

EFFECT OF BIOGEIN, RHIZOBACTERIEN AND VIVA ROSE FERTILIZATION ON GROWTH AND CHEMICAL COMPOSITION OF *Cupressus sempervirens*, L. SEEDLINGS.

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Abstract

In this study, seedlings one year old of *Cupressus sempervirens*, planted in the nursery of Woody Trees Res. Dept., Hort. Res. Inst., Giza, Egypt. For two successive years (2011 and 2012) to investigate the best bio-fertilization treatments and the suitable doses on growth of *Cupressus* by "Biogein or Rhizobacterien at the rates of 2.50, 5.00 and 7.50 g/ bag as well as chemical fertilizer with "Viva Rose" as a foliar spray at the rates of 1.00, 2.00 & 4.00 g/l .Seedlings were grown in 14 cm diameter black polyethylene bag filled with a mixture of sand plus loam (1:1, v. /v.) were used in this investigation . Results indicated that plant height and stem diameter, showed a significant increased by adding Biogien at the rate 2.50g/bag, compared to control. Spraying the seedlings with "Viva Rose" at the rate of 4.00ppm induced utmost means of number of branches and leaves /plant, compared with the control and other treatments. While untreated plants gave the longest of root length /plant. Combination treatment "Biogein plus Rhizobacterien at the rate 2.50 plus 1.00ppm "Viva Rose" recorded high increases of fresh and dry weights of aerial parts "stem and leaves". However, fresh and dry weights of roots were increased significantly by addition Biogien at the rate of 5.00g/bag and 1.00ppm of "Viva Rose". Chemical compositions were highly variance in response to all fertilizer treatments compared with the untreated plants. Biogien at the rate of 2.50g/bag level increased significantly chlorophyll a, total chlorophyll" a +b", N% and nutrient uptake. Meanwhile, Rhizobacterien at the rate of 2.50g/bag elevated contents of leaves from chlorophyll b, carotenoids, and total indoles. Total phenols increased by "Viva Rose" at the rate of 4.00ppm, but spraying "Viva Rose" at the rate of 2.00 ppm increased P%. While, P, K % were highly increased at the rate of 5.00g/bag Rhizobacterien.

From the obtained results it could be recommended, that the addition of Biogien at 2.5 g/bags to *Cupressus sempervirens* seedlings (grown in 14 cm diameter bags thrice with one month interval from "March to May" during the growing season) resulted in the best and strong seedlings, besides to short the time in nursery in order to realization the coast of seedlings producing and to save our environment.

Keywords: Bio-fertilizer, "Biogein- Rhizobacterien", Foliar Fertilization, "Viva Rose", Vegetative growth, Chemical composition, *Cupressus sempervirens*.

INTRODUCTION

Italian Cypress Tree -*Cupressus sempervirens*, L. family Cupressaceae is also sometimes known as the Mediterranean Cypress or the Tuscan Cypress. No other tree epitomises the Tuscan landscape as this tall, narrow coniferous tree. Its pencil shape is often used in Italian garden design and in formal gardens around the world. *Cupressus sempervirens* is a beautiful evergreen upright conifer that is easy to grow in the UK climate, it is hardy and tolerates all conditions, both as a specimen tree in its own right or if a group are grown alongside each other it makes for an extremely effective, yet very elegant evergreen screen. The vast majority of the trees in cultivation are selected cultivars with a fastigate crown, with erect branches forming a narrow to very narrow crown often less than a tenth as wide as the tree is tall. The dark green 'exclamation mark' shape of these trees is a highly characteristic signature of Mediterranean towns and village landscapes. Formerly, the species was sometimes separated into two varieties, the wild *C. sempervirens* var. *sempervirens* (syn. var. *horizontalis*), and the fastigate *C. s.* var. *pyramidalis* (syn. var. *fastigiata*, var. *stricta*), but the latter is now only distinguished as a Cultivar Group, with no botanical significance. It is also known for its very durable, scented wood, used most famously for the doors of St. Peter's Basilica in the Vatican City, Rome. Cypress used to be used in distilleries as staves to hold mash ferments to make alcohol before the invention of stainless steel. Commonly seen throughout New Mexico, the Mediterranean Cypress is also known as the "drama tree" because of its tendency to bend with even the slightest of breezes. In cosmetics, it is used as an astringent, firming, antiseborrheic, antidandruff, antiaging and as fragrance, according to Bailey (1976) and Carrasco (2009). Subba Rao (1993) affirmed that *Azotobacter chroococcum* "which is found in Biogien fertilizer" synthesizes antifungal antibiotics that gives it a special advantage for use in field production. El-Sayed *et al.*, (2007) on *Ficus binnendijkii*, L. 'Amstel King' plants indicated that, using Biogien at the rate of 10 g/plant improve all plant characteristics studied (plant height, stem diameter, number of leaves and branches/plant, fresh and dry weights of leaves, stems and roots, and chlorophyll a, b and carotenoids content, and N, P and K percentages of the leaves). Moreover, treating the plants with Biogien at the rate of 5 g/plant or rizobacterene at the rate of 10 g/plant revealed also great influence on plant parameters in both seasons. Abdel-Fattah *et al.*, (2009a) indicated that (bio-fertilizer as a soil drench at the rate of 10 ml/l plus spraying it at the rate of 5ml/l.) significantly improved all vegetative and root growth parameters, of *Dracaena marginata* "Bicolor" Lam. and *Ruscus hypoglossum*.

