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BIO-FERTILIZERS AS A PARTIAL ALTERNATIVE TO CHEMICAL NPK FERTILIZATION OF JOJOBA (*Simmondsia chinensis* Link.) PLANTS GROWN IN DIFFERENT SOIL TYPES

E.I. El-Maadawy and Kh. S. Moursy*
Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
** Plant Nutrition Department, National Research Center, Dokki, Giza, Egypt.*

ABSTRACT

This study was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the two successive seasons of 2002/2003 and 2003/2004, with the aim of investigating the feasibility of using bio-fertilizers as a partial alternative to chemical NPK fertilization for producing jojoba (Simmondsia chinensis Link.) plants in three types of soil (sand, clay and calcareous sand). Plants grown in each type of soil received the following fertilization treatments: (1) Control (unfertilized), (2) NPK fertilizer (21:7:7, N:P_2O_5:K_2O) at 2 g /pot/2 months, (3) half the NPK rate (i.e., 1 g /pot/2 months) + Bacillus megatherium var. Phosphaticum, (4) ½ NPK + Azotobacter chroococcum, (5) ½ NPK + Azospirillum lipoferum, (6) ½ NPK + Azotobacter chroococcum + B. megatherium var. Phosphaticum, (7) ½ NPK + Azospirillum lipoferum + B. megatherium var. Phosphaticum, (8) ½ NPK + Azotobacter chroococcum + B. megatherium var. Phosphaticum + Pseudomonas aeruginosa, (9) ½ NPK + Azospirillum lipoferum + B. megatherium var. Phosphaticum + P. aeruginosa, or (10) ½ NPK + Azotobacter chroococcum + Azospirillum lipoferum + B. megatherium var. Phosphaticum + P. aeruginosa.

In general, plants grown in sand or clay gave better results (with sand giving the best vegetative growth, and clay giving the best chemical composition) than those grown in calcareous sand. In both seasons, plants grown in sand gave the highest mean values for number of leaves/plant, leaves fresh and dry weights, fresh weights of stems and roots, N percentage in leaves, and K percentage in roots. Plants grown in clay gave the highest mean values (in both seasons) for total carbohydrates percentage in leaves, chlorophylls "a", "b" and total chlorophyll contents in leaves, and P percentages in all plant parts (leaves, stems and roots). The best results for most of the other morphological and chemical characteristics (viz., plant height, stem diameter, number of branches, leaf area, dry weights of stems and roots) were obtained with sand in one season, and with clay in the other. In contrast, calcareous sand gave the highest mean values for the N percentage in stems and roots, and the K percentage in stems (in both seasons), as well as the highest carotenoids content and K percentage in leaves in the second season. Most of the fertilization treatments had a favorable effect on the different growth and chemical composition characteristics, with NPK giving generally lower values than combinations of ½ NPK and bio-fertilization. Also, in many cases combining ½ NPK with two *or more bacterial strains (i.e. combining nitrogen-fixing and phosphorus-solubilizing bacteria) gave higher values than using ½ NPK with only one bacterial strain. The relative effectiveness of the fertilization treatments, compared to each other, varied from one plant characteristic to the other. For example, treatment (5) gave the greatest plant height, while treatments (7) and (10) gave the highest stem diameter, and treatments (8) and (7) gave the highest number of branches (in the first and second seasons, respectively). Accordingly, the recommended fertilization treatment varies depending on the effect that is required*.

Keywords: Bio-fertilizers, partial alternative, chemical NPK fertilization, jojoba (S*immondsia chinensis* link.) plants, soil types.

INTRODUCTION

Jojoba (*Simmondsia chinensis* Link.), a member of the family *Simmondsiaceae*, is a spreading shrub that has a life span of over 100 years, and may exceed 200 years (Gentry, 1958 and Kearney *et al*.,1960)**.** It grows to a height of 2-6 ft (rarely to 16 ft) and a width of 3-6 ft. The simple, smooth-edged leaves are thick, leathery, bluish-green in colour, oblong, opposite; with a length of about 2.5-3.5 cm. Jojoba flowers bloom in winter and are greenish-yellow in colour. The fruit is acorn-shaped, and the mature seeds contain oil (which is really a liquid wax) that resembles sperm whale oil in composition and properties.

Approximately 90% of the seed oil harvested is utilized by the cosmetics industry, and has a natural moisturizing and healing effect on the skin. The oil may also be used in many industrial processes and for the production of pharmaceuticals and commercial products such as lubricants, waxes, candles, and rubber compounds such as varnishes, rubber adhesives, and linoleum. In addition, the seed oil is a good source of straight-chain alcohols and acids used in detergents, disinfectants, emulsifiers, and bases for creams and ointments. Jojoba foliage can also be used as forage for livestock and wildlife. Rofail *et al*. (2000**)** also conducted field and laboratory studies which indicated that jojoba oil can be used as an insecticide for control of newly hatched larvae of certain strains of cotton bollworm.

Jojoba shrubs have an exceptionally deep root system which helps the plant to survive in drought conditions **(**Ayanoglu, 2000a). This feature makes it possible to grow jojoba (for landscape purposes or for oil production) in the newly reclaimed desert areas in Egypt. However, the sandy soil in such areas is often calcareous, with an alkaline pH and high contents of soluble salts. Ayanoglu (2000b) reported that some types of jojoba grow very well on soils with salinity and alkalinity problems. Some types even grow satisfactorily and set flowers at a soil-water salinity of about 7000 ppm.

