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Influence of some Biofertilizers and Chemical Fertilizers on some Vegetative and Root Growth Characteristics of *Casuarina equisetifolia* Seedlings Grown in *Sandy Soil*

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ABSTRACT

A pot experiment was carried out at the Experimental Farm and Laboratory of Horticulture Department, Faculty of Agriculture, Benha University Egypt during the 2019 and 2020 successive growing seasons. The study was carried out to define the efficiency of various biofertilizers Minia Azotein (MA), Frankia and Vesicular Arbuscular Mycorrhiza. each alone or combined with two doses of chemical fertilizer NPK at 75% and 50% from the recommended dose (350:200:100 kg/ fed) and/or the full dose of 100% NPK for improving some vegetative and root growth characteristics of *Casuarina equistifolia* seedlings grown in sandy soil. Results showed that all treated seedlings of casuarina with Frankia 50g/ plant, and Vesicular Arbuscular Mycorrhiza (VAM) 50 ml/plant in combination with 75% NPK T8 and T10 and or NPK 100% recommended dose (10:5:2.5g/plant) T2 recorded significant increases in plant height, stem diameter, number of branches, biomass fresh and dry weights compared to the other treatments and control. The highest rooting values of root length, root fresh, and dry weights of casuarina plants were found with the abovementioned treatments followed by Minia Azotein (MA) combined with 75%NPK (T6) in both seasons while the other treatments did not attain any significant variances in vegetative and root growth parameters. It can be concluded that Frankia 50g/plant, Vesicular Arbuscular Mycorrhiza (VAM) 50 ml/plant with 75% of the recommended dose (7.5:3.75:1.875g/plant)T6, T8, and/ or 100% of the recommended dose of NPK (10:5:2.5g/plant) T2 were the best in improving vegetative and root characteristics of *Casuarina equistifolia* seedlings.

Keywords: *Casuarina equisetifolia*, Minia Azotein (M.A), Frankia, Arbuscular Mycorrhiza Fungi (AMF), chemical fertilizer NPK, vegetative and root growth.

INTRODUCTION

Casuarina is a needle tree an upright, fast-growing, evergreen tree with many branches. native to Asia where they call it (Jemara or Simara) and a length of up to 35m they multiplied by seeds belonging to the family (Casuarinaceae) coexisting with radial root nodules (Karthikeya, 2016). These trees tolerate drought, salinity, and heavy metal pollution (Pooja and Katharina, 2021) including three species, and their hybrids of the three species, *C. equisetifolia*, and *C. glauca* are highly salt-tolerant fall with strong winds (Wheeler *et al.*, 2011). *Casuarina equisetifolia* is an important tree and suffers from bacterial wilt caused by *Ralstonia solanacearum* (Yongcheng *et al.*, 2021). It acts as a shelter for ants, bees, and birds (Alok, 2021). *Casuarina equisetifolia* has traditionally been used to treat neurological problems, acne, sore throats, stomach ulcers, constipation, cough, diabetes, and dysentery (Vain *et al.*, 2022). It helped to improve the ecosystems in agricultural systems and helps reduce negative environmental impacts such as erosion, runoff, evaporation, and increases soil organic matter (Ian, 2021). It has the ability to treat wastewater by removing the methylene blue dye from it (Helly *et al.*, 2021). The actinorhizal plant *Casuarina equisetifolia* L. has a mutualistic relationship with Frankia and promotes nitrogen fixation in root nodules (Yujin *et al.*, 2021).

The role of N_2-fixing bacteria has been demonstrated by several researchers (Dadarwall *et al.*, 1997; Hedge *et al.*, 1999; Hauwaka, 2000). N_2-fixing microorganisms have a function of fixing N_2 in the atmosphere, their effect on photosynthetic hormones such as indole acetic acid, gibberellins, and cytokinin, and their role in promoting the absorption of many nutrients while improving water quality, which enhances the characteristics of vegetative growth. Anti-bacterial and anti-fungal protect the host plants from infection (Soliman, 2019).

For N fixation, Frankia relates to *Casuarina equisetifolia*, and it is predicted that Frankia fixes up to 362 kg N/ha./Year, which is an important nutrient for all plant metabolic processes and development (Shantharam and Mattoo, 1997 and Ojha *et al.*, 2018). N_2 fixation can be catalyzed by Frankia by adding nitrogen at the recommended rate, actinorhizal plants may fix nitrogen in a symbiotic manner (Mackay *et al.*, 1987). In addition to Mycorrhiza and the nitrogen-fixing bacteria Frankia, actinorhizal plants are also able to form a unique type of roots called "proteoid roots" or "cluster roots" in response to the detrimental effects of nutrient deficiencies in the soil (Malajczuck and Lamont, 1981; Crocker and Schwintzer, 1993).