So this investigation aims to limit the best type and suitable dose of some bio-fertilizer "Biogien or Rizobacterien" or "Viva Rose" as a foliar spray to realize the high quality of *Cupressus sempervirens* seedlings growth.

MATERIALS AND METHODS

This investigation was carried out on Woody Trees nursery of the Experimental Farm of Hort. Res. Inst., Giza, Egypt during 2011 and 2012 years, to study the effect of bio-fertilizer "Biogien or Rizobacterien" or chemical-fertilizer "Viva Rose" as a foliar sprays at various rates on growth and chemical composition of *Cupressus sempervirens*. One year-old seedlings of *Cupressus* were transplants with height initial of 10-12 cm and carrying 8-10 leaves were planted in both seasons, individually in black polyethylene bags of 14 cm diameter filled with equal mixture sand and loam (1:1 v: v).

The physical and chemical properties of the used mixture soil are shown in Table (a) which, was analyzed according to Champan and Pratt (1978).

Table a. Physical and chemical properties of the used mixture medium in the two years (2011 and 2012).

Season	Particles size distribution (%)						E.C. (dS/m)	pH	Cations (meq/L)			
	Coarse sand	Fine sand	Silt	Clay	Organic matter	CaCO ₃			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺
2011	15.2	25.5	18.4	36.4	1.70	2.80	2.99	7.58	7.55	2.34	10.90	0.75
2012	15.3	24.7	17.8	38.0	1.50	2.70	2.78	7.50	10.33	1.56	8.67	0.75
	Anions (meq/L)			Macro-and micro-elements (ppm)								
	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	N	P	K	Fe	Zn	Mn	Cu		
2011	3.94	8.64	8.96	164.20	17.01	370.50	12.00	3.70	7.62	8.76		
2012	4.08	7.96	9.27	173.16	15.78	361.76	15.80	4.36	8.03	8.80		

After a month from planting "on 20th of March", the seedlings received monthly the following treatments three times for both seasons:

- 1-No fertilization, referred to as control.
- 2-Biofertilizer with either Biogien (a commercial product that contains a specific clone of *Azotobacter chroococcum* bacteria, conc.10⁶cells/ml) and Rhizobacterien (a Commercial product containing a specific strain of *Rhizobium sp.* bacteria, conc. 10⁷- 10⁸ cells/m) at the rates of 2.50, 5.00 and 7.50 g /seedling.
- 3-Liquid fertilizer (Table (b) clear the content's of "Viva Rose" fertilizer according to the production company "UAD" Union for Agric. Development w/w. was sprayed on the foliage till run-off, point at the levels of 1.00, 2.00 and 4.00 g/l. of water.

4- A combination of Biogien a t 2.5g/seedling + Rhizobacterien at 2.5g/seedling+Viva Rose at 1.00g/l.