Another factor to consider when cultivating new desert areas is the feasibility of using organic agricultural methods. During the last several years, the use of chemicals for fertilization or pest control in agricultural crops (including medicinal plants) has been increasingly criticized, on the basis that chemicals may adversely influence the chemical composition of agricultural products that are consumed by humans or animals, in addition to having an unfavorable impact on the environment. Several studies have confirmed the usefulness of bio-fertilizers as an alternative to conventional NPK fertilization in a number of ornamental and medicinal crops (El-Kashlan , 2001) on roselle, (Nofal *et al.*, 2001) on *Ammi visnaga*, (Gad , 2001) on *Foeniculum vulgare* and *Anethum graveolens*, and many others]. Nitrogen-fixing bacteria (such as *Azotobacter chroococcum* and *Azospirillum lipoferum*) and phosphorus-solubilizing bacteria (such as *Bacillus megatherium* and *Pseudomonas aeruginosa*) are among the most common strains used for bio-fertilization of horticultural crops. Free living nitrogen-fixing bacteria have not only the ability to fix nitrogen, but also to release certain phytohormones (similar to GA_3 and IAA) which could stimulate plant growth, absorption of nutrients and photosynthesis **(**Fayez *et al*., 1985).

The aim of this study was to investigate the feasibility of using bio-fertilizers (including nitrogen-fixing and phosphorus-solubilizing bacteria) as a partial alternative to chemical NPK fertilization to produce jojoba *Simmondsia chinensis* Link.), in different types of soil.

MATERIALS AND METHODS

This study was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the two successive season of 2002/2003 and 2003/2004, with the aim of comparing the effects of conventional chemical fertilization and bio-fertilizers on the growth and chemical composition of jojoba (*Simmondsia chinensis* Link.) plants grown in different types of soil.

Jojoba seeds were obtained from the Horticultural Research Center in El-Qassasseen. On 15th August 2002 and 2003 in the first and second seasons, respectively, the seeds were sown in 12-cm plastic pots filled with sand (100%). On 15th February 2003 and 2004 in the first and second seasons, respectively, the plants (with heights of 15-20 cm) were transplanted into 30-cm clay pots filled with three different media (sand, clay or calcareous sand). The physical and chemical compositions of the three types of soil are shown in Tables A and B.

Plants grown in each type of soil received the following fertilization treatments:

(1) Control (unfertilized) 0
(2) NPK fertilizer (21:7:7, N: P_2O_5: K_2O) at 2 g /pot/2 months0
Every treatments treated with half the NPK rate (i.e., 1 g /pot/2 months) +
(3) *Bacillus megatherium* var. Phosphaticum. (*B.*)
(4) *Azotobacter chroococcum,* (*Azot.*)
(5) *Azospirillum lipoferum*. (*Azos.*)
(6) *B. +Azot.*
(7) *B. +Azos.*
(8) *B. +Azot.* + *Pseudomonas aeruginosa*.
(9) *B. +Azos.* + *P. aeruginosa*.
(10) *B. +Azot. +Azos.* + *P. aeruginosa*.

The chemical NPK fertilizer used in this study (Haisol Blue, imported by Agroland Co., Giza) was added every two months as a top dressing, followed by irrigation. Each of the different bacterial strains was applied separately as a liquid inoculums (10 ml /pot), which was added to the soil after 15 days from transplanting. An additional dose (10 ml /pot) was applied after two months from the first application. The bacterial inoculums were prepared by diluting the bacteria in a Nitron Pross medium at the concentration of 1 mg x 10^9.

Table A. The mechanical analysis of the three types of soil (sand, clay and calcareous sand) used for growing jojoba (*Simmondsia chinensis* Link.) plants.

Soil types	Coarse sand (%)	Fine Sand (%)	Silt (%)	Clay (%)	Textural class
Sand	48.85	42.84	5.66	2.65	Sand
Clay	12.30	20.00	35.40	32.3	Clay loam
Calcareous sand	36.52	60.34	2.11	1.03	Calcareous sand

Table B. The chemical characteristics of the three types of soil (sand, clay and calcareous sand) used for growing jojoba (*Simmondsia chinensis* Link.) plants.

Soil types	pH	E.C. (dS/m)	$CaCO_3$ (%)	Cations (meq/L)				Anions (meq/L)		
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	Cl^{-}	SO_4^{--}
Sand	7.4	0.80	1.53	0.97	0.39	3.6	0.20	0.99	0.90	1.80
Clay	8.0	0.50	1.01	1.95	1.20	1.90	0.10	2.00	1.10	1.35
Calcareous sand	8.37	2.38	2.3	14.0	7.00	29.04	0.85	---	20.5	24.0

The layout of the experiment was a split plots design, with the main plots assigned to the soil types, while the sub-plots were assigned to the fertilization treatments. The main plots were arranged in a randomized complete blocks design. The experiment included a total of 30 treatments (3 types of soil X 10 fertilization treatments, including the control) and 3 blocks (replicates), each consisting of 5 pots /treatment.

At the termination of each season (on 1st August 2003 and 2004 in the first and second seasons, respectively), data were recorded on plant vegetative growth characteristics, including plant height (cm), stem diameter (cm) at a height of 5 cm from the soil surface, number of branches/ plant, number of leaves/ plant, area (cm^2) of the 4th leaf from the top of the plant (using a Licor portable area meter, model LI 3000), as well as the fresh and dry weights of leaves, stems and roots/ plant. The data recorded on vegetative growth were statistically analyzed. An analysis of variance (ANOVA) was carried out, and the means were compared using the "Least Significant Difference (LSD)" test at the 0.05 level, as described by Steel and Torrie (1980).