Vesicular Arbuscular Mycorrhiza (VAM)) secrete the enzyme phosphatase that dissolves organic phosphorous and works to increase the activity of phosphorous-dissolving bacteria, which helps to increase the amount of it in the soil (Yang *et al*., 2022). The fungus absorbs phosphorous through a variety of mechanisms, including the secretion of the

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enzyme Phosphates by the fungus hyphae, which works to dissolve organic phosphorous and convert it into forms suitable for plant absorption, and the secretion of hydroxyl acids, which seize the elements calcium, iron, and aluminum while leaving the phosphorous element dissolved in the soil solution. Because of their mutually beneficial relationship, mycorrhizal fungi increase the activity of phosphate-dissolving bacteria. As a result, the amount of dissolved phosphorus in the soil solution rises (Dinkelaker and his associates, 1995).

Mineral NPK fertilizer promotes the vegetative growth, root development, flower development, seed development, and fruit development of tree seedlings. Because nitrogen is found in amino acids, proteins, alkaloids, coenzymes, and certain vitamins, it plays an important role in metabolism, heredity, reproduction, and growth, according to experts (James and Michael, 2009). Trees need nutrients in order to carry out the various biological processes inside them. Nitrogen enters the formation of proteins, amino acids, enzymes, nucleic acids, RNA, DNA, chlorophyll, vitamins, and alkaloids (Curtis, 1996). Nitrogen is absorbed in the form of nitrate ions NO_3 or ammonia NH_4 (Anthony and Glass, 2010). A moving element that shows symptoms of deficiency on the lower leaves, the plant needs it by 1-5%. Its absence hinders the growth of the plant by exceeding the permissible limit and the root total is more than the vegetative (Arnold, 2015). Moreover, phosphorous enters the formation of phospholipids and nucleic acids, in addition to its role in the processes of oxidation, reduction, photosynthesis and respiration (James, 2020). In addition to its entry into the synthesis of ATP, the carrier of energy, it is absorbed in the form of mono- and di-phosphate, which is a mobile element. Symptoms of deficiency appear on the aged leaves (Wei *et al.*, 2021). Potassium is found in the plant in the form of soluble inorganic salts. It has a role as an activator of enzymes, the representation of carbohydrates and proteins, and the opening and closing of stomata (Kaan and Ramazan, 2022). Chemical fertilizers have much damage to humans, plants, soil, and the environment, including what is caused by an increase in nitrogen fertilizers, from the conversion of nitrates to nitrites that cause bowel cancer and the increase in the incidence of pests, insects, and diseases in plants and the accumulation of heavy elements in the soil beside the polluting soils of the environment resulting from the phosphate industry. (Nikita and Puneet, 2020and Masindi and Muedi, 2018).

The research aims to define the efficiency of various biofertilizers Minia Azotein (MA), Frankia and visicular Arbuscular Mycorrhiza (VAM) each alone or combined with tow doses of chemical fertilizer NPK at 75% and 50% from the recommended dose (350:200:100 kg/fed) and / or the full dose of 100% NPK for improving some vegetative characteristics i.e. plant height, stem diameter, number of branches, biomass fresh and dry weights , and root growth characteristics i.e. root length, root fresh and dry weights of *Casuarina equistifolia* seedlings grown in sandy soil.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm and Laboratory of Horticulture Department, Faculty of Agriculture, Moshtohor, Benha University, Egypt, during two successive seasons of 2019 and 2020 on *Casuarina equistifolia* seedlings to evaluate the effectiveness of biofertilizer Minia Azotein(MA), Frankia and Vesicular Arbuscular Mycorrhzial (VAM)) each alone or combined with two doses of chemical fertilizer NPK at 75% and 50% of the recommended dose (350:200:100 kg/fed) and or the full dose of 100% NPK for improving some vegetative growth and root growth characteristics i.e. plant height, stem diameter, number of branches, biomass fresh and dry weights, root length, root fresh and dry weights of *Casuarina equistifolia* seedlings grown in sandy soil. seedlings of one-year-old with an average height of 10-15 cm diameter of 3-6mm were purchased from EL- Orman Garden, Ministry of Agriculture, Egypt. On March 1st week for both experimental seasons, the seedlings were repotted in 40cm plastic pots filled with 30kg of sandy soil, one seedling in each pot.

Physical and chemical properties of the used soil were done according to the method described by Okalebo *et al.* (2002) and listed in Table (a)

Table a. Physical and chemical analysis of the experimental soil before the application of any fertilizers in both seasons 2019 and 2020.

Soil analysis	2019	2020
Sand %	81.04	80.92
Silt %	12.28	11.83
Clay %	6.86	25.7
Soil texture	Sandy	Sandy
Organic matter %	0.11	0.09
$CaCO_3$	3.35	3.51
pH (1:2.5)	8.01	7.92
E. C (m mhos /cm)	1.23	1.19
Total N (%)	0.001	0.001
Available P (mg/kg)	3.00	2.50
Available K (mg/kg)	94.00	105.00
Fe (mg/kg)	10.10	9.80
Mn (mg/kg)	8.50	8.30
Cu (mg/kg)	1.10	0.90
Zn (mg/kg)	0.88	1.00

Biofertilizer:- 1- Minia Azotein MA (commercial name) was used. It contains live cells of N-fixing bacteria (1 ml = 10^7 cells of bacteria), were obtained from the Laboratory of Biofertilizers, Department of Genetic, Fac. of Agric., Minia University according to Abdou *et al.* (2006) The three biofertilizers were used at the concentrations of 50 ml/pot of Minia Azotein MA, 50g/ pot of Frankia, and 50ml/pot of Vesicular Arbuscular Mycorrhiza VAM, three times on 16th April, 16th June, and 16th August one week after chemical fertilizer treatments had been done and immediately after irrigated pots with 75% of its field capacity(2.25L/pot) in both seasons of 2019 and 2020.