Table b. The content's of "Viva Rose" fertilizer:

N%	P% "P ₂ O ₅ "	K% "K ₂ O"	Fe%	Zn%	Mn%	Cu%	Mg %	MI%	Citric acid%	sucrose%
22.00	5.00	11.00	0.05	0.07	0.24	0.05	0.3	0.5	0.002	0.07

-All plants under the various treatments received the usual agricultural practices such as weeding ...etc. whenever needed.

- Data were taken at the end of each year (on 8th September) as follows:

Plant height (cm.), stem diameter (cm.), number of branches/plant, number of leaves/plant, length of roots/plant (cm.), as well as, fresh and dry weights of aerial parts" stems and leaves" and fresh and dry weights of roots (g) .

- Meanwhile, in fresh leaf samples they were taken from the middle parts of the plants, photosynthetic pigments (chlorophyll a, b and carotenoids, mg/g F.W.) were determined according to Moran, R. (1982).

- In fresh leaf the total indoles and total soluble phenols were determined colourimetrically by using Folin Ciocaltea reagent A.O.A.C. (1990).

-While in dry aerial parts, the percentages of nitrogen was determined using micro-Kjeldahle method as described by Jackson (1973), phosphorus, was measured colorimetrically as indicated by Cottenie *et al.*, (1982) in addition to potassium using a Flamephotometer set as reported by Jackson (1973). Nutrient uptake (g/plant) Jackson,(1973) were calculated by using formula as follows.

$$\text{Nutrient uptake (g/plant)} = \text{Nutrient content (\%)} * \text{dry matter (g)} / 100$$

The layout of the experiment in the two seasons was a randomized complete block design (RCBD) with three replicates (Mead *et al.*, 1993), as each replicate consisted of nine plants.

-Data were then tabulated and subjected to analysis of variance according to SPSS Program (Levesque, 2007) using Duncan's Multiple Range Test (1955) was used to verify the significance level among means of various treatments.

RESULTS AND DISCUSSION

Table (1) show that plant height was significantly increased by plants fertilized with Biogien at the rate of 2.5g/bag or sprayed with "Viva Rose" at the rate 2.00ppm and untreated plant compared to other treatments in the first season whereas the utmost means were recorded in the second year by addition Biogien at the rate of 2.5g/bag. Meanwhile stem diameter significantly increased by using Biogien at the rate of 2.5g/bag. Number of branches /plant and number of leaves /plant recorded much increases by Viva Rose fertilizer at the rate of 4.00ppm in the two year. The

control gave the highest mean of root length /plant comparing to all other treatments. Results of growth parameters were in agreement with those obtained by Abdel-Fattah *et al* (2009) on *Dracaena marginata* and *Ruscus hypoglossum*.

Table (2) It is clear that the treating plants with Biogien at the rate of 5.00 g/bag, "Viva Rose" at the rate of 4.00ppm and Biogien plus Rhizobacterien at the rate of 2.5g/bag plus " Viva Rose" at the rate of 1.00ppm gave highly values of fresh and dry weights of aerial parts. On the other hand, fresh and dry roots were increased by plants treated with Biogien bio-fertilizer at the rate of 5g/bag or "Viva Rose "at the rate of 1.00ppm treatments which were insignificant for the two seasons under study. Rhizobacterien recorded less water amount between fresh and dry weights. This may be attributed to the capability of Biogein in fixing more atmospheric N and secrete more vitamins and growth promoting substances necessary for good and healthy growth Subba Rao,(1993) reported the same on *Azotobacter chroococcum* .On the same line were those results of Abdel-Fattah *et al* (2009) on *Dracaena* and *Ruscus*.

Table 1. Effect of Biogein, Rhizobacterien and "Viva Rose" treatments on some vegetative growth parameters of *Cupressus sempervirens* seedling during 2011 and 2012 years.