Chemical analysis of fresh leaf samples was also conducted to determine their contents of pigments [chlorophyll "a", chlorophyll "b", total chlorophylls (a+b) and carotenoids], using the method described by Saric *et al.* (1967). In addition, samples of leaves, stems and roots were oven-dried at a temperature of

70°C for 24 hours, and their content of total carbohydrates was determined using the method outlined by Dubois *et al*. (1956). Also, the nutrients were extracted from dried tissue samples (of leaves, stems and roots) using the method described by Piper (1947), then the nutrient extracts were chemically analyzed to determine their contents of nitrogen (using a modified Micro-Kjeldahl apparatus, as described by Pregl, 1945), phosphorus (using the method described by Jackson (1967), and potassium [using an atomic absorption, flame-photometer (Philips, model PU 9100X), as recommended by Chapman and Pratt (1961).

RESULTS AND DISCUSSION

I. Vegetative growth

1. Plant height

The results recorded in the two seasons (Table 1) show that the type of soil had a significant effect on the height of jojoba plants. In both seasons, plants grown in the calcareous sandy soil were significantly shorter than those grown in clay. Also, plants grown in calcareous sand were significantly shorter in the second season, compared to plants grown in sand, but in the first season, the difference between the heights of plants grown in these two types of soil was statistically insignificant. On the other hand, no significant difference was detected between the heights of plants grown in sand or in clay, with clay giving the tallest plants in the first season, and sand giving the tallest plants in the second season. The unfavorable effect of calcareous soil on shoot growth may be attributed to the relatively high pH value, which leads to the conversion of some nutrients (such as phosphorus and boron) to an insoluble form which is unavailable to the roots.

The data presented in Table 2 also show that the different fertilization treatments had a generally favorable effect on plant height, i.e. they gave taller plants than the unfertilized control. In the first season, treatments including *Pseudomonas aeruginosa* caused only slight (insignificant) increases in plant height, whereas all other treatments gave significantly taller plants than the control. The favorable effect of the fertilization treatments was generally more pronounced in the second season, with only one treatment (NPK fertilization) giving an insignificant increase in plant height, while all other treatments increased plant height significantly, compared to the control. It is also clear that in both seasons, combinations of NPK and bio-fertilization which included the use of *Pseudomonas aeruginosa* were generally less effective than combinations without *P. aeruginosa*. Among the different fertilization treatments, the most effective one for increasing plant height in both seasons was the application of ½ NPK + *Azospirillum lipoferum*, followed by ½ NPK + *Azotobacter chroococcum*, then ½ NPK + *Bacillus megatherium* var. Phosphaticum. The favorable effect of *Azotobacter* and *Azospirillum* bacteria on plant height has been reported by Gad (2001) on *Foeniculum vulgare* and *Anethum graveolens*, Kandeel *et al.* (2001) on *Foeniculum vulgare*, Nofal *et al*. (2001) on *Ammi visnaga*, and Gadagi *et al.* (2004) on *Gaillardia pulchella* plants. In contrast, the least effective treatment in the first season was ½ NPK + *Azotobacter chroococcum* + *Bacillus megatherium* var. *phosphaticum* + *Pseudomonas aeruginosa*, whereas the least effective treatment in the second season was the use of chemical NPK fertilization alone.

Regarding the interaction between the types of soil and the fertilization treatments, it can be seen in Table 1 that the tallest plants in the first season were those grown in sand and fertilized with ½ NPK + *Azospirillum lipoferum*, whereas the tallest plants in the second season were those that had been grown in sand and fertilized with ½ NPK + *Azotobacter chroococcum*.

2. Stem diameter

Measurements of stem diameter recorded in the two seasons (Table 1) have shown that no significant difference was found between the values obtained from plants grown in sand and those from plants grown in clay, with clay giving the thickest stems in the first season, while sand gave the thickest stems in the second season. In general, both of these growing media (sand and clay) were significantly more suitable for stem thickening in jojoba plants, compared to calcareous sand. In most cases, plants grown in calcareous sand gave significantly thinner stems than those grown in sand or clay.

It is also clear from the data in Table 1 that, in most cases, the different fertilization treatments gave thicker stems than those of the unfertilized control plants. Moreover, data recorded in the first season has shown that combinations of ½ NPK with more than one bacterial strain was generally more effective for increasing stem thickness, compared to using the full NPK rate (alone), or using combinations of ½ NPK with only one bacterial strain. No significant difference was detected in the first season between the stem diameters of plants fertilized with NPK alone, and those of plants receiving ½ NPK combined with only one bacterial strain. On the other hand, using NPK alone gave significantly thinner stems in the second season, compared to all the treatments in which using ½ NPK was combined with using bio-fertilization. The data in Table (1) also show that the thickest stems (in both seasons) were those of plants fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum, or ½ NPK + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *B. megatherium* var. *phosphaticum* + *P. aeruginosa*, with no significant difference between the values resulting from these two treatments. Similar increases in stem diameter as a result of fertilization using N-fixing bacteria have been reported by Sharma *et al*. (1996) on *Acacia auriculiformis*. Significant differences in stem thickness were also detected as a result of the interaction between the soil types and the fertilization treatments. In general, plants grown in calcareous sand and supplied with the different fertilization treatments had thinner stems than those of plants grown in sand or clay, and supplied with the same fertilization treatments. The thickest stems were those of plants grown in sand and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa* (in the first season), or ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum (in the second season).