2- Frankia the double layer technique was used to separate Frankia sp. from the collected Casuarina root nodules (Murry *et al.*, 1984)**.** The chosen nodules were washed several times in water to remove most dirt and organic material adhered to the surface, then thoroughly cleaned under the dessecting microscope to remove any soil and organic particles left adhered to the nodule surface or inserted between the lobes. Surface lobes were sterilized in sodium hypochlorite (2.0 percent) and HgCl (0.1 percent in 0.5 percent HC) for 15 minutes, then washed multiple times with sterile distilled water. Individual lobes were grown in nutrient broth for one week at 29+2°C to assess sterilization quality (Carrasco *et al.*, 1992). Using a sterile scalable, the sterilized lobes were sliced into little pieces. The little lobe pieces were eventually distributed over a 1.59 percent agar modified-Q-mod bottom layer in a Petri plate. 3 ml of semi-solid modified Q-medium was placed on top of the overlayer, covering the lobe pieces (Caru, 1993), and cultured for 2-3 months at 29+2°C. On modified -Q-mod, Frankia colonies that grew out of nodule fragments and had normal hyphae and sporangia were separated (Lalonde and Calvert, 1979). Purification tests were performed on Frankia isolates (Diem and Dommergues 1983). The pure Frankia isolates were kept at 29+2°C on a modified BAP liquid medium, and subculturing was done on the same medium every week.

3-**Vesicular Arbuscular Mycorhizal (VAM)** was collected from the Biofertilizer Laboratory of Ain Shams University, Genetics Department, (AMF) pollen was originally extracted from the soil around wheat roots in the experimental field of Fac. Agriculture, Ain Shams University, Shubra El-Kheima, Cairo, Egypt, according to Gerdmann and Nicolsan (1963).

Chemical fertilizer N,P and K as recommended dose 350 kg/fed of amonium sulfate (20.6% N), 100 kg/fed of potassium sulfate (48% K_2O) and 200 kg/fed of calcium superphosphate (15.5%P_2O_5) all obtained from Kema factory of Aswan. The NPK doses were applied as dressing inside each pot in three equal portions added at 9th April, 9th June and 9th August at one week interval prior to each biofertilizer treatments. Each pot was received (10:5:2.5g) as recommended dose of NPK and (7.5:3.75:1.875g) as 75% of recommended dose of NPK and (5:2.5:1.25g) as 50% of recommended dose of NPK.

The experiment was arranged in a randomized complete block design (RCBD) with three replicates for each treatment each replicate contained 3 Casuarina seedlings (11 treatments × 3 replicates × 3 seedlings) =99 seedlings every season.

1- Control-: without any fertilizer T1.
2- 100 % NPK as recommended dose:(10:5:2.5g/ pot) T2.
3- Minia Azotein MA: (50ml/pot) T3.
4- Frankia: (50g/pot) T4.
5- Vesicular Arbuscular Mycorrial VAM: (50ml/pot) T5.
6- 75% NPK + MA:(50ml/pot) T6.
7- 50% NPK + MA:(50ml/pot) T7.
8- 75% NPK + Frankia: (50g/pot) T8.
9- 50% NPK + Frankia :(50g/pot) T9.
10- 75% NPK +VAM: (50ml/pot) T10.
11- 50% NPK +VAM: (50ml/pot) T11.

All horticultural practices including irrigation and weed management were done as recommended to this respect.

At the end of each season on November 1st in both seasons, the data were recorded on vegetative growth characteristics of casuarina plants i.e. plant height(cm), stem diameter(cm), number of branches, biomass fresh and dry weights(g/plant). On root growth characteristics i.e. root length(cm), root fresh and dry weights (g/plant).

Statistical analysis

Data were obtained from treatments of the factors under study for analyzes of *variance* (ANOVA) was used to assess the significance of the data at $P \leq 0.05$ and differences were evaluated using least significant differences (L.S.D) according to (Ssnedecor and Cochran, 1989) using MSTAT-C statistical software package(1986).

RESULTS AND DISCUSSION

Vegetative characteristics

Plant height (cm):

The data in Table (1) showed a significant increase in plant height compared to control. The tallest seedlings was used recorded when mineral NPK fertilizer as a recommended dose (10:7.5:2.5g/pot) was used and attained the highly percentages of increases reached to 47.78 and 51.27 % over control in the first and second season respectively. Followed by treatment 75% of NPK with Frankia (50g/plant) which gave a significant increase in plant height reached to 46.46 and 51.16 % over control in the first and second season respectively, then treatment by Mycorrhizae (50ml/pot) plus 75% NPK, which gave a significant increase in length reached to 46.46 and 51.16 % over control in the first and second season. respectively. According to this data the differences between the highest three treatments had no significant.