Treatments	Plant height (cm)	Stem diameter (cm)	No. of Branches/ plant	No. of Leaves/ plant	Root length / plant (cm)
Control	25.67A	0.31AB	0.67C	23.34BC	12.67A
Biogien at 2.5g/bag	25.67A	0.34A	0.00C	21.00B-D	11.34AB
Biogien at 5g/bag	19.00AB	0.27AB	0.00C	21.34B-D	9.34D-F
Biogien at 7.5g/bag	21.67AB	0.27AB	0.00C	20.67B-D	9.67C-F
Rhizobacterien at 2.5 g/ bag	19.00AB	0.31AB	0.00C	14.67D	9.51D-F
Rhizobacterien at 5g/bag	21.34AB	0.24AB	0.00C	16.67CD	8.67F
Rhizobacterien at 7.5 g/ bag	22.67AB	0.21AB	0.00C	19.00B-D	11.00BC
Viva Rose at 1.00ppm	17.34B	0.24AB	0.00C	14.00D	10.34B-E
Viva Rose at 2.00ppm	25.34A	0.27AB	0.00C	18.00CD	9.00EF
Viva Rose at 4.00ppm	19.34AB	0.24AB	4.67A	44.67A	11.67AB
Biogien + Rhizobacterien at 2.5g/bag+Viva Rose at1.00ppm	19.00AB	0.17B	3.67B	26.67B	10.67B-D
Second year :2012					
Control	25.00AB	0.24A	1.00C	23.00BC	12.00A
Biogien at 2.5g/bag	27.34A	0.37A	0.00D	19.67BC	10.67A-C
Biogien at 5g/bag	23.00A-C	0.31A	0.00D	20.67BC	9.00CD
Biogien at 7.5g/bag	20.00B-D	0.37A	0.00D	19.34BC	9.34B-D
Rhizobacterien at 2.5 g/ bag	21.00B-D	0.37A	0.00D	14.00BC	9.00CD
Rhizobacterien at 5g/bag	22.00A-D	0.31A	0.00D	16.00BC	8.00D
Rhizobacterien at 7.5 g/ bag	20.00B-D	0.37A	0.00D	18.67BC	10.67A-C
Viva Rose at 1.00ppm	17.00D	0.34A	0.00D	12.67C	10.00BC
Viva Rose at 2.00ppm	25.67AB	0.37A	0.00D	17.00BC	8.00D
Viva Rose at 4.00ppm	18.67CD	0.27A	4.34A	41.34A	11.00AB
Biogien + Rhizobacterien at 2.5g/bag+ Viva Roseat1.00ppm	20.00B-D	0.27A	3.00B	26.34B	9.67B-D

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5% level.

Table 2. Effect of Biogein, Rhizobacterien and "Viva Rose" treatments on fresh and dry weights of different parts of *Cupressus sempervirens* seedling during 2011 and 2012 years.

Treatments	Fresh weight of Aerial parts (g)	Dry weight of Aerial parts (g)	Fresh weight of Roots (g)	Dry weight of Roots (g)
	First year : 2011			
Control	3.98A	2.67AB	0.78A	0.41A
Biogien at 2.5g/bag	3.72A	2.48B-E	0.67A	0.36A
Biogien at 5g/bag	4.46A	2.86AB	1.04A	0.45A
Biogien at 7.5g/bag	3.43A	2.56B-D	0.57A	0.31A
Rhizobacterien at 2.5 g/ bag	3.15A	2.49B-E	0.54A	0.29A
Rhizobacterien at 5g/bag	2.69A	2.14E-G	0.62A	0.22A
Rhizobacterien at 7.5 g/ bag	2.86A	2.28C-F	0.54A	0.34A
Viva Rose at 1.00ppm	2.77A	1.76G	0.63A	0.31A
Viva Rose at 2.00ppm	2.91A	2.19D-F	0.64A	0.41A
Viva Rose at 4.00ppm	5.19A	2.97A	0.38A	0.29A
Biogien + Rhizobacterien at 2.5g/bag+ Viva Rose at1.00ppm	3.19A	2.15E-G	0.38A	0.21A
Second year :2012				
Control	3.74AB	2.34AB	0.64A	0.34A
Biogien at 2.5g/bag	3.34AB	2.00AB	0.74A	0.31A
Biogien at 5g/bag	2.67AB	2.67A	0.74A	0.44A
Biogien at 7.5g/bag	3.00AB	2.00AB	0.54A	0.27A
Rhizobacterien at 2.5 g/ bag	3.00AB	2.00AB	0.51A	0.21A
Rhizobacterien at 5g/bag	2.34B	2.00AB	0.57A	0.21A
Rhizobacterien at 7.5 g/ bag	2.34B	2.00AB	0.47A	0.31A
Viva Rose at 1.00ppm	2.34B	1.67B	0.57A	0.27A
Viva Rose at 2.00ppm	3.67AB	2.00AB	0.61A	0.37A
Viva Rose at 4.00ppm	5.00A	2.67A	0.34A	0.27A
Biogien + Rhizobacterien at 2.5g/bag+ Viva Rose at1.00ppm	3.00B	1.67B	0.31A	0.14A

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5% level.

