry weight.

9. Determination of infection level (%)

The infection level (%) was determined using light microscope by measuring the length of colonized feeder roots with any VAM fungal structure either hyphae, vesicule or arbuscule relative to the total length of the root artificially inoculated using the following equation:

I L= C L/ TL× 100

Where:

I L = Infection level,

C L = A colonized length

T L = The total length of the feeder root examined

Ten feeder roots were examined for each sample, then the average was computed.

10. Ultrastuctural examination of infected feeder roots with the symbiotic and pathogenic fungi

Feeder root samples were collected, washed free from debris, cut into small pecies (3 mm length) then soaked in chain of ethanol solution, 10, 20, ----, 100% (absolute), then in xylol. The specimens were soaked in each concentration for 1.0 hour, dried and fixed for scanning electron microscope (SEM) examination, according the method described by Hayat , (1991).

11. The experimental design

Randomized complete block design (RCBD) was used in this experiment. The split- split plot technique was used in analyzing the data obtained, where the main plot was for phosphorus application, the sub plot was for inoculation with pathogenic fungus and the sub-sub plot was for symbiotic agent according to Snedecor, (1956).

RESULTS

1. Survival% (S %)

After two months of the inoculation with *R. solani*, the plants were tested for survival and presence of symbiotic and the pathogenic agents in feeder roots and their impacts on the plants.

Data presented in Table (1) show the following:-

1-Unfertilized seedlings with rock phosphate displayed the highest survival rate (49%).

2- The inoculated seedlings with EC+VAM has brought about the highest S% (68.75%), followed by the inoculated ones with ectomycorrhiza only, (53.75%), but there were no significant differences among the rest treatments.

3-The inoculation with symbiotic agents either singly with ectomycorrhiza (EC) or dually with vesicular arbuscular mycorrhiza (VAM), has brought about the highest S%, since it was 63.75 and 75%, respectively.

The inoculation with EC+VAM for the seedlings uninoculated with *R. solani* and unfertilized with rock phosphate brought about the highest S% (90%), yet the uninoculated seedlings with any symbiotic agent and with pathogenic fungus and fertilized with rock phosphate displayed the lowest S% (0%).

2. Mycorrhization

2.1. Qualitative characteristics

After three months of the artificial inoculation singly with EC as well as dually with VAM in both growing seasons of this study, it was found that the feeder roots of pine seedlings displayed the typical mycorrhizal structures. However, light and scanning electron microscopy revealed the presence of extrametrical hyphae, endometrical hyphae, arbuscules and vesicules in the cortex tissue of the inoculated roots (Figs. 1 and 2). At the end of the experiment span (after 9 months of the artificial inoculation), masses of clamydospores were detected in the old feeder roots for both growing seasons (2013-2014) (Fig.1). As for ectomycorrhiza, scanning electron microscopy revealed the presence of extrametrical hyphae (Fig. 3).

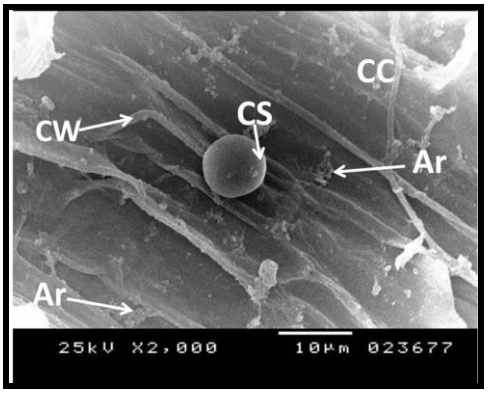

Fig. 1: Scanning electron micrograph (SEM) indicates that feeder root of *Pinus roxburghii* contained chlamydospors of *Glomus fasciculatum* CS: Chlamydospors, CC: cortex cell. Ar: Arbscule, CW: Cell wall

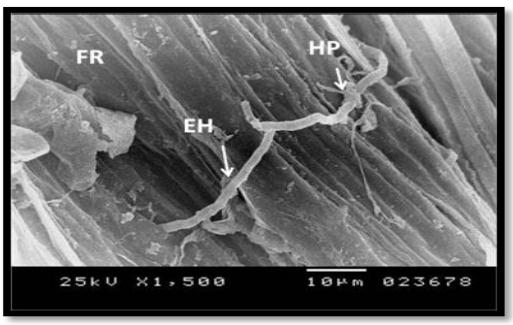

Fig. 2: Scanning electron micrograph indicates feeder root (FR) of *Pinus roxburghii* invaded by VAM/ extrametrical (EH) Hp: Hyohopodium of VAM. EH: Extramatrical hyphea.