Stem Diameter (cm):

The data in Table (1) showed that the recommended dose of 100% NPK T2 and Frankia (50g/pot) combined with 75% of NPK T8 followed by Mycorrhizae (50ml/pot) combined with 75% of NPK T10 produced the thickest diameter seedlings of Casuarina with non-significant differences between them whereas Minia Azotein (50ml/pot) combined with 75% ranked the fourth in this concern while the other treatments did not attain any significant variances in stem diameter compared to control (00.0) T1.

Number of branches:

The data in Table (1) showed that the recommended dose (10:7.5:2.5g/pot) *of* 100% mineral NPK fertilizer T2 and Frankia (50g/pot) combined with 75% of mineral NPK fertilizer (7.5:3.75:1.875g) T8 followed by Mycorrhiza (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced the highest values of branch number of seedlings of casuarina with non significant differences between them whereas Minia Azotein (50ml/pot) combined with 75% of mineral NPK fertilizer T6 ranked the fourth in this concern while the other treatments did not attain any significant variens in number of branches compared to control (00.0)T1.

Biomass fresh weight(g):

The data in Table (1) showed a significant increase in biomass fresh weight compared to control. the heaviest weight of biomass fresh weight was recorded when the mineral NPK fertilizer as a recommended dose was used and attained the highly percentages of increases reached to48.13 and 48.99 % over control in the first and second season respectively. Followed by treatment 75% of NPK with Frankia (50g/plant) which gave a significant increase in biomass fresh weight reached to 45.36 and 46.17 % over control in the first and second season respectively, then treatment by Mycorrhiza (50ml/pot) plus 75% NPK, which gave a significant increase in biomass fresh weight reached to 43.28 and 44.07 % over control in the first and second season respectively. According to this data the differences between the highest three treatments had no significance.

Biomass dry weight(g):

The data in Table (1) showed that the recommended dose (10:7.5:2.5g/pot) of 100% mineral NPK fertilizer T2 and Frankia (50g/pot) combined with 75% of mineral NPK fertilizer (7.5:3.75:1.875g) T8 followed by Mycorrhiza (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced The heaviest biomass dry weight of seedlings of casuarina with non significant differences between them whereas Minia Azotein (50ml/pot) combined with 75% of mineral NPK fertilizer T6 ranked the fourth in this concern while the other treatments did not attain any significant variens in biomass dry weight compared to control (00.0)T1.

The increase in vegetative characteristics i.e. plant height, diameter, number of branches, biomass fresh weight, biomass dry weight fertilizer NPK are in parallel with those obtained by Abo El-Wafa (2014) and Abdou et al., (2014) on Populus nigra mentioned that fertilizing seedling with NPK at a rate 4:2:2 g/ pot improved its vegetative characteristics. On Swietenia mahagoni seedlings, Youssef et al. (2019) evaluated the effect of different NPK fertilizer rates (0, 4, and 6 g/plant) on vegetative growth. Results showed that clay media plus 6 gm NPK gave the highest values of plant height, leaves fresh and dry weights, and leaves number /plant. While, clay and sand (1:1) media plus 6 gm NPK recorded the highest values of leaves number/ plant compared with other treatments. The stimulating effect of chemical fertilization on Vegetative growth parameters It may be due to the role of chemical fertilization in supplying plants with the nutrients they need for production more carbohydrate and protein necessary for the vegetative growth of casuarina seedlings Vasanthakrishna, et al. (1994) on Casuarina equisetifolia Jihane et al. (2016) on Casuarina spp Frosi et al. (2016). On Poincianella pyrmidalis, and Cnidoscolus quercifolius Abdou and Badran (2018) on Delonix regia, Kohan et al. (2000) and Abdou et al. (2014) on Populus nigra, Abd El-Aziz (2000) on Azadirachta indica, El-Mahrouk (2000) and Youssef et al. (2019) on Swietenia spp. Sayed (2001), Abdou et al. (2006) and Agera et al. (2019) on Khaya senegalensis, Abd El-Dayem (2003) on Taxodium distichum, Al-Menaie et al. (2010) on Cassia nodosa and Cassia fistula, Adejobi et al. (2015) on cocoa, Saravanakumar and Shanthinipriya (2017) on Populus deltoids. The increase in vegetative characteristics due to Chemical fertilizer NPK is in parallel with those obtained by Vasanthakrishna et al. (1994) on Casuarina equisetifolia and Sanginga et al. (1989) on Allocasuarina (A. torulosa and A. littoralis) and Casuarina equisetifolia since they found that the effect of phosphorous on seedling growth varies with plant species and whether nitrogen was derived by symbiotic fixation or given nitrate. The effects of nitrogen treatments (pollinating plants and plants) were fertilized with nitrogen) and phosphorous on the growth of C. equisetifolia significantly between 0 and 30 mg/kg of soil. Increasing the phosphorous level in the soil from 0 to 30 mg/kg led to an increase in the vegetative characteristics.