3. Number of branches/plant

The number of branches formed on jojoba plants was not significantly affected by the type of soil (Table 1). In both seasons, the lowest number of branches was found on plants grown in calcareous sand. On the other hand, the highest number of branches in the first season was formed on plants grown in sand, whereas plants grown in clay had the highest number of branches in the second season. The data in Table 1 also show that, in most cases, the tested fertilization treatments significantly promoted the branching of jojoba plants, compared to the unfertilized control, which had the lowest number of branches/plant (in both seasons). In both seasons, no significant difference was detected between the effects of *Azotobacter chroococcum* and *Azospirillum lipoferum*, when used separately in combination with ½ NPK. It is also clear that plants fertilized with NPK alone, or with ½ NPK + one bacterial strain had generally fewer branches than plants fertilized with ½ NPK + a combination of nitrogen-fixing and phosphorus-solubilizing bacteria. The highest value recorded in the first season was obtained from plants fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*, followed by plants fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. *phosphaticum*. On the other hand, the highest number of branches in the second season was found on plants fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. *phosphaticum*, followed by plants fertilized with ½ NPK + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*. The favorable effect of bio-fertilizers on branching has been described by Gad (2001) on *Foeniculum vulgare* and *Anethum graveolens*, and Gadagi *et al.* (2004) on *Gaillardia pulchella* plants.

Regarding the interaction between the effects of types of soil and fertilization treatments on the number of branches, the data in Table 1 show that the various treatment combinations resulted in significant differences between the recorded values. It is also worth mentioning that in unfertilized plants, or plants receiving NPK fertilization only, using clay as the growing medium gave higher values than sand or calcareous sand. This may be attributed to the higher cation exchange capacity of clay (compared to sand, or calcareous sand), which helps the soil to prevent the leaching of nutrients, and to maximize their uptake by the roots and their use in vegetative growth, including the formation of branches. Among the different combinations of soil types and fertilization treatments, using a calcareous sandy soil and fertilization with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum gave the highest value in the first season, whereas the highest value in the second season was obtained from plants grown in clay and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

4. Number of leaves/plant

It can be seen from the data presented in Table 1 that the number of leaves formed on jojoba plants was significantly affected by the type of soil. In both seasons, plants grown in sand had the highest number of leaves, followed by plants grown in clay, with no significant difference between these two types of soil. On the other hand, plants grown in calcareous sand had significantly fewer leaves (in most cases) than those grown in sand or clay. It is also clear from the data in Table 1 that, in both seasons, all the tested fertilization treatments significantly increased the number of flowers produced by jojoba plants, compared to the unfertilized control plants. Supplying the plants with NPK fertilization alone gave lower values in both seasons, compared to values obtained from plants receiving the various bio-fertilization treatments. Also, combining ½ NPK with only one strain of bacteria was generally less effective for increasing the number of leaves, compared to combining ½ NPK with two or more bacterial strains (especially in the first season). Among the different fertilization treatments, supplying the plants with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. *phosphaticum* gave the highest number of leaves/plant (in both seasons). Similar increases in the number of leaves as a result of using bio-fertilizers have been reported by Gad (2001) on *Foeniculum vulgare* and *Anethum graveolens*, and Gadagi *et al.* (2004) on *Gaillardia pulchella* plants.

The interaction between the effects of soil types and fertilization treatments caused significant differences in the number of leaves formed on jojoba plants. In the first season, the highest number of leaves was produced by plants grown in sand and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. *phosphaticum* + *P. aeruginosa*, whereas in the second season the highest number of leaves was produced by plants grown in sand and fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

5. Leaf area

Measurements of leaf area that were recorded in the two seasons (Table 2) revealed that, in most cases, plants grown in sand or clay had significantly larger leaves than plants grown in calcareous sand. In the first season, plants grown in sand had the largest leaves. However, these leaves were not significantly larger than those formed by plants grown in clay. On the other hand, plants grown in clay gave significantly larger leaves in the second season, compared to plants grown in sand or in calcareous sand. The data presented in Table 2, also show that in the first season, supplying the plants with ½ NPK + *Azospirillum lipoferum* was the only fertilization treatment that significantly increased leaf area, compared to the unfertilized control plants, or to plants receiving any other fertilization treatment. The favorable effect of fertilization on leaf growth was more pronounced in the second season, with most fertilization treatments causing significant increases in leaf area, compared to that obtained from control plants. Among the different fertilization treatments that were tested, the most effective one in the second season (i.e., giving the largest leaves) was supplying the plants with ½ NPK + *B. megatherium* var. *phosphaticum*, followed by fertilization with ½ NPK + *Azospirillum lipoferum*. On the other hand, the smallest leaves in he second season were produced by plants fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum.

Regarding the interaction between the effects of soil types and fertilization treatments, the data in Table 2 show that in the first season, the largest leaves were produced by plants grown in clay and fertilized using ½ NPK + *Azospirillum lipoferum*, whereas the largest leaves in the second season were formed on plants grown in clay and fertilized using ½ NPK + *Azotobacter chroococcum*.

6. Fresh and dry weights of leaves/plant.

The fresh and dry weights of leaves/plant were significantly affected by the type of soil in which the plants were grown. In both seasons, the highest values were obtained from plants grown in sand, followed by plants grown in clay, whereas the lowest fresh and dry weights of leaves/plant were obtained as a result of growing the plants in calcareous sand. In the first season, the mean values obtained from plants grown in sand (for both fresh and dry weights of leaves/plant) were insignificantly higher than those obtained from plants grown in clay, but were significantly higher than those from plants grown in calcareous sand. The beneficial effect of growing the plants in sand was more pronounced in the second season, with plants grown in sand giving significantly higher fresh and dry weights of leaves/plant, compared to values obtained from plants grown in any of the other two types of soil (clay or calcareous sand).