Chemical composition:

Data in Table (3) and "Fig. 1 and 2" showed a significant increased in chlorophyll a, b and carotenoids contents in leaves by using bio-fertilizer with Biogien or Rhizobacterien and "Viva Rose" fertilization. Treating plants with Biogien the rate of 2.5g/bag raised the means of chlorophyll a (2.67mg/g) over the control means 1.37 mg/g F.W. and total chlorophyll "a + b". Meanwhile, Rhizobacterien at the rate of 2.5 g/bag significantly increased chlorophyll b, carotenoids and total indole contents in leaves of cupressus seedlings.

Total phenols, showed a highly increased by "Viva Rose" at the rate of 4.00 ppm . The percentage of N, P and K were markedly increased due to the different treatments of fertilization, the highest significant percentage from N induced by addition Biogien at the rate of 2.50g/bag and Rhizobacterien at the rate of 5.00 g/bag .Spraying plants with "Viva Rose" at 2.00ppm recorded high amount of P%. While, K percentage significantly increased by Rhizobacterien at the rate of 5.00g/bag treatment compared to its untreated plant or all the other treatments. However,

Biogien at the rate of 2.50g/bag recorded significantly increased of nutrient uptake contents in leaves.

These results due to the role of N in chlorophylls and amino acids synthesis, and P which contributes in regulating the opening and closing of stomata and possibly membrane turgor that affect chlorophyll formation through its radioactive properties. Phosphorus would activate various metabolic processes and it is involved in energy transfer process during building of phospholipids and nucleic acids Marschner, (1995). Moreover, P provides plant metabolic process with phosphate bonds, which are necessary for building pigments and other constituents Baek, *et al* (1999).

Table 3. Effect of Biogien, Rhizobacterien and "Viva Rose" treatments on chemical composition of *Cupressus sempervirens* seedling during 2012 year.

Treatments	Chlorophyll (a) (mg/g f.w.)	Chlorophyll (b) (mg/g f.w.)	Carotenoids (mg/g f.w.)	Total indoles (mg/g f.w.)	Total phenols (mg/g f.w.)	N%	P%	K%
Control	1.37B	0.48C	0.75A	0.02B	0.02I	0.42D	2.69C	1.01F
Biogien at 2.5g/bag	2.67A	0.13E	0.26C	0.04B	0.02I	0.70B	0.34I	1.44B
Biogien at 5g/bag	0.28F	0.26D	0.63AB	0.08B	0.02I	0.84A	0.20J	1.24D
Biogien at 7.5g/bag	0.27F	0.15E	0.32C	0.31A	0.08G	0.42D	0.81G	1.12E
Rhizobacterien at 2.5 g/ bag	0.41D	1.65A	0.75A	0.32A	0.09F	0.84A	2.31E	1.55AB
Rhizobacterien at 5g/bag	0.18G	0.15E	0.62AB	0.02B	0.10E	0.56C	0.47H	1.61A
Rhizobacterien at 7.5 g/ bag	0.59C	0.12E	0.45BC	0.02B	0.13D	0.56C	3.95B	1.49B
Viva Rose at 1.00ppm	0.61H	0.17E	0.16C	0.02B	0.19C	0.56C	3.02D	1.32C
Viva Rose at 2.00ppm	0.19G	1.32B	0.35BC	0.02B	0.18C	0.42D	4.95A	1.21D
Viva Rose at 4.00ppm	0.38E	0.13E	0.18C	0.02B	0.32A	0.42D	0.96F	1.15E
Biogien + Rhizobacterien at 2.5g/bag+Viva Rose at1.00ppm	0.19G	0.12E	0.32C	0.02B	0.21B	0.42D	2.34E	1.22D

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test at 5% level.

