Journal of Plant Production

Journal homepage & Available online at: www.jpp.journals.ekb.eg

Impact of Biofertilizers and Chemical Fertilizers on some Chemical Characteristics of *Casuarina equisetifolia* Seedlings and Sandy Soil Macronutrient's Content

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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 , uring 2019 and 2020 successive growing seasons. The study was carried out to define the efficiency of various biofertilizers; Minia Azotein, Frankia and Visicular 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 to improving some chemical characteristics of *Casuarina equistifolia* seedlings grown in sandy soil and improving its macronutrient's content N, P and K. Results showed that all treated seedlings of casuarina with Frankia 50g/ plant, and Visicular Arbuscular Mycorrhiza 50 ml/plant in combination with 75% NPK T8 and T10 and or NPK 100% recommended dose (10:5:2.5g/plant) recorded significant increases in total chlorophylls a,b, carotenoids, N, P , K and carbohydrate percentage compared to the other treatments and control. The highest macronutrient content N, P and K values were found with the abovementioned treatments followed by Minia Azotein combined with 75%NPK in both seasons while the other treatments did not attain any significant variens in soil's macronutrient content N, P and K and chemical characteristics parameters. It can be concluded that Frankia 50g/plant , Visicular Arbuscular Mycorrhiza 50 ml/plant with 75% of recommended dose (7.5:3:75:1.875g/plant) and/ or 100% of recommended dose of NPK (10:5:2.5g/plant) were the best in improving soil's macronutrient and chemical characteristics of *Casuarina equistifolia* seedlings.

Keywords: *Casuarina equisetifolia*, Minia Azotein (M.A) , Frankia, Visicular Arbuscular Mycorrhiza (VAM) , Chemical fertilizer NPK, Soil's macronutrient, Chemical characteristics.

INTRODUCTION

Casuarina equistifolia belongs to the Family Casuarinaceae which includes four genera (*Allocasuarina*, *Casuarina*, *Ceuthostoma*, and *Gymnostoma*) and 96 species. native to Australia, Southeast Asia, and the Pacific Islands. They are drought and salinity resistant and grow- swiftly (Karthikeya, 2016). It is well-known for its capacity to withstand salt stress. it can form symbiotic relationships with Visicular Arbuscular Mycorrhiza (VAM) and soil bacteria Frankia (Pape *et al.*, 2022). Casuarina species that are monoecious and dioecious have been identified male flowers are gathered in rings among grey scales, while spherical and lateral female flowers are clustered in light brown clusters1,3. the breeze pollinates the female flowers. (Pape *et al.*, 2020). *Casuarina equistifolia* trees reach a height of 10-25 m for 10-12 years (Balasubramanian 2001). Very susceptible to cold and is one of the preferred trees in coastal areas (Liu *et al.*, (2014). *Casuarina equisetifolia* trees planted on the shores have helped reduce their erosion and block the salty sea water spray, as well as reducing the harmful effects of dust storms and dry winds in some arid areas. (Xin Yang *et al.*, 2022). *Casuarina equisetifolia* seeds can germinate up to 300 mM NaCl while their seedlings inoculated with Frankia Ceq1 strain can form root nodules, grow and survive up to 500 mM NaCl due to the Frankia's tolerance to extreme salinity can be used for revegetation in saline areas (Chauchan, and Pokhriyal, 2020). The lignin composition of the Syringyl and Guaiacyl rings and cellulose and hemicellulose in high proportions helped them be used well in the paper industry. (Akash *et al.*, 2021). Its wood has a high density of 83.0 g / cm3, which is difficult to use in industry for cracking and warping, and is good as fuel, yielding 500 kcal/kg. (Ashok *et al.*, 2020; Hasan and Talal, 2020). it has traditionally been used to treat neurological problems, acne, sore throats, stomach ulcers, constipation, cough, diabetes, and dysentery (Vain *et al.*, 2022)

Biofertilizers such as Minia Azotein are from low-cost sources that do not pollute the environment compared to chemical fertilizers. Biological fertilizers contain microorganisms, which help in the growth of crops through different mechanisms, as they work on the biological fixation of nitrogen, which promotes growth or the addition of enzymatic or hormonal substances that help to increase soil nutrients (Galal and Aly, 2004).