The increase in vegetative characteristics due to Minia Azotein fertilizer was deduced by Badran et al., (2003) on Acacia spp and Abo El-Wafa (2014) on Populus nigra mentioned that fertilizing Plants with Minia Azotein at a rate 50ml/pot improved its vegetative characteristics.

The mentioned results of vegetative characteristics concerning Frankia are coincided with those obtained by Saravanan et al. (2012) on Casurina equistifolia since fertilizing Casurina equistifolia plants with Frankia at a rate of 50 g/ seedling improved its vegetative characteristics. The results of bio-fertilizer with Frankia are in parallel with those obtained by Sanginga et al. (1989). In addition, seedlings of C. equisetifolia inoculated with Frankia had 7-10 times increase in the seedlings shoot (Karthieyan et al., 2012). They found that the inoculated Casuarina equisetifolia seedlings increased in vegetative growth parameters by between 90% -100% to the seedlings not inoculated with Frankia. The stimulating effect of bio-fertilizer with Frankia on vegetative growth parameters is due to its ability to convert atmospheric nitrogen into ammonia via the enzyme nitrogenase, a process known as nitrogen fixation. They do this while living in root nodules on actinic plants. Bacteria can provide most or all of the nitrogen requirements of the host plant. As a result, actinic plants colonize and often thrive in soils low in phytonutrients (Sanginga *et al.*, 1989). Similar trends were obtained by several authors like Diem *et al.* (1982), Vasanthakrishna *et al.* (1994) on *Casuarina equisetifolia*, Wheeler *et al.* (2000) on four species of casuarinas *C. cunninghamiana, C. equisetifolia, C. glauca, C. junghuniana*.

The increase in vegetative characteristics due to Mycorrhiza fertilizer was deduced by Abo EL-Leleil (2016) on *Swieteiana mahagony* mentioned that fertilizing Plants with Mycorrhiza at a rate of 150 spores improved its vegetative characteristics. The stimulating effect of bio-fertilizer with mycorrhizal on vegetative growth parameters may be due to the successful fungal symbiosis with the plant where the plant is a source of energy for the fungus in the form of fixed carbon, while the fungus gives nutrients to plants, the most important of which is phosphorous, which is present in the soil solution in very low concentrations as mentioned by Hayman (1983), Rajendran and Devaraj (2004) on *Casuarina equisetifolia*, Jihane *et al.* (2016) on *Casuarina spp*, Trappe (1982) on many important forest tree species, such as *liquidambar* (sweetgum), *fraxinus* (ash), *liriodendron* (poplar), Schmitz-zeitz (1995) on *Fagus sylvatica.* The results of biofertilizer with mycorrhizal fungi are parallel to those obtained by Karthieyan *et al.* (2012) and they found that the inoculated seedlings increased in vegetative characteristics by between 90%-100% over the non-inoculated seedlings.

Root growth characteristics

Root length (cm):

The data in Table (1) showed a significant increase in root length of Casuarina equisetifolia seedlings compared to control. The longest root length were recorded when mineral NPK fertilizer as a recommended dose (10:7.5:2.5g/pot) was used and attained the highly percentages of increases reached to 105.26 and 77.77 % over control in the first and second season respectively. Followed by treatment 75% of NPK with Frankia (50g/plant) which gave a significant increase in root length reached to 101.31 and 74.49 % over control in the first and second season respectively, then treatment of Mycorrhiza (50ml/pot) plus 75% NPK, which gave a significant increase in length reached to 83.94 and 72.22 % over control in the first and second season respectively. According to this data the differences between the highest three treatments had no significance.

Root fresh weight (g):

The data in Table (2) showed that the recommended dose (10:7.5:2.5g/pot) of 100% mineral NPK fertilizer T2 and Frankia (50g/pot) combined with 75% of mineral NPK fertilizer (7.5:3.75:1.875g) T8 followed by Mycorrhiza (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced The heaviest root fresh weight of seedlings of casuarina with non significant differences between them whereas Minia Azotein (50ml/pot) combined with 75% of mineral NPK fertilizer T6 ranked the fourth in this concern while the other treatments did not attain any significant variens in root fresh weight compared to control (00.0)T1.

Root dry weight (g):

The data in Table (1) showed a significant increase in root dry weight compared to the control. The heaviest weight of root dry weight was recorded when the mineral NPK fertilizer as a recommended dose was used and attained the high percentages of increases reached to 30.85 and 33.05 % over control in the first and second season respectively. Followed by treatment 75% of NPK with Frankia (50g/plant) which gave a significant increase in root dry weight reached to 29.88 and 31.33 % over control in the first and second season respectively, then treatment by Mycorrhiza (50ml/pot) plus 75% NPK, which gave a significant increase in root dry weight reached to 29.67 and 29.92 % over control in the first and second season respectively. According to this data, the differences between the highest three treatments had no significance.

The increase in root growth characteristics i.e. root length, root fresh and dry weight due to NPK fertilizer are in parallel with those obtained by Abo El-Wafa (2014) and Abdou *et al.* (2014) on *Populus nigra* who mentioned that fertilizing seedlings with NPK at a rate 4:2:2 g/ pot improved its root characteristics.