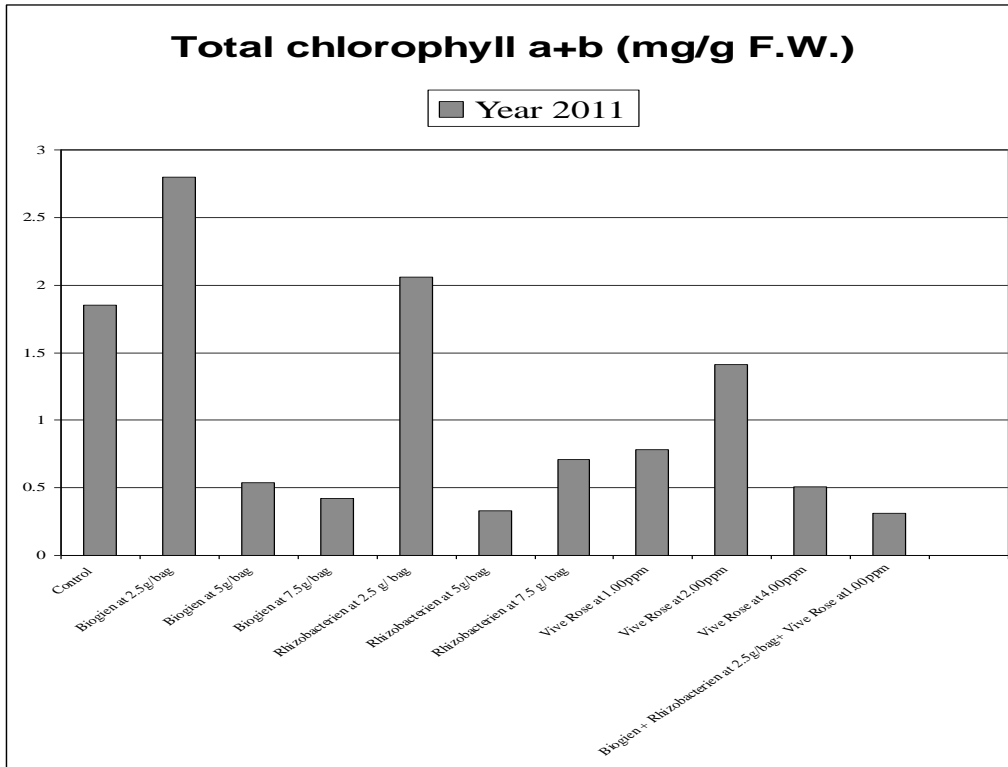


Figure 1. Histogram showing the effect of bio and chemical fertilization on total chlorophyll "a+b" (mg/g) of *Cupressus sempervirens* seedlings.