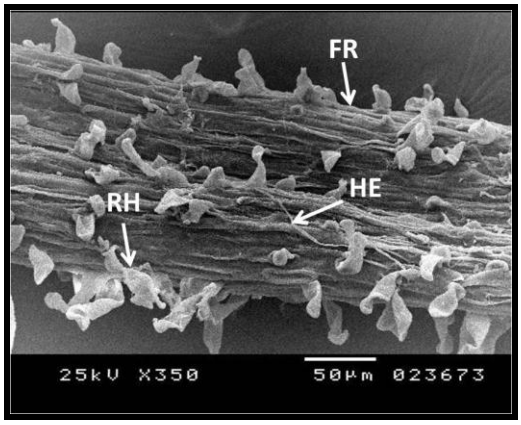

Fig. 3: Scanning electron micrograph indicates feeder root (FR) of *Pinus roxburghii* invaded by hyphae of ectomycorrhiza (HE). RH: Root hairs. CW: Cell wall.

2.2. Quantitative characteristics (inoculation level (IL) by ectomycorrhiza).

Examination of feeder roots of *Pinus roxbjurghii*, showed that the inoculation level was increased with dual inoculation, i.e., the synergistic relationship between both symbiotic agents took place. However, the IL of dually inoculated seedlings with EC+VAM and singly inoculated ones with (EC) was averaged 40 and 20%, respectively. From data obtained, it can be concluded the VAM has a promotive effect compared with that inoculated with only ectomycorrhizal alone.

3. Growth parameters

3.1. Shoot height (SH)

Data presented in Table (2) show the following:

1-The uninoculated seedlings with *R. solani* displayed the highest SH in both growing seasons (15.74, 17.19 cm, respectively).

2-EC- inoculated seedlings and those dually inoculated ones, i.e. with EC+VAM exhibited the highest SH (18.35, 17.67, 18.63 and 18.47cm, respectively in both growing seasons).

3-However, there were no significant differences between SH of inoculated seedlings with VAM (12.67 and 14.66 cm, respectively) and those treated with rhizolex (12.38 and13.25 cm respectively) in both growing seasons.

4-The uninoculated with *R. solani* displayed the highest SH (18.04 cm), but with no significant differences between those fertilized ones in the first growing season.

5-The fertilized seedlings with rock phosphate, and uninoculated with *R. solani* displayed the highest SH (19.79 cm), but there were no significant differences between those unfertilized ones in the second season.

3.2. Shoot growth rate (SGR) (cm/ month)

Data Table presenting in (3) show the following:

1-However, the uninoculated seedlings with *R. solani* displayed the highest SGR (1.74 and 1.91cm/ month), followed by inoculated ones with *R. solani* (1.41 and 1.54 cm/ month, resprctively in the both growing seasons).

2-The uninoculated seedlings which were fertilized with phosphate displayed the highest SGR (2.00 cm/ month), followed by those inoculated ones with pathogenic fungus and unfertilized with rock phosphate (1.65 cm/ month).

3-The inoculated seedlings with EC either singly or dually with VAM displayed the highest SGR (2.32 and 2.39 cm/ month, respectively). The uninoculated seedlings with any symbiotic agents showed the lowest value of SGR, since it was 0.91 cm/ month. in the first growing season.

The significant triple interaction among all factors studied showed that the inoculated seedlings of *P. roxburghii* with EC either singly or dually with VAM, fertilized with rock phosphate and uninoculated with *R. solani* displayed the highest SGR (2.92 and 3.12 cm/ month, respectively in the first growing season), but in the second ones it was 2.80, 2.99 cm/ month, respectively.

3.3. Live shoot ratio based on dry weight (LSRDW)

Data presented in Table (4) declared that:

1-Uninoculated *P. roxburghii* seedlings with *R. solani* displayed the highest LSRDW (0.405g) which averaged as much 1.5 fold as that obtained in inoculated ones (0.269g) in the first growing season. On the other hand, in the second growing season it was 0.718g. Which averaged as much 4 fold as that in inoculated ones.

2-Inoculation with ectomycorrhiza alone and the dually with VAM has brought about the highest LSRDW 0.469g and 0.441g, respectively, i.e., 2 fold that of the control in the first growing season, but in the second one it was 0.883 and 0.877g respectively.

3-The uninoculated seedlings with *R. solani* and fertilized with rock phosphate showed the highest LSRDW of 0.524 and 1.233g, respectively, in both growing seasons.

4-Considering the significant interaction between tested fungus and symbiosis type, it is obvious that the symbiotic agents, i.e., EC, and EC+VAM induced the highest LSRDW in fertilized seedlings with rock phosphate, since averaged 0.616 and 0.619g, respectively in the first growing season , but in the second ones averaged (1.537 and 1.584g, respectively).