The stimulating effect of chemical fertilization on root growth characteristics may be due to the role of chemical fertilization in supplying plants with the nutrients they need for production more carbohydrate and protein necessary for the root growth of casuarina seedlings as mentioned by several authors like Vasanthakrishna *et al.* (1994) on *Casuarina equisetifolia* Jihane *et al.* (2016) on *Casuarina spp* Frosi *et al.* (2016) on *Poincianella pyrmidalis*, and *Cnidoscolus quercifolius* Abdou and Badran (2018) on *Delonix regia*, Kohan *et al.* (2000) and Abdou *et al.* (2014) on *Populus nigra*, Abd El-Aziz (2000) on *Azadirachta indica*, El-Mahrouk (2000) and Youssef *et al.* (2019) on *Swietenia* spp. Sayed (2001), Abdou *et al.* (2006) and Agera *et al.* (2019) on *Khaya senegalensis*, Abd El-Dayem (2003) on *Taxodium distichum*, Al-Menaie *et al.* (2010) on *Cassia nodosa* and *Cassia fistula*, Adejobi *et al.* (2015) on cocoa, Saravanakumar and Shanthinipriya (2017) on *Populus deltoids*.

The results of chemical fertilization are in parallel with those obtained by Vasanthakrishna *et al.* (1994) on *Casuarina equisetifolia*. Likewise, Sanginga *et al.* (1989) work on Allocasuarina (*A. torulosa* and *A. littoralis*) and *Casuarina equisetifolia* and found that the effect of phosphorous on seedling growth varies with plant species and whether nitrogen was derived by symbiotic fixation or given nitrate. The effects of nitrogen treatments (pollinating plants and plants) were fertilized with nitrogen) and phosphorous on the growth of *C. equisetifolia* significantly between 0 and 30 mg/kg of soil. Increasing the phosphorous level in the soil from 0 to 30 mg/kg led to an increase in the dry weight of the plant and the dry weight of the roots.

The increase in root characteristics due to Mycorrhiza fertilizer was deduced by Abo EL- Leleil (2016) on *Swieteiana mahagony* mentioned that fertilizing plants with Mycorrhiza at a rate (150spores) improved its root characteristics. The stimulating effect of bio-fertilizer with Mycorrhiza on root characteristics parameters It is due to the successful fungal symbiosis with the plant where the plant is a source of energy for the fungus in the form of fixed carbon, while the fungus gives nutrients to plants, the most important of which is phosphorous, which is present in the soil solution in very low concentrations (Hayman, 1983). Rajendran and Devaraj (2004) on *Casuarina equisetifolia*, Jihane *et al.* (2016) on *Casuarina spp*, Trappe (1982). on many important forest tree species, such as *liquidambar* (sweetgum), *fraxinus* (ash), and *liriodendron* (poplar), Schmitz-zeitz (1995). on *Fagus sylvatica* were in the same way.

The mentioned results of root characteristics concerning Frankia coincide with those obtained by Saravanan *et al.* (2012) on *Casurina aquistifolia* who revealed that fertilizing *Casurina equistifolia* plants with Frankia at a rate of 50 g/seedling improved its root characteristics. The results of bio-fertilizer with Frankia are in parallel with those obtained by Sanginga *et al.* (1989) who found that seedlings of *C. equisetifolia* inoculated with Frankia had 7-10 times the dry weight of un inoculated seedlings in terms of shoot and root dry weight. Karthieyan *et al.* (2012) found that the inoculated *Casuarina equisetifolia* seedlings increased in root characteristics parameters by between 90% -100% than the seedlings not inoculated with Frankia. The stimulating effect of bio-fertilizer with Frankia on root characteristics parameters may be due to its ability to convert atmospheric nitrogen into ammonia via the enzyme nitrogenase, a process known as nitrogen fixation. They do this while living in root nodules on actinic plants. Bacteria can provide most or all of the nitrogen requirements of the host plant. As a result, actinic plants colonize and often thrive in soils low in phytonutrients (Sanginga *et al.*, 1989). Our findings were supported by those which obtained by Diem *et al.* (1982), Vasanthakrishna *et al.* (1994) on *Casuarina equisetifolia*, Wheeler *et al.* (2000) on four species of casuarinas *C. cunninghamiana, C. equisetifolia, C. glauca, C. junghuniana*

Table 1. Effect of biofertilizers (Minia Azotein (MA) , Frankia and Vesicular Arbuscular Mycorrhiza (VAM) and chemical fertilizer NPK on Plant height (cm), Stem diameter (cm), No. of branches, Biomass fresh & dry weights (g) of *Casuarina aquisetifolia* seedlings in both seasons 2019 and 2020.