The plant becomes infected with Frankia by penetrating the root of the hair in the meristem area and infection strands are formed to fill the cells of the cortex. Root nodules are formed, which, taking on an increase in size, fix nitrogen depending on the organic carbon contained in the plant cells and on the enzyme nitrogen contained within them (Karthieyan *et al.*, 2012, Marie Claver, *et al.*, 2020, Karthikeyan, 2016). N fixation by Actinomycetes Frankia ranges from 15 to 80% which helps to increase soil nutrients from nitrogen (He and

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DOI: 10.21608/jpp.2022.141660.1119

Critchley, 2008). Frankia infective efficacy is influenced by *host habitat, ambient conditions*, and related plant species that Frankia is associated with Casuarina *equisetifolia* and can supply More than 300 kg N/ha/year, (Marie Clavier *et al.*, 2020). The survival of casuarina trees in challenging regions is determined by their association with atmospheric nitrogen-fixing Frankia, (Sayed, 2011). Frankia fixes atmospheric nitrogen essential for all plant metabolism and growth activities (Shantharam and Mattoo, 1997). Frankia infection depends on the habitat of the host, the prevailing environmental conditions, and the types of companion plants. External symbionts of the external root, internal root, and Frankia can occur in the same rootstock of the plant (Wang and Qiu, 2006).

Visicular Arbuscular Mycorrhiza (VAM) can act as a biofertilizer by increasing plant uptake of phosphate ions, as well as N, P, K, Mg, and many micro-nutrients, thereby increasing plant yield and stimulating growth (Smith, and Reed, 1997; Veresoglou *et al.*, 2011). As a result, Moghith *et al.*, (2021) concluded that plants inoculated with mycorrhizal fungus had the most significant improvements in all characteristics of vegetative growth and seed yield compared to non-inoculated (control) plants of (*Salvia hispanica L*). The fungus absorbs phosphorous through a variety of mechanisms, including the secretion of the 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; Nathalie *et al.*, 2020).

Mineral NPK fertilizer aids the vegetative growth of tree seedlings as well as the development of roots, flowers, seeds, and fruits. Nitrogen is required for the metabolism, inheritance, reproduction, and development of amino acids, proteins, alkaloids, co-enzymes, and a variety of vitamins (James and Michael, 2009). macronutrients N, P, K and some micronutrients boron, iron, copper and zinc stimulate tree growth (Veresoglou *et al.*, 2011; Smith *et al.*, 2011 and Van der Heijden *et al.*, 2008) The use of chemical fertilizers leads to an increase in agricultural production by 35 - 77% in the field of agriculture (Fageria and Baligar, 2005). However, it sometimes leads to a decrease in soil fertility, because of its increased use (Zargar *et al.*, 2020). The use inorganic fertilizers such as urea and triple superphosphate helped to increase the vegetative characteristics of *Casuarina equisetifolia* seedlings because of their influence on the processes inside the seedling and their role in the photosynthesis process (Bhuiyan, *et al.*, (2000) . Nitrogen has an important role in the formation of nucleic acids, proteins, enzymes, chlorophyll, vitamins, and nitrogenous organic compounds involved in the vital processes within the plant (Hayashi. and Chino, 1985). Phosphorous is included in the synthesis of nucleic acids, DNA, RNA, photosynthesis, respiration, and the formation of phosphorous compounds and cell membranes. It reduces the harmful effect of increasing nitrogen in the soil (Hina *et al.* 2018). Potassium is important in the formation and functioning of enzymes and the opening and closing of stomata, its deficiency leads to the accumulation of chlorine. (Mirza *et al.* 2018). Chemical fertilizers require a large amount of energy to produce, but their use shows many damages to plants, soil, humans, animals and the environment, for example, the toxic pyrite in nitrogen fertilizers, the problems resulting from the volatilization of urea to the eyes and respiratory system, and the pollution resulting from the process of manufacturing phosphate fertilizers and the process of interaction of phosphate rock with acid Sulfuric and harmful HF emission as well as the harmful effect of accumulation of heavy metals such as cadmium, chromium, lead, arsenic, (Nikita and Puneet, 2020; Masindiand,2018).