Data obtained showed that triple interaction of the all studied factors showed that the inoculation with ectomycorrhiza either singly or dually with VAM and unfertilized with rock phosphate gave 0.957 and 0.901g, respectively, which is considered as much 4 fold as that of the control (0.201g) in the first growing season .

3.4. Root dry weight (RDW)

Data presented in Table (5) declared the followings:

1-The fertilized seedlings with rock phosphate displayed the highest RDW 0.788 and 0.711g, respectively in both growing seasons, but there was a significant negative effect of the inoculation with the tested *R. solani*, whilst uninoculated seedlings with such fungus displayed the highest RDW (0.845 and 0.769g respectively in the both growing seasons.

2-The inoculation with EC, either singly or dually with VAM, exhibited the highest RDW 1.053 and 1.013g, respectively in the first growing season , but in the second ones it was . (0.945 and 0.936g, respectively) .

3-The uninoculated seedlings with *R. solani* and fertilized with rock phosphate showed the highest RDW of 1.475g in the first growing season, but in the second growing season the uninoculated seedlings with *R. solani* and unfertilized with rock phosphate displayed the highest RDW (1.326 g).

4-The inoculation with ectomycorrhiza either singly or dually with VAM of unfertilized seedlings with rock phosphate had brought about the highest RDW, (1.864 and 1.846g, respectively), yet the uninoculated seedlings (control) displayed the lowest RDW, since it was 0.072 in the first growing season.

Finally, it can be concluded that the significant triple interaction among the effects of the three studied factors has manifested the positive impact of the inoculation with ectomycorrhiza either singly or dually with VAM in case of the fertilized seedlings with rock phosphate and uninoculated with *R solani* as compared with the other treatments, since it was 3.481 and 3.559 g, respectively in the first growing season, but in the second seasons it was 3.064 and 3.262 g, respectively.

3.5. Total dry weight (TDW)

1-The fertilized seedlings with rock phosphate displayed the highest TDW of 1.24 and 1.37g, respectively in the both growing seasons.

2-Regardless the effect of rock phosphate addition and inoculation with the symbiotic agent, there were significant differences among uninoculated seedlings (control) and inoculated ones with the symbiotic agents; since it`s TDW was 1.332 and 0.494g, respectively in the first growing season , but in the second ones since their TDW were1.485 and 0.295g, respectively.

3-Despite the effect of rock phosphate application and the inoculation with the *R. solani*, the seedlings inoculated with EC either singly or dually with VAM exhibited the highest TDW (1.575 and 1.52g, respectively).

4-The significant interaction between the effect of rock phosphate application and the inoculation with the tested fungus revealed that the uninoculated seedlings with *R. solani* which were unfertilized with rock phosphate displayed the highest TDW (2.06g), with non significant differences when compared with those unfertilized ones.

5-The significant interaction between the effect of the inoculation with the *R. solani* and with the symbiotic fungi revealed that the inoculation with ectomycorrhiza either singly or dually with VAM and unfertilizing with rock phosphate has brought about the highest TDW (2.52 and 2.53g, respectively).

Finally, it can be concluded that the significant triple interaction among the three studied factors has manifested the positive impact of the dually inoculation with ectomycorrhiza either singly or with VAM in case of fertilized seedlings with rock phosphate, which wee uninoculated with *R. solani* as compared with the other treatments, since their TDW values were 4.46 and 4.46 g, respectively in the first growing season, while in the second growing season they were 5.904 and 6.3g, respectively.

3.5. Total dry weight (TDW)

Data presented in Table (6) showed the following:

The fertilized seedlings with rock phosphate displayed the highest TDW of 1.24 and 1.37g, respectively in the both growing seasons.

Regardless the effect of rock phosphate addition and inoculation with the symbiotic agent, there were significant differences among uninoculated seedlings (control) and inoculated ones with the symbiotic agents; since it`s TDW was 1.332 and 0.494g, respectively in the first growing season, but in the second ones since their TDW were1.485 and 0.295g, respectively.

Despite the effect of rock phosphate application and the inoculation with the *R. solani*, the seedlings inoculated with EC either singly or dually with VAM exhibited the highest TDW (1.575 and 1.52g, respectively).

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The significant interaction between the effect of the inoculation with the *R. solani* and with the symbiotic fungi revealed that the inoculation with ectomycorrhiza either singly or dually with VAM and unfertilizing with rock phosphate has brought about the highest TDW (2.52 and 2.53g, respectively).