Treatments	Plant height (cm)		Stem diameter (cm)		No. of branches		Biomass fresh weight (g)		Biomass dry weight (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1	122.0	129.1	1.17	1.53	36.1	36.4	469.7	481.25	140.80	148.94
T2	180.3	195.3	2.27	2.33	49.7	51.4	695.8	717.04	258.80	273.75
T3	128.5	130.0	1.26	1.61	36.2	38.2	471.7	483.34	141.80	149.99
T4	133.7	130.5	1.37	1.77	36.3	40.3	472.8	484.48	142.30	150.52
T5	133.7	130.5	1.37	1.77	36.3	40.3	472.8	484.48	142.30	150.52
T6	165.5	180.3	1.93	2.18	44.4	47.2	607.5	624.95	212.70	224.99
T7	144.9	160.5	1.64	1.93	40.8	43.5	535.4	553.12	175.10	185.22
T8	179.3	195.0	2.20	2.30	48.9	50.3	682.8	703.48	252.00	266.56
T9	159.3	166.8	1.81	2.06	42.7	44.9	548.6	563.53	181.90	192.41
T10	174.6	189.0	2.18	2.26	48.3	49.6	673.0	693.26	246.80	261.06
T11	150.6	166.1	1.75	2.01	41.1	44.2	547.1	561.97	181.20	191.67
L.S.D. at 5%	5.7	6.5	0.12	0.11	1.4	1.8	51.5	53.5	16.8	18.2

T1=Control, T2=100 % NPK, T3= Minia Azotein at 50 ml/plant, T4= Frankia at 50 g /plant, T5= Mycorrhiza at 50 ml /plant, T6= 75 % NPK+ Minia Azotein, T7=50 % NPK+ Minia Azotein, T8=75 % NPK+ Frankia, T9=50 % NPK+ Frankia, T10=75 % NPK+ Mycorrhiza, T11=50 % NPK+ Mycorrhiza

Table 2. Effect of biofertilizers Minia Azotein (MA), Frankia, Vesicular Arbuscular Mycorrhiza (VAM), and chemical fertilizer NPK on root length(cm), root fresh and dry weights(g) of *Casuarina equisetifolia* seedlings in both seasons 2019 and 2020.

Treatments	Root length(cm)		Root fresh weight (g)		Root dry weight(g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
T1	38.00	39.6	247.22	248.00	137.14	137.42
T2	78.00	70.4	363.85	373.18	179.46	182.85
T3	38.30	45.2	254.00	255.15	139.60	140.01
T4	47.30	48.6	271.44	282.51	145.93	149.94
T5	38.50	46.2	260.17	270.14	141.84	145.45
T6	66.70	57.9	321.28	323.57	164.01	164.84
T7	48.90	50.0	283.37	284.76	150.25	150.76
T8	76.50	69.1	360.16	366.42	178.12	180.39
T9	57.50	53.1	309.38	311.31	159.69	160.39
T10	69.9	68.2	359.36	361.31	177.83	178.54
T11	49.20	52.8	297.57	299.06	155.41	155.95
L.S.D. at 5%	8.4	2.3	11.22	12.11	4.05	4.35

T1=Control T2=100 % NPK T3= Minia Azotein at 50 ml/plant T4= Frankia at 50 g /plant T5= Mycorrhiza at 50 ml /plant T6= 75 % NPK+ Minia Azotein T7=50 % NPK+ Minia Azotein T8=75 % NPK+ Frankia T9=50 % NPK+ Frankia T10=75 % NPK+ Mycorrhiza T11=50 % NPK+ Mycorrhiza

Mycorrhizal Fungi play a role in the process of plant growth as a result of their secretion of some organic compounds and the intertwining of their cells with the roots of the host, which helps to stabilize the soil granules, and increase its ability to retain water, which helps to increase agricultural production (Barrios, 2007; Smith and Read, 2008). VAM inoculation causes an increase in the vegetative characteristics and production of various crops, and it works to improve the properties of sandy soil and increase its content of nitrogen, phosphorous, and potassium. It also works to increase the amount of humus and thus expands its ability to retain water.

Frankia strains are nitrogen-fixing actinomycetes that were isolated for the first time in 1978. They form root nodules. The vesicles contain the enzyme nitrogenase, which is important in the process of atmospheric nitrogen fixation, which helps to solve many soil problems and increase reclaimed areas. (Fontaine *et al.*, 1986; Sayed *et al.*, 2000: Hahn *et al.*, 2003). Frankia inoculation causes an increase in the vegetative characteristics and production of various crops and it works to improve the properties of sandy soil and increase its content of nitrogen, phosphorous, and potassium. It also works to increase the amount of humus and thus expands its ability to retain water.