MATERIALS AND METHODS

This study was carried out at the Expermental Farm and Laboratory of Horticulture Department, Faculty of Agriculture , Moshtohor , Benha University, Egypt, during two successive seasons of 2019 and 2020 on *Casuarina equistifolia* seedling to evaluate the effectiveness of biofertilizer; Minia Azotein(MA), Frankia and visicular Arbuscular Mycorrhiza (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 soil's macronutrient content N, P and K and some chemical characteristics i.e. Photosynthetic pigments (chlorophyll a,b, carotenoids), Nitrogen, phosphorus, potassium and carbohydrates *Casuarina equistifolia* seedling grown in sandy soil . seedlings of one year old an average height of 10-15 cm diameter 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
DTPA Fe (mg/kg)	10.10	9.80
DTPA Mn (mg/kg)	8.50	8.30
DTPA Cu (mg/kg)	1.10	0.90
DTPA 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), which 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 Visicular Arbuscular Mycorrhzia VAM, three times in 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 dissecting 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-Visicular ArbuscularMycorrhizal (VAM) were 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 intervals prior to each biofertilizer treatment. Each pot was received (10:5:2.5g) as recommended dose of NPK and (7.5:3.75:1.875g) as 75% of the recommended dose of NPK and (5:2.5:1.2g) as 50% of the 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- Visicular Arbuscular Mycorrhiza 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 in this respect.

At the end of each season on November 1st in both seasons, the data were recorded on soil's macronutrient content N, P and K and some chemical characteristics i.e. total chlorophylls a,b, carotenoids (mg/g F.W.), Nitrogen (N%), phosphorus(P%), potassium(K%) and total carbohydrate (%).

Recorded data

Chemical characteristics parameters (Photosynthetic pigments (chlorophyll a,b , carotenoids) , percentage of carbohydrates, and Soil analysis after harvesting

- Nitrogen (%) was determined according to the modified Micro kjeldahel method as described by (Wilde *et al.* 1985).
- Phosphorus (%) was determined colorimetrically by the spectrophotometer at wave length of 650 µm according to the method of (Chapman and Pratt 1975).
- Potassium (%) was determined using Flame–photometry method according to (Cottenie *et al.* 1982).
- Total chlorophylls contents: Total chlorophylls content (mg/100g f.w.) was determined in fresh leaves according to (Moran, 1982).
- Total carbohydrates content: Total carbohydrates content was determined in dry powder material according to (Herbert *et al.*, 1971).
- Soil analysis after harvesting according to (Okalebo *et al.*, 2002).

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 (Snedecor and Cochran, 1989) using MSTAT-C statistical software package(1986).

RESULTS AND DISCUSSION

Chemical composition determinations:

Photosynthetic pigments (mg/g. F,W,)

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 Mycorrhizae (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced the highest contents of chlorophyll a, b and carotenoids 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 variances in Photosynthetic pigments i.e. chlorophyll a, b and carotenoids compared to control (00.0)T1.

Table 1. Effect of biofertilizers (Minia Azotein (MA), Frankia and Visicular Arbuscular Mycorrhiza (VAM) and chemical fertilizer NPK on total chlorophylls a,b, carotenoids content (mg/g F.W.) of *Casuarina aquisetifolia* seedlings in both seasons 2019 and 2020 .