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DISCUSSION

The obtained data declared that the fertilization with rock phosphate under tetrapartite symbiotic system significantly increased survival %, root dry weight, live shoot ratio based on dry weight and total dry weight. According to this was in a parallel line with Omar 1998 who found that the fertilization with rock phosphate and inoculation with *Glomus consticume* had greatest positive effect on growth, dry weight and phosphorus contents of wheat.

The inoculation with native AM fungi significantly decreased the seedling mortality during the first four weeks of the plantation. It is well known that the transplanting shock and the damage suffered by the seedlings could be critical to the success of the plantation. As inoculated seedlings were stronger than the uninoculated ones, they could survive outplanting by showing better resistance to the environmental conditions and the obtained results are in agreement with those of Duponnois *et al.* (2006) on Lavedula species and cypress.

From the obtained data, it could be concluded that the seedlings of *Pius roxburghii* responded to the inoculation with symbiotic agents positively and notably; inoculation with EC, VAM and EC+VAM in terms of shoot height, shoot growth rate, shoot dry weight, roots dry weight, total dry weight may be due to root lignifications as mentioned by Hassan, *et al.*, (2011) in blue pine roots, or because of nutrient competition, with emphasis on competition for carbon, has been suggested as a mechanism of the arbuscular mycorrhizal fungi - mediated biocontrol though not much evidence is found in the literature (Jung *et al.*, 2012). The carbon transfer from the host plant to the AMF is estimated to range from 4 to 20% of the total assimilated carbon (Hammer *et al.*, 2011). Arbuscular mycorrhizal fungi are known to be able to increase the uptake of water and mineral nutrients for their host plant, such as phosphate and nitrogen (Parniske, 2008 and Baum *et al.*, 2015) but probably also micro-elements such as zinc (Smith and Smith, 2011a,b). In return, they receive photosynthetic carbon from their host (Gianinazzi *et al*., 2010). Arbuscular mycorrhizal fungi supported the plant against various abiotic stress factors such as drought, cold or heavy metal toxicity (Singh *et al.*, 2011).

Although compounds typically involved in plant defence reactions are elicited by arbuscular mycorrhizal fungi only in low amounts, they could act locally or transiently by making the root more prone to react against pathogens. Based on molecular, immunological and histochemical techniques provided new insights into these mechanisms (Barea and Azcón 1996).

Furthermore, the dually inoculation with the symbiotic agents in this study has brought about the highest growth parameters as compared with single inoculation with any symbiotic partener particularly, in *P. roxburghii* seedlings which wewre fertilized with rock phosphate and uninoculated with pathogenic fungus. Enhanced height may also be attributed to alterations in the root structural design such as an increase in lateral root development according to Fitter (1985), Yano (1996), Harrison (2005). They reported that lateral roots are highly colonized and it appears that the symbionts trigger alterations in root structural design to create the most favored sites of interaction.

The Inoculation with ectomycorrhiza suppressed the growth of *R. solani* . Hassan, *et al.*, (2011) found that *Pisolithus tinctorius* and *Laccaria laccata*, significantly inhibited the growth of *R. solani* and *F. oxysporum* by 46.2, 45.4 ,44.7 and 43.7%, respectively.

The Inoculation of *Cupressus atlantica* with AM fungi increased growth and survival in greenhouse and field (Duponnois, *et al.*, 2007). The enhancement of infection by VAM expressed as the growth of plant organs and/ or its dry weight was reported by several authors, notably; Charles *et al*., (1982); Standrberg and Johnsson (1999); Gaur *et al*., (2000); Forestier *et al*., (2001) and Sohn *et al*., (2003), even though under salinity stress (Evelin *et al*., 2009), drought stress (El-Settawy, 2008) and polluted soil (Kaya *et al*., 2009). The phenomon of positive effects of VAM on mycorrhization, not vice versa, in terms of growth and dry weight of the host plant in tripartite regime was reported by Lokshman and Kadam (2011). It has been reported that the dual inoculation with N-fixing Frankia and VAM induced the growth and increased dry weight of *Casuarina equisetifolia* seedlings as compared with sole inoculation of either symbiont (Vasanthakrishna *et al*., 1994) and of *Casuarina glauca* (El-Settawy and El-Gamal, 2003 and El-Settawy, 2008).

CONCLUSIONS

The results obtained showed that the inoculation with EC either singly or with VAM led to enhance significantly the survival (%) under inoculation with the pathogenic fungus, *Rhizoctonia solani*, growth significantly (in terms of shoot height, shoot growth rate, and total dry weight) of *Pinus roxburghii* seedlings. The synergistic relationship between effects of EC and VAM was evident. The dual inoculation has brought about the highest growth and dry weight of seedlings than the inoculation with either symbiont singly did. The negative impacts of the inoculation with *R. solani* were noticed in terms of growth parameters. Rock phosphate application had positive effect on total dry weight and live shoot ratio based on dry weight.