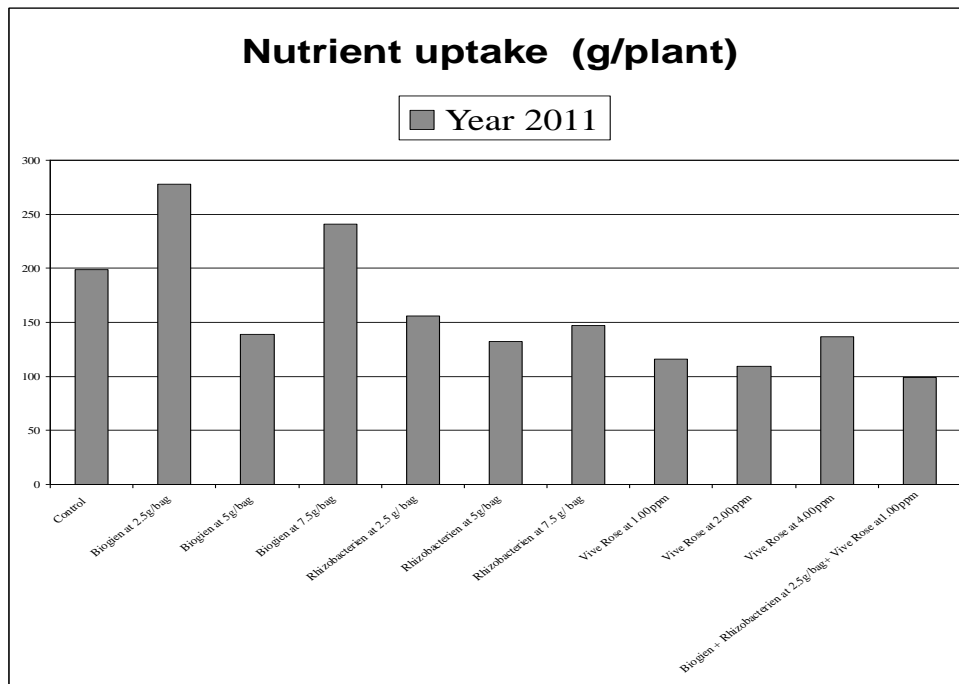


Figure 2. Histogram showing the effect of bio and chemical fertilization on nutrient uptake (g/plant) of *Cupressus sempervirens* seedlings.

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تأثير معاملات التسميد بالبيوجين والريزوبكتريين والفيفا روز على النمو والتركييب الكيماوى لشتلات السرو

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قسم بحوث نباتات الزينه وتنسيق الحدائق و قسم بحوث الأشجار الخشبيه
معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة - مصر

أجري هذا البحث على شتلات السرو عمرسنه بمشئل قسم بحوث الأشجار الخشبيه - معهد بحوث البساتين، الجيزة، خلال عاميين متتاليين ٢٠١١ و ٢٠١٢ لدراسه أفضل سماد وكذلك الجرعه المناسبه لكلا من السماد الحيوي (البيوجين والريزوبكتريين) بمعدل صفر و ٢.٥ , ٥ , ٧.٥ جرام / كيس والسماد الكيماوى " فيفا روز كسماد ورقى بمعدل ١ و ٢ و ٤ جزء فى المليون" على النمو والتركييب الكيماوى لشتلات السرو الناميه فى أكياس بلاستيك قطر ١٤ سم مملوءة ب٧٠٠ جرام من الطمى والرمل بمعدل (١:١- بالحجم).

وقد أوضحت النتائج المتحصل عليها على ما يلى:-

- ارتفاع النبات وسمك الساق زادت معنوياً بإضافه البيوجين بمعدل 2.5 جرام لكل كيس.
 - فى حين رش النباتات بالفيفا روز بمعدل ٤ جزء فى المليون أدى الى زياده عدد الأفرع والأوراق /نبات مقارنة بالكنترول والمعاملات الأخرى.
 - أستخدم "البيوجين + الريزوبكتريين بمعدل ٢.٥ جرام لكل كيس + الفيفا روز بمعدل ١ جزء فى المليون أدت لزياده الوزن الطازج والجاف للأجزاء الخضريه "الأوراق والسيقان".
 - الوزن الطازج والجاف للجذور زاد معنوياً بإضافه البيوجين بمعدل 5 جرام لكل كيس وكذلك الفيفا روز بمعدل ١ جزء فى المليون.
 - البيوجين بمعدل ٢.٥ جرام لكل كيس أدى لزياده كلوروفيل أ والكلوروفيل الكلى والنسبه المئويه للنتروجين وكذلك النتروجين الممتص .
 - الريزوبكتريين بمعدل ٢.٥ جرام لكل كيس أدى لزياده محتوى الأوراق من كلوروفيل ب والكاروتينويدات و الأندولات الكليه.
 - زادت الفينولات الكليه عند رش الفيفا روز بمعدل ٤ جزء فى المليون .
 - الريزوبكتريين بمعدل ٥ جرام /كيس أدى إلى زياده النسبه المئويه للبوئاسيوم.
- التوصية:** ينصح بتسميد شتلات السرو المنزرعة فى أكياس بلاستيك سواد قطرها ١٤ سم ب ٢.٥ جرام بيوجين/ كيس مرة كل شهر من مارس إلى مايو ينتج شتلات قويه ذات جودة عاليه النمو الى جانب تقليل فتره بقائها فى المشئل مما يقلل من تكاليف الإنتاج بلأضافه إلى المحافظه على نظافه البيئه.