Treatments	chlorophyll a content (mg/g F.W.)		chlorophyll b content (mg/g F.W.)		carotenoids content (mg/g F.W.)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
T1	1.987	2.270	0.756	0.780	0.907	0.945
T2	2.451	2.463	0.823	0.847	0.986	1.026
T3	2.253	2.306	0.768	0.792	0.922	0.960
T4	2.301	2.387	0.780	0.816	0.940	0.971
T5	2.289	2.319	0.772	0.796	0.926	0.965
T6	2.417	2.436	0.811	0.836	0.973	1.014
T7	2.357	2.376	0.791	0.820	0.949	0.989
T8	2.444	2.461	0.820	0.845	0.984	1.025
T9	2.402	2.421	0.806	0.831	0.967	1.008
T10	2.439	2.451	0.818	0.843	0.980	1.022
T11	2.384	2.403	0.800	0.825	0.960	1.000
L.S.D. at 5%	.012	.001	.005	.001	0.006	0,005

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

Nitrogen, phosphorus and potassium percentages:

Nitrogen percentage (N%):

The data in Table (2) showed a significant increase in nitrogen percentage of *Casurina equistifolia* seedlings compared to control. The highest concentration of nitrogen was recorded when the mineral NPK fertilizer as a recommended dose and attained the high percentages of nitrogen increases reached to 38.39 and 36.59 % 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 nitrogen percentage reached to 38.24 and 36.45 % over control in the first and second season respectively, then treatment Mycorrhiza (50ml/pot) plus 75% NPK, which gave a significant increase in nitrogen percentage reached to 37.76 and 36.07 % over control in the first and second season respectively. According to this data the differences between the highest three treatments had no significant.

Phosporous percentage (P):

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 Mycorrhizae (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced The highest percentages of phosporous 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 variance in percentages of phosporous compared to control (00.0)T1.

Potassium percentage (K):

The data in Table (2) showed a significant increase in potassium percentage of *Casurina equistifolia* seedlings compared to control. The highest concentration of nitrogen was recorded when the mineral NPK fertilizer as a recommended dose and attained the high percentages of increases reached to 38.39 and 36.59 % over control in the first and second season respectively. Followed by treatment of 75% of NPK with Frankia (50g/plant) which gave a significant increase in potassium percentage reached 38.24 and 36.45 % over control in the first and second season respectively, then treatment Mycorrhiza (50ml/pot) plus 75% NPK, which gave a significant increase in potassium percentage reached to 37.76 and 36.07 % over control in the first and second season respectively. According to this data the differences between the highest three treatments had no significance.

The stimulatory effect of Mycorrhiza on percentages of nitrogen, phosphorus, potassium may be due to it causes an increase in soil phosphorous that is important in cell growth and leads to an increase in percentages of carbohydrates (Trappe, 1982). The increase in percentages of nitrogen, phosphorus, potassium due to Mycorrhiza fertilizer was deduced by Abo EL- Leleil (2016) on *Swieteiana mahagony* mentioned that fertilizing Plants with Mycorrhiza improved its carbohydrates content

The inoculation with Frankia leads to an increase in percentages of nitrogen, phosphorus, potassium which play an important role in physiological processes inside plant leads to an increase in photosynthetic pigments. The mentioned results of percentages of N, P and K due to Frankia have coincided with those obtained by Saravanan *et al.*, (2012). On *Casurina aquistifolia* revealed that fertilizing *Casurina equistifolia* plants with Frankia at a rate of 50 g/ seedling improved its percentages of N, P and K.

The stimulatory effect of mineral NPK on percentages of N, P and K may be due to the role of N, P and K and cytokinins, as well as, enzymes, produced by them, thus helping to produce more photosynthetic pigments and delaying leaf senescence improved leads to an increase in percentages of N, P and K.The increase in percentages of N, P and K due to Chemical fertilizer NPK are in parallel with those obtained by Abo El-Wafa (2014) and Abdou *et al.*, (2014) on *Populus nigra* mentioned that fertilizing seedlng with NPK at a rate 4:2:2 g/ pot improved its content of chlorophylls and are in parallel with those obtained by Sanginga *et al.*, (1989) on (*All casuarina (A. torulosa and A. littoralis*) and *Casuarina equisetifolia*