The different fertilization treatments also had a considerable effect on the fresh and dry weights of leaves in jojoba plants (Table 2). In most cases, the different fertilization treatments gave significantly higher values than the control. Among the tested treatments, using NPK fertilization alone was the least effective treatment. In fact, fertilization with NPK was the only treatment which gave an insignificant increase in the values recorded in the second season, compared to the control. All other fertilization treatments gave significantly higher values than the control. In the first season, the highest fresh weight of leaves was obtained from plants fertilized using ½ NPK + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *B. megatherium* var. *phosphaticum* + *P. aeruginosa*, while the highest dry weight of leaves was obtained from plants fertilized using ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. *phosphaticum*. In the second season, the highest values for both the fresh and dry weights of leaves were obtained from plants fertilized using ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*. These results are in agreement with the findings of Gad (2001) on *Foeniculum vulgare* and *Anethum graveolens* plants.

The interaction between the types of soil and the fertilization treatments resulted in significant differences in the recorded values. However, the relative effects of the different treatment combinations, compared to each other, differed from one season to the other. In the first season, the highest fresh and dry weights of leaves were obtained from plants grown in sand and fertilized with ½ NPK + *Azospirillum lipoferum*. As previously mentioned, this combination of treatments also gave the largest leaves, which indicates that leaf area was more important than the number of leaves in affecting the fresh and dry weights of leaves/plant. In the second season, the highest fresh weight of leaves was obtained from plants grown in sand and fertilized with ½ NPK + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*, while the highest dry weight of leaves was obtained from plants grown in sand and fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

7. Fresh and dry weights of stems/ plant.

As shown in Table 3, the fresh and dry weights of stems were significantly affected by the type of soil in which the plants were grown. In most cases, the highest values were those of plants grown in sand, followed by plants grown in clay, whereas the lowest values were obtained from plants grown in the calcareous sandy soil. This trend was generally more pronounced in the second season than in the first.

The different fertilization treatments also had a significant effect on the fresh and dry weights of stems (Table 3). In both seasons, all the tested fertilization treatments significantly increased the fresh weight of stems, compared to the control. On the other hand, fertilization with NPK alone significantly increased the dry weight of stems in the first season (but not in the second season), compared to

the control, whereas combining ½ NPK with any of the bio-fertilization treatments increased the stems dry weight significantly in both seasons. It is also clear from the data in Table 3 that the relative effectiveness of the different fertilization treatments, compared to each other, varied from one season to the other. In the first season, the most effective treatments for increasing the fresh and dry weights of stems were supplying the plants with ½ NPK + *Azospirillum lipoferum*, or ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum. Plants fertilized with ½ NPK + *Azospirillum lipoferum* also gave the highest stems dry weight in the second season, whereas the highest fresh weight of stems in the second season was that of plants fertilized using ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

The interaction between the effects of soil types and fertilization treatments caused significant differences in the fresh and dry weights of jojoba stems (Table 3). In the first season, the best combination of treatments in terms of increasing the fresh and dry weights of stems was growing the plants in sand and fertilizing them with ½ NPK + *Azospirillum lipoferum*. However, the highest stems fresh weight in the second season was obtained from plants grown in sand and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum, while the highest stems dry weight was obtained from plants grown in sand and fertilized with ½ NPK + *Azotobacter chroococcum*.

8. Fresh and dry weights of roots/plant.

It is clear from the data in Table 3 that the, in most cases, plants grown in sand gave the highest fresh and dry weights of roots, followed by plants grown in clay, whereas the lowest values were obtained from plants grown in calcareous sand. The only exception to this general trend was detected in the first season, with plants grown in clay giving a roots dry weight which was insignificantly higher than that obtained from plants grown in sand.

The results recorded in the two seasons (Table 3) also show that fertilization had a significant effect on the fresh and dry weights of jojoba roots. In most cases (especially with the roots fresh weight), the different fertilization treatments significantly increased the values recorded in both seasons, compared to those obtained from control plants. Among the tested fertilization treatments, the most effective ones appeared to be ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. *phosphaticum* (which gave the highest roots fresh weight in the first season, and the highest roots dry weight in the second season), and ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var Phosphaticum (which gave the highest roots dry weight in the first season, and the highest roots fresh weight in the second season). The data presented in Table 3, also show that as a result of the interaction between the effects of soil types and fertilization treatments, significant differences were detected between the roots fresh and dry weights of plants receiving the various treatment combinations. In the first season, the highest roots fresh weight was that of plants grown in calcareous sand and fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum, whereas the highest roots dry weight was that of plants grown in clay and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum. In the second season, the highest values for both the fresh and dry weights of roots were obtained from plants grown in sand and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

II. Chemical composition.

1. Leaf pigments (chlorophylls and carotenoids) contents.

Chemical analysis of fresh leaf samples have shown that the highest chlorophyll "a", chlorophyll "b" and total chlorophyll (a + b) contents were obtained in leaves of plants grown in clay, followed by plants grown in sand, whereas the lowest values were obtained in leaves of plants grown in calcareous sand (Table 4). On the other hand, the effect of soil types on the carotenoids content was not clear, as the results differed from one season to the other.

It is also clear from the data in Table 4 that, in both seasons, the different fertilization treatments increased the contents of chlorophyll "a", chlorophyll "b" and total chlorophyll, but decreased the carotenoids content, compared to the values recorded in leaves of control plants. Among the tested fertilization treatments, supplying the plants with ½ NPK + *Azotobacter chroococcum* appeared to be the most favorable treatment for promoting chlorophyll synthesis and accumulation in jojoba leaves. This treatment gave the highest chlorophyll "a" and total chlorophyll (a + b) contents in both seasons, as well as the highest chlorophyll "b" content in the first season, but gave a relatively low carotenoids content. On the other hand the highest chlorophyll "b" content in the second season was found in the leaves of plants fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