It is recommended; however, the inoculation the seedlings of *P. roxburghii* Sarg. dually with convenient species of EC and VAM to control or to mitigate the detrimental effects of soil borne fungi, *R. solani*, so that, exaggerate its survival%, growth and performance as an important nursery practice in the frameworks of afforestation and reforestation programs, even though in poor soils.

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الملخص العربى

تأثير فطريات الميكوريزا وإضافة الفوسفور على نمو بادرات الصنوبر المصابة بفطر الرايزوكتونيا سولانى

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أجري هذا البحث فى موسمي نمو بدءاً من ١٠ فبراير ٢٠١٣ حتى ٥ ديسمبر ٢٠١٣ ومن١٠ فبراير ٢٠١٤ حتى٥ ديسمبر٢٠١٤ وذلك بمشتل قسم الغابات وتكنولوجيا الأخشاب بمحطة البحوث الزراعية– كلية الزراعة– جامعة الإسكندرية بمنطقة ابيس وكذلك معامل قسم الغابات وتكنولوجيا الأخشاب بالكلية. بغرض دراسة تأثير التلقيح بفطر الميكوريزا الخارجية(EC) والميكوريزا الداخلية(VAM) وكلاهما معاً على تكوين الميكوريزا وحيوية النبات ومكافحة مرض الذبول الطرى المتسبب عن الفطر الرايزوكتونيا سولانى ومقارنتها بإستخدام المبيد الفطرى الريزولكس وتقدير النمو والوزن الجاف لشتلات الصنوبر *Pinus roxburghii* والمسمدة بصخر الفوسفات. وكانت المعاملات الحيوية المستخدمة هى فطر الميكوريزا الخارجية *Laccaria laccata*، فطر الميكوريزا الداخلية vesicular arbuscular mycorrhizae (VAM) *Glomus fasciculatum* وخليط من الإثنين..أظهرت النتائج المتحصل عليها زيادة مستوى الإصابة بالميكوريزا الخارجية بصورة معنوية عندما لقحت باللقاح المزدوج مقارنة باللقاح الفردى لأى منهما وذلك يرجع إلى التأثير المحفز للميكوريزا الداخلية حيث بلغت نسبة الإصابة ٢٠ و٤٠% للتلقيح الفردى بالميكوريزا الخارجية والتلقيح المزدوج بالميكوريزا الخارجية والداخلية على التتابع. وبناءاً على التداخل المعنوى بين إضافة صخر الفوسفات والتلقيح بالمتكافلات، أظهرت النتائج المتحصل عليها أن الشتلات المسمدة بصخر الفوسفات أظهرت أعلى قيمة فى حيوية النباتات وكذلك وزن الجذر ونسبة الجزء الحى من المجموع الخضرى على أساس الوزن الجاف والوزن الكلى الجاف. أما فيما يخص مقاييس النمو، فقد أظهرت النتائج أن المعاملة بالميكوريزا الخارجية فقط وكذلك المعاملة الثنائية قد أعطت أعلى قيمة لكل من الإرتفاع، معدل النمو، الوزن الجاف للمجموع الخضرى، للجذور، الوزن الكلى الجاف ونسبة الجزء الحى من المجموع

الخضرى على أساس الوزن الجاف. وبناءاً على التداخل المعنوى بين إضافة صخر الفوسفات والتلقيح بالمسبب المرضى، أثبتت النتائج المتحصل عليها أن النباتات المسمدة بصخر الفوسفات وغير المعداه بفطر *R. solani* قد أعطت أعلى قيمة فى الإرتفاع، معدل النمو، وعلى أساس الوزن الكلى الجاف. أظهرت الشتلات الملقحة بنوعى الميكوريزا وغير المعداه بالرايزوكتونيا سولانى والمسمدة بصخر الفوسفات أعلى قيمة فى كل من حيوية النبات، الإرتفاع، معدل النمو، ونسبة الجزء الحى من المجموع الخضرى على أساس الوزن الجاف والوزن الجاف للجذور.

وعموماً فقد أظهرت النتائج المتحصل عليها أن التلقيح المزدوج بنوعى الميكوريزا الخارجية والداخلية يعد أفضل وسيلة لتعظيم النمو وزيادة حيوية النباتات وتثبيط نمو المسبب المرضى *R. solani* وتعظيم مقدار الوزن الجاف لشتلات أشجار الصنوبر وكذلك التأثيرات الإيجابية للمتكافلات موضع الدراسة.