Table 2. Effect of biofertilizers (Minia Azotein (MA), Frankia and Visicular Arbuscular Mycorrhiza (VAM) and chemical fertilizer NPK on nitrogen, phosphorus, potassium, carbohydrates percentages of *Casuarina aquisetifolia* seedlings in both seasons 2019 and 2020

Treatments	N %		P %		K %		Carbohydrates %	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
T1	2.089	2.115	0.246	0.271	1.608	1.658	10.72	12.88
T2	2.891	2.889	0.459	0.505	2.503	2.561	23.52	23.76
T3	2.257	2.285	0.277	0.305	1.777	1.832	12.96	12.96
T4	2.522	2.553	0.324	0.356	2.151	2.218	16.88	16.08
T5	2.415	2.445	0.283	0.311	1.89	1.949	14.72	13.04
T6	2.777	2.811	0.411	0.452	2.444	2.443	20.16	19.52
T7	2.666	2.612	0.326	0.357	2.172	2.240	16.96	16.64
T8	2.888	2.886	0.434	0.477	2.488	2.546	22.80	23.12
T9	2.757	2.791	0.367	0.404	2.369	2.391	18.32	18.16
T10	2.878	2.878	0.423	0.465	2.474	2.529	22.08	22.64
T11	2.739	2.773	0.361	0.397	2.319	2.318	18.00	16.88
(L.S.D.) at 5 %	.019	.002	.041	.045	.038	.043	1.44	1.12

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

Carbohydrates percentage

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 Mycorrhizae (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced The highest percentages of carbohydrates 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 variance in percentages of carbohydrates compared to control (00.0) T1.

The stimulatory effect of Mycorrhiza on percentages of carbohydrates may be due to it causes an increase in soil phosphorous that is important in cell growth and leads to an increase in percentages of carbohydrates (Trappe, 1982). The increase in percentages of N, P and K due to Mycorrhiza fertilizer was deduced by Abo EL- Leleil (2016) on *Swieteiana mahagony* mentioned that fertilizing Plants with Mycorrhiza improved its content of carbohydrate .The increase in carbohydrates content due to Mycorrhiza fertilizer was deduced by Abo EL- Leleil (2016) on *Swieteiana mahagony* mentioned that fertilizing Plants with Mycorrhiza improved its carbohydrates content.

The inoculation with Frankia leads to an increase in soil nutrients such as nitrogen which plays an important role in physiological processes inside plant leads to an increase in carbohydrates content. The increase in carbohydrates content due to Frankia are coincided with those obtained by Saravanan *et al.*, (2012). On *Casuarina equisetifolia* revealed that fertilizing *Casuarina equisetifolia* plants with Frankia at a rate of 50 g/ seedling improved its carbohydrates content

The stimulatory effect of mineral NPK on percentages of carbohydrates may be due to the role of N, P and K and cytokinins, as well as, enzymes, produced by them, thus helping to produce more photosynthetic pigments and delaying leaf senescence. The increase in carbohydrates content due to Chemical fertilizer NPK are in parallel with those obtained Badran *et al.* (2003) on *Acacia* spp. and Abo El-Wafa (2014) on *Populus nigra* and are in parallel with those obtained Danso. (1990). on *Casuarina equisetifolia.*

Effect of chemical and biofertilizers on sandy soil after planting

Total N, P and K content in soil:

Nitrogen percentage (N) in soil:

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 Mycorrhizae (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced the highest contents of nitrogen in soil 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 contents of nitrogen compared to control (00.0) T1.

Phosphorous content (mg/kg) in soil:

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 Mycorrhizae (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced the highest contents of phosphorous in soil 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 contents of phosphorous compared to control (00.0) T1.

Potassium content (mg/kg) in soil:

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 Mycorrhizae (50ml/pot) combined with 75% of mineral NPK fertilizer T10 produced the highest contents of potassium 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 contents of potassium compared to control (00.0)T1.

The effect of mineral NPK on the contents of phosphorous , potassium and nitrogen percentage may be due to direct addition of these elements to the soil. The increase in the contents of nitrogen, phosphorous, and potassium in sandy soil was inferred due to treatment with NPK mineral fertilizer and biofertilizer treatment (Senjobi, 2012 and Swaefy, *et al.*, 2007) on sandy soil.