Regarding the interaction between the soil types and the fertilization treatments, the data presented in Table 4 show that the most favorable treatment combination was growing the plants in clay, combined with fertilization using ½ NPK + *Azotobacter chroococcum*. Leaves of plants grown using this combination of treatments had the highest chlorophyll "a" content in both seasons, as well as the highest total chlorophyll (a + b) content in the second season, whereas the highest total chlorophyll content in the first season was obtained found in the leaves of plants grown in clay and fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*. Results recorded regarding the chlorophyll "b" content varied from one season to the other, with plants grown in clay and fertilized with ½ NPK + *Azospirillum lipoferum* giving the highest value in the first season, whereas the highest value in the second season was obtained from plants grown in sand and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

2. Total carbohydrates percentage in leaves.

Results of chemical analysis of dried leaf samples (Table 5) have shown that the type of soil had a significant effect on the total carbohydrates percentage in the leaves of jojoba plants. In both seasons, plants grown in clay had the highest total carbohydrates percentage, followed by plants grown in sand. In the first season, a significant difference was detected between values obtained from plants grown in these two types of soil, but in the second season, the difference between them was insignificant. On the other hand, plants grown in calcareous sand gave significantly lower values in both seasons, compared to plants grown in sand or clay.

Table (5): Effect of soil type, chemical NPK fertilization and bio-fertilizers on the total carbohydrates content in leaves of jojoba (*Simmondsia chinensis* Link.) during the 2002/2003 and 2003/2004 seasons.

Fertilization Treatments (F)	Total carbohydrates (% D.W.)							
	First Season				Second Season			
	Soil type (A)			Mean	Soil type (A)			Mean
	Sand	Clay	*Cal. sand		Sand	Clay	*Cal. and	
Control	10.1	12.6	4.30	**9.00**	10.05	9.81	4.01	**8.10**
NPK	12.4	15.9	7.82	**12.04**	11.11	10.09	5.74	**8.98**
½ NPK + B	11.7	15.0	6.33	**11.31**	12.06	14.12	5.37	**10.51**
" + *Azot.*	12.2	16.1	6.15	**11.48**	10.01	12.07	4.13	**8.73**
" + *Azos.*	10.2	12.9	8.70	**10.60**	9.67	13.90	5.23	**9.60**
" + B + *Azot.*	8.40	10.4	7.64	**8.81**	10.08	11.10	7.96	**9.71**
" + B + *Azos.*	9.10	11.3	7.49	**9.29**	12.13	12.81	8.45	**11.13**
" + B + *Azot.*+ P	8.10	9.50	8.09	**8.56**	10.90	11.91	5.80	**9.53**
" + B + *Azos.*+ P	9.10	10.9	9.60	**9.86**	12.60	13.52	6.93	**11.01**
" +*Azot.* +*Azos.* +B + P	8.90	10.6	9.14	**9.58**	15.80	14.29	8.34	**12.81**
Mean	**9.22**	**11.12**	**6.61**	**---**	**11.44**	**12.36**	**6.19**	**---**
L.S.D. at 0.05								
A	1.090				1.309			
F	0.604				0.692			
A x F	1.890				2.208			

*Cal. Sand = Calcareous sand

B = *Bacillus megatherium* var. Phosphaticum *Azot*. = *Azotobacter chroococcum* *Azos*. = *Azospirillum lipoferum* P = *Pseudomonas aeruginosa*

The data in Table 5, also show that in general, most of the tested fertilization treatments significantly increased the total carbohydrates percentage in jojoba leaves, compared to the control (especially in the second season). However, the relative effectiveness of the fertilization treatments, compared to each other, varied from one season to the other. In the first season, the highest value was obtained in plants receiving NPK fertilization alone. Also, data recorded in the first season showed that combining ½ NPK with only one bacterial strain (*B. megatherium* var. Phosphaticum, *Azotobacter chroococcum* or *Azospirillum lipoferum*) was generally more effective for increasing the total carbohydrates percentage, compared to treatments in which ½ NPK was combined with two or more bacterial strains. In contrast, results recorded in the second season showed that the highest total carbohydrates percentage was obtained when NPK was combined with all the tested bacterial strains (i.e., in plants fertilized with ½ NPK + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*), whereas using NPK alone, or combining ½ NPK with only one bacterial strain gave relatively low values (especially with *Azotobacter chroococcum* and *Azospirillum lipoferum*).

Results recorded in the two seasons have also shown that significant differences were found between the total carbohydrates percentages in leaves of plants grown with different combinations of soil types and fertilization treatments (Table 5). In most cases (especially in the second season), plants grown in calcareous sand and supplied with the different fertilization treatments had lower total carbohydrates percentages than plants grown in sand or clay and receiving the same fertilization treatments. On the other hand, the highest value in the first season was obtained from plants grown in clay and fertilized with NPK alone, whereas in the second season the highest value was obtained from plants grown in sand and fertilized with ½ NPK + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *B. megatherium* var Phosphaticum + *P. aeruginosa*.

3. Nutrient percentage in leaves, stems and roots.

a. Nitrogen.

The effect of the soil types on the N percentage differed from one part of the plant to the other (Table 6). In both seasons, plants grown in sand had the highest N percentage in the leaves, followed by plants grown in calcareous sand, whereas the lowest N percentage was found in the leaves of plants grown in clay. On the other hand, the highest N percentages in the stems and roots were obtained in plants grown in calcareous sand, followed by plants grown in clay, whereas the lowest values were obtained from plants grown in sand (in most cases).