Chemical fertilizers enter most of the processes that enter the plant, where nitrogen enters the components of most organic compounds such as nucleic acids (RNA and DNA), amino acids, alkaloids, vitamins, and enzymes. Phosphorous is necessary for plants as it enters as one of the components of the cell nucleus and is of great importance in cell division and development of meristem cells and enters the formation of phospholipids nucleic acids and has an important role in the phosphorylation process that causes the production of energy compounds (ADP and ATP). Most of the physiological processes, such as carbohydrate formation, photosynthesis and other various processes within the plant. Potassium is necessary for most of the physiological processes that take place inside the plant, as it has an important role in the process of nitrogen metabolism, and has a role in the work of enzymes. On the osmotic pressure of cells Ingels (1994), Yagodin (1984), Mengel and Kirkby (1987) and Bhuiyan, *et al.* (2000). Mineral fertilization causes an increase in the chemical characteristics and production of various crops. The stimulating effect of chemical fertilization may be due to the role of chemicals in supplying the plants with their required nutrients for more carbohydrates and protein production which are necessary for the increase in total chlorophyll contents of *Casurina equistifolia* seedlings. Sanginga *et al.* (1989) on *Allocasuarina (A. torulosa and A. littoralis*) and *Casuarina equisetifolia.* Danso (1990) *on Casuarina equisetifolia.* and Vasanthakrishna *et al.* (1994) on *Casuarina equisetifolia.*

Finally, the results showed that all treated seedlings of casuarina with frankia, Vesicular Arbuscular Mycorrhiza (VAM) in combination with 75% NPK (T8 and T10) and or NPK, 100% recommended dose (T2) recorded significant increases in vegetative characteristics i.e. Plant height, Stem diameter, Number of branches, Biomass fresh and dry weights compared to the other treatments and control. The highest rooting values of root length, root fresh, and dry weights of casuarina plants were found with the abovementioned treatments followed by Minia Azotein (MA) combined with 75%NPK (T6) in both seasons. while the other treatments did not attain any significant variances in vegetative and root growth parameters. It can be concluded that Frankia 50g/plant, Vesicular Arbuscular Mycorrhiza (VAM) 50 ml/plant with 75% of the recommended dose (7.5:3:75.5:1.875g/plant)T6, T8, and/ or 100% of the recommended dose of NPK (10:5:2.5g/plant) T2 were the best in improving vegetative and root characteristics of *Casuarina equistifolia* seedlings.

CONCLUSION

From the results, it is recommended to fertilize *Casuarina equistifolia* seedlings grown in a pot of sandy soil with 7.5 g of nitrogen fertilizer (ammonium sulfate 20.5% N), 3.75 g of phosphate fertilizer (calcium superphosphate 15.5%) P_2O_5 and 1.875 g of potassium fertilizer (potassium sulfate 48 % K2O) with the addition of one of the biofertilizers (Frankia at 50 g / seedling or Mycorrhiza at 50 ml/seedling in order to decline 25% of chemical fertilizer and to improve the vegetative and root growth of *Casuarina equisetifolia* seedlings.

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تاثير الاسمدة الحيوية والمعدنية على النمو الخضري والجذري لشتلات *Casuarina equisetifolia* النامية في الاراضي الرملية.

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[1]قسم البساتين بالكلية. الزراعة ، جامعة بنها ، مصر

[2]قسم الاراضي بالكلية. الزراعة ، جامعة بنها ، مصر

أجريت هذه الدراسة في مزرعة ومختبرات قسم البساتين بكلية الزراعة بمشتهر ، جامعة بنها ، خلال موسمين متتاليين لعامي 2019 و 2020 على شتلات الكازورينا تمت الدراسة من خلال استخدام بعض الاسمدة الحيوية والأسمدة الكيماوية NPK بهدف تقييم فاعلية المنيا آزوتين MA والفرانكيا وفطريات الميكورايزا الجذرية (VAM) كلا بمفردها او بالتداخل بينهم على الصفات الخضرية والجذرية لشتلات الكازورينا المزروعة في تربة رملية . أظهرت النتائج زيادة في الصفات الخضرية محل الدراسة وهي ارتفاع الشتلة وسمكها وعدد فروعها وكتلتها الحيوية الطازجة والجافة وكذلك الصفات الجذرية وهي طول الجذر ووزنه الطازج والجاف مقارنة بالشتلات الغير معاملة خلال موسمي التجربة حيث أعطت الشتلات المسمدة بالجرعة الموصى بها من السماد الكيماوي NPK بمعدل (2.5 :5 :10 / جم / اصيص) أعلى قيمة في الصفات المذكورة أعلاها تلتها الشتلات المخصبة بالفرانكيا بمعدل(50 جم / للأصيص) بالتداخل مع 75٪ من الجرعة الموصى بها من السمادي الكيماوي (1.875 :3.75 :7.5) كما سجلت الشتلات المخصبة بالميكورايزا بمعدل 50 مل / اصيص + 75٪ من الجرعة الموصى من NPK القيمة التالية بدون اي فروق معنوية بين المعاملا ت المذكورة اعلاها وتم الحصول على أقل القيم بواسطة الشتلة الغير معاملة باي من الاسمدة الكيماوية او الحيوية . وعليه يمكن التوصية بتسميد شتلات الكازوارينا المنزرعة بالتربة الرملية بالتخصيب بالفرانكيا بمعدل(50جرام / اصيص) + 75٪ من الجرعة الموصى بها من NPK (1.875 :3.75 :7.5 جرام) اوبالتخصيب بالميكورايزا بمعدل(50 مل/ اصيص) + 75٪ من الجرعة الموصى منNPK لتحسين الصفات الخضرية والجذرية لشتلات الكازورينا (*Casuarina equistifolia*) .