The effect of mycorrhizal fungi on the contents of phosphorous , potassium and nitrogen percentage may be due 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) Barrios, 2007; Smith and Read., 2008) The results obtained of increase in contents of nitrogen, phosphorous, and potassium in sandy soil are in parallel with those obtained Djajadi, and Nurul (2011) and Bryan, *et al.* (2015) on sandy soil.

The effect Frankia of on the contents of soil of phosphorous, potassium and nitrogen percentage, may be due to containing 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)

Table 2. Effect of biofertilizers (Minia Azotein (MA), Frankia and Visicular Arbuscular Mycorrhiza (VMF) each alone or combined with two doses of chemical fertilizer NPK at 75% and 50% from the recommended dose(10:7.5:2.5g/pot) and/or the full does of 100% NPK on nitrogen, phosphorus, potassium, carbohydrates percentages of *Casuarina aquisetifolia* seedlings in both seasons 2019 and 2020.

Treatments	N %		P (mg/kg)		K (mg/kg)	
	Agric. Seasons		Agric. Seasons		Agric. Seasons	
	1st	2nd	1st	2nd	1st	2nd
T1	0.002	0.002	6	7	98	113
T2	0.11	0.10	12	11	243	254
T3	0.002	0.002	7	6	110	100
T4	0.002	0.002	6	7	116	108
T5	0.002	0.002	7	6	109	114
T6	0.10	0.09	8	7	204	195
T7	0.09	0.10	8	7	136	129
T8	0.09	0.10	9	8	189	201
T9	0.08	0.09	9	7	140	145
T10	0.08	0.07	9	9	197	200
T11	0.08	0.07	8	9	137	146

The use of both chemical and biofertilizers led to an increase in the chemical composition of casuarina seedlings, as it succeeded in improving the content of different plant pigments and ratios of nitrogen, phosphorous, potassium and total carbohydrates content, and increase in the total content of phenols compared to untreated plants, as well as an increase in the content of the soil from nitrogen, phosphorous and potassium.

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 chemical 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 chemical 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.

The research aims to study effect of treating sandy soil with some chemical and biofertilizers and the interaction between them to show its effect on its natural properties and its content of basic nutrients from phosphorous, nitrogen and potassium, and to show the effect of those treatments on the chemical characteristics of *Casuarina equisetifolia* seedlings grown in it, which is total content of chlorophyll, nitrogen, phosphorous, potassium and Carbohydrates.

Chemical fertilizers enter into 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 stimulated effect of chemical fertilization may be due to the role of chemical on supplying the plants with their required nutrients for more carbohydrates and proteins production which are necessary for increase in total chlorophylls contents of *Casurina equistifolia* seedlings .Sanginga *et al.* (1989) on Allocasuarina *(A. torulosa and A. littoralis*) and *Casuarina equisetifolia* Danso (1990). *on Casuarina equisetifolia.* Vasanthakrishna *et al.* (1994) on *Casuarina equisetifolia*

The role of N2-fixing bacteria has been elucidated by some researchers (Rashed (2017), Ganaw (2017) and Soliman (2019). They found a role for N2-fixing bacteria in: Its role in fixing nitrogen from the atmosphere, whether symbiotic or non-symbiotic, by a process called Nitrogen fixation, in addition to its production of some photosynthetic hormones such as cytokinin, indole acid and gibberellins, and helps to enhance the absorption of other nutrients such as phosphorous which helps to increase growth in the plant and secretes some antibiotics that reduce some pathogens. Biofertilizers causes an increase in the chemical 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

Finally, the results showed that the highest values of nitrogen, phosphorous and potassium were obtained in the soil and the highest values of the three elements mentioned in the seedling in addition to the total content of chlorophyll and carbohydrates percentage when treated with the recommended amount of chemical fertilizer NPK (treated T2). followed by T9: the combined of Frankia at 50 g /pot and 75 % of recommended dose of NPK (7.5:3.75:1.825). Meanwhile T11: the combined of Mycorrhiza at 50 ml /pot and 75 % of the recommended dose of NPK ranked the third values in parameters mentioned. the least values were obtained by cotrol (0.0) T1.