It is also clear from the data in Table 6 that all the tested fertilization treatments increased the N percentage in the different parts of the plant (leaves, stems and roots), compared to the unfertilized control. However, fertilization with NPK alone, or combining ½ NPK with one strain of bacteria, gave generally lower N percentages in the different plant parts (especially the leaves and stems), compared to using treatments in which ½ NPK was combined with two or more bacterial strains. Among the different fertilization treatments, supplying the plants with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa* was clearly the most effective treatment for increasing the N percentage in the different parts of the plant. This treatment gave the highest N percentage in the stems in seasons, and the highest N percentage in the leaves in the first season, as well as the highest N percentage in the roots in the second season. The general increase in the N percentage in leaves of plants supplied with bio-fertilizers is in agreement with results reported by El-Kashlan (2001) on roselle, and Gad (2001) on *Foeniculum vulgare* and *Anethum graveolens*.

Regarding the interaction between soil types and fertilization treatments, the data in Table 6 show that the highest N percentage in the leaves was obtained from plants grown in sand and fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa* (in the first season) or ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var Phosphaticum (in the second season). On the other hand, the highest N percentage in the stems (in both seasons) was obtained from plants grown in calcareous sand and fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*, while the highest N percentage in the roots was obtained from plants grown in calcareous sand fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum (in the first season) or ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum (in the second season).

b. ***Phosphorus***

The data presented in Table 7 show that the P percentage in the different parts (leaves, stems and roots) of jojoba plants was significantly affected by the type of soil in which the plants were grown. In both seasons, plants grown in clay

had the highest P percentages in the different plant parts, followed by plants grown in sand, whereas the lowest P percentages were found in the tissues of plants grown in calcareous sand.

The P percentage in the different plant parts was also significantly affected by the tested fertilization treatments (Table 7). In general, most of the fertilization treatments significantly increased the P percentages in the leaves, stems and roots, compared to the control (especially in the second season). Among the different fertilization treatments, using NPK alone gave lower P percentages in the stems and roots, compared to most of the other treatments. Moreover, combining ½ NPK with one bacterial strain gave generally lower P contents in the different plant parts, compared to combining ½ NPK with two or more bacterial strains. Using a combination of ½ NPK with all the tested bacterial strains (i.e., ½ NPK + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*) gave the highest P percentage in the leaves in the first season, and the highest P percentage in the roots in the second season. These results are in agreement with the findings of El-Kashlan (2001) on roselle, and Gad (2001) on *Foeniculum vulgare* and *Anethum graveolens*. On the other hand, fertilization with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa* gave the highest P percentage in the roots in the first season, and the highest P percentage in the leaves in the second season. The highest P percentage in the stems was found in plants fertilized using ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum (in the first season) or ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum (in the second season).

Significant differences in the P percentages in different plant parts were also detected as a result of the interaction between the different soil types and fertilization treatments (Table 7). In general, plants grown in clay and receiving the different fertilization treatments had higher P percentages in the different plant parts, compared to plants grown in sand or calcareous sand, and receiving the same fertilization treatments. In both seasons, plants grown in clay and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa* had the highest P percentage in the leaves, while the highest P percentage in the stems was found in plants grown in clay and fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum (in the first season) or ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. *phosphaticum* (in the second season). Plants grown in clay and fertilized with ½ NPK + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa* had the highest P percentage in the roots in the first season, whereas in the second season the highest P percentage in roots was found in plants grown in clay and fertilized with ½ NPK + *Azotobacter chroococcum* + *Azospirillum lipoferum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

c. *Potassium*

Chemical analysis of samples taken from different parts of jojoba plants has revealed that, in both seasons, the K percentages in the leaves and roots were not significantly affected by the type of soil in which the plants were grown (Table 8). On the other hand, the K percentage in the stems was significantly higher when

the plants were grown in calcareous soil, compared to values obtained from plants grown in sand or clay (in both seasons). Results recorded in the two seasons (Table 8) also show that plants receiving any of the tested fertilization treatments had higher K percentages in the different plant parts, compared to values obtained from the unfertilized control plants. Among the different treatments, the least effective one for increasing the K percentage was fertilization with NPK alone. In fact, this treatment caused only slight (insignificant) increases in the K percentages in leaves and roots, compared to the control (in both seasons), whereas most of the other treatments gave significantly higher values than those obtained from the control. Similar increases in the K percentage in leaves as a result of applying bio-fertilizers have been obtained by El-Kashlan (2001) on roselle, Gad (2001) on *Foeniculum vulgare* and *Anethum graveolens*, Kandeel *et al.* (2001) on *Foeniculum vulgare*, and Nofal *et al*. (2001) on *Ammi visnaga*. Fertilization with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum appeared to be the most effective treatment for increasing the K percentage in the leaves and roots, since it gave the highest K percentage in the leaves in the first season, and the highest K content in the roots in the second season, as well as relatively high K contents in the leaves in the second season, and in the roots in the first season. On the other hand, the K percentage in the stems was generally more affected by the fertilization treatments than the K percentage in the leaves or roots. In both seasons, all the fertilization treatments (even using NPK alone) caused significant increases in the K percentage in stems, compared to the control, with the highest value resulting from fertilization using ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum + *P. aeruginosa*.

The data presented in Table 8, also show that plants grown with the various combinations of soil types and fertilization treatments showed significant differences in the K percentages within each of the different plant parts. However, the relative effects of the different combinations, compared to each other, varied from one season to the other. For example, the highest K content in the leaves in the first season was obtained from plants grown in calcareous sand and fertilized with ½ NPK + *Azotobacter chroococcum* + *B. megatherium* var. Phosphaticum, whereas in the second season the highest K percentage in leaves was obtained from plants grown in clay and fertilized with ½ NPK + *Azotobacter chroococcum*. Similar differences between the results recorded in the two seasons were also detected with the K percentages in the stems and roots.

Conclusively, from the above results, it is clear that growing jojoba plants in sand or clay gave better results than growing them in calcareous sand, with sand giving the most vigorous vegetative growth, while clay had the most favorable effect on the chemical composition. Most of the fertilization treatments had a favorable effect on the different vegetative growth and chemical composition characteristics, with combinations of ½

NPK and bio-fertilization giving generally higher values than using NPK alone. Also, in many cases, combining ½ NPK with two or more bacterial strains (i.e. combining nitrogen-fixing and phosphorus-solubilizing bacteria) gave higher values than using ½ NPK with only one bacterial strain. However, the relative effectiveness of the fertilization treatments, compared to each other, varied from one plant characteristic to the other. Accordingly, the recommended fertilization treatment varies depending on the effect that is required.

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الأسمدة الحيوية كبديل جزئى للتسميد الكيماوى NPK فى نباتات الجوجوبا المنزرعة فى أنواع مختلفة من التربة

عفت إسماعيل المعداوى- *خالد سيد مرسى
قسم بساتين الزينة، كلية الزراعة، جامعة القاهرة، الجيزة.ج.م.ع.
* قسم تغذية النبات، المركز القومى للبحوث، الدقى، الجيزة- ج.م.ع.

أجريت هذه التجربة في مشتل التجارب بقسم بساتين الزينة، كلية الزراعة، جامعة القاهرة، الجيزة، خلال الموسمين المتتاليين ٢٠٠٣/٢٠٠٢ و ٢٠٠٤/٢٠٠٣ بهدف دراسة جدوى استخدام الأسمدة الحيوية كبديل جزئي للتسميد الكيماوي ((NPK لإنتاج نباتات الجوجوبا ((*Simmondsia chinensis* Link. في ثلاثة أنواع مختلفة من التربة (رملية، طميية أو تربة جيرية).

عوملت النباتات المنزرعة في كل نوع من التربة بالمعاملات السمادية التالية:

(١) معاملة المقارنة (غير مسمدة)٠

(٢) تسميد كيماوي NPK (٢١:٧:٧ ، ن : فو٢أ٥ : بو٢أ) بمعدل ٢ جم/ إصيص/شهرين٠

نصف معدل التسميد الكيماوي (½ NPK) مع كل من:

(٣) *Bacillus megatherium* var. *Phosphaticum* (*B.*)

(٤) *Azotobacter chroococcum*٠ (*Azot.*)

(٥) *Azospirillum lipoferum* ٠ (*Azs.*)

(٦) *B.* . + *Azot.*

(٧) *B.* + *Azos.*

(٨) *Pseudomonas aeruginosa*+ *B.* + *Azot.*

(٩) *P. aeruginosa*+ *B.* +*Azos.*

(١٠) *P. aeruginosa*+ *B.* + *Azos.* +*Azot.*

بصفة عامة أعطت زراعة النباتات في تربة رملية أو طمي نتائج أفضل من الزراعة في التربة الجيرية (و أعطى الرمل أفضل نمو خضري، في حين أعطى الطمي أفضل تركيب كيماوي). وفى كل من الموسمين أعطت النباتات المنزرعة في رمل أعلى المتوسطات لعدد الأوراق/نبات، والأوزان الطازجة والجافة للأوراق، والأوزان الطازجة للسيقان والجذور، والنسبة المئوية للنتروجين في الأوراق، والنسبة المئوية للبوتاسيوم في الجذور. أما النباتات المنزرعة في طمي فأعطت أعلى المتوسطات (في الموسمين) للنسبة المئوية للكربوهيدرات الكلية في الأوراق، ومحتوى الأوراق من الكلوروفيللات "أ" و "ب" والكلوروفيل الكلى، والنسبة المئوية للفوسفور في أجزاء النبات المختلفة (الأوراق والسيقان والجذور). هذا وتم الحصول على أفضل النتائج للصفات المورفولوجية والكيماوية الأخرى (ارتفاع النبات، قطر

الساق، عدد الأفرع، مساحة الورقة، الأوزان الجافة للسيقان والجذور) عند الزراعة في الرمل في أحد الموسمين، وعند الزراعة في طمي في الموسم الآخر. وبعكس النتائج السابقة أعطت التربة الجيرية أعلى المتوسطات للنسبة المئوية للنتروجين في السيقان والجذور، والنسبة المئوية للبوتاسيوم في السيقان (في الموسمين)، وكذلك أعلى محتوى من الكاروتينويدات وأعلى نسبة مئوية للبوتاسيوم في الأوراق في الموسم الثاني. أعطت أغلب المعاملات السمادية تأثيراً جيداً على الصفات المختلفة للنمو والتركيب الكيماوي، وأعطى التسميد الكيماوي NPK بصفة عامة قيماً أقل بالمقارنة بالجمع ما بين استخدام نصف معدل التسميد الكيماوي، والتسميد الحيوي. كذلك فإنه في الكثير من الحالات أدى الجمع ما بين نصف معدل التسميد الكيماوي واستخدام أثنين أو أكثر من السلالات البكتيرية (أي الجمع ما بين بكتيريا مثبتة للنتروجين وأخرى مذيبة للفوسفور) إلى نتائج أفضل من استخدام نصف معدل التسميد الكيماوي مع سلالة واحدة بكتيرية فقط. هذا و اختلفت الفعالية النسبية للمعاملات السمادية، مقارنة ببعضها البعض، باختلاف الصفة النباتية موضع الدراسة، فعلى سبيل المثال أعطت المعاملة (١) أعلى القيم لارتفاع النبات، في حين أعطت المعاملتان (٧) و (١٠) أكبر قطر للساق، وأعطت المعاملتان (٨) و(٧) أكبر عدد من الأفرع (في الموسم الأول والموسم الثاني، على التوالي). وبالتالي تختلف المعاملة السمادية الموصى بها تبعاً للتأثير المطلوب.