CONCLUSION

From the results, it is recommended to fertilize *Casuarina equistifolia* seedling grown in 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 % K_2O) with 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 to improve the chemical properties of *Casuarina equisetifolia* seedlings, and increase its chemical content, nitrogen, phosphorous, potassium, and carbohydrates percentage , improve the natural properties of sandy soils and increase their content of nutrients and production of various crops and 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

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تاثير الاسمدة الحيوية والاسمدة الكيماوية على بعض الصفات الكمياوية لشتلات *Casuarina equisetifolia* ومحتوي التربة الرملية النامية بداخلها من المغذيات

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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 *Casuarina equisetifolia.*تمت الدراسة من خلال استخدام بعض الاسمدة الحيوية والأسمدة الكيماوية NPKبهدف تقييم فاعلية المنيا آزوتين MA والفرانكيا وفطريات الميكورايزا الجذرية (VAM) كلا بمفردها او بالتداخل بينهم على الصفات الكيماوية لشتلات *Casuarina equistifolia* من الكلوروفيلات أ ، ب ، الكاروتينات وإجمالي كلا من النيتروجين و الفوسفور و البوتاسيوم و الكربوهيدرات المزروعة في تربة رملية واثره علي محتوي التربة من العناصر المغذيات من النيتروجين والفسفور والبوتاسيوم . أظهرت النتائج الصفات الكيماوية محل الدراسة المذكورة اعلاه حيث اعطت الشتلات المخصبة بالجرعة الموصى بها من NPK (2.5 :5 :10 / جم / اصيص) أعلى قيم تلتها الشتلات المعاملة بمزيج من الفرانكيا بمعدل(50جرام / اصيص) و 75٪ من الجرعة الموصى بها من NPK و احتلت الشتلات المعاملة بالميكورايزا عند (50 مل / اصيص)+ 75٪ من الجرعة الموصى من NPK القيمة الثالثة في المعاملا ت المذكورة وتم الحصول على أقل القيم بواسطة الشتلات الغير معاملة باي من الاسمدة الكيماوية او الحيوية مع زيادة في نسب (النيتروجين والفوسفور والبوتاسيوم) في التربة الرملية بعد الزراعة مقارنة بالتربة الرملية قبل التسميد والزرعة خلال موسمي التجربة حيث أعطت التربة المخصبة بالجرعة الموصى بها بمعدل (2.5 :5 :10 / جم / وعاء) من السماد الكيماوي NPK أعلى تركيز من العناصر الثلاثة الرئيسية المذكورة أعلاه وتلتها التربة المعاملة بالفرانكيا بمعدل(50 جم / اصيص) + 75٪ من الجرعة الموصى به االسمادي الكيماوي في الوقت نفسه احتلت التربة المعاملة بالميكورايزا بمعدل (50 مل / اصيص) +75٪ من الجرعة الموصى من NPK القيمة الثالثة في المعاملا ت المذكورة وتم الحصول على أقل القيم بواسطة التربة الغير معاملة باي من الاسمدة الكيماوية او الحيوية وعليه يمكن التوصية بمعاملة شتلات الكازوارينا المنزرعة بالتربة الرملية بالتخصيب بالفرانكيا بمعدل (50جرام / وعاء) + 75٪ من الجرعة الموصى بها من NPK او بالتخصيب بالميكورايزا بمعدل (50 مل / اصيص) + 75٪ من الجرعة الموصى منNPK لتحسين المكونات الكيميائية من الكلوروفيل و النيتروجين و الفوسفور والبوتاسيوم و الكربوهيدرات لشتلات الكازورينا (*Casuarina equistifolia*) وتحسين خواص التربة الرملية وزيادة تماسكها واحتفاظها بالماء وزيادة محتوها من المغذيات الكبري من النيتروجين والفسفور والبوتاسيوم .