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RESPONSE OF ARTEMISIA PLANTS (*Artemisia cina* L.) TO BIOORGANIC AND INORGANIC FERTILIZERS UNDER CULTIVATION ON CALCAREOUS AND SANDY SOILS.

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ABSTRACT

In this study, a greenhouse pot experiment was designed to investigate the response of Artemisia plants to fertilization with different nine treatments as a combination of biofertilizers (*Azotobacter* + *Azospirillum* + phosphate dissolving bacteria) with different forms of organic and/or inorganic fertilizers in two different soil types (sandy soil from Wadi El-Natrun and calcareous soil from El-Khatataba), which are commonly found in the Egyptian desert soils.

Obtained data showed that the tested Artemisia plants significantly responded to biofertilization treatment, which was very clear in the enhancing the values of both fresh and dry weights of roots and shoots of this herb, total nitrogen and phosphorous content, and its quality as indicated by the percentage of the main active substances (cineol and myrecen) characteristic of this medicinal plant. Again, the antimicrobial activities of artemisia essential oil against some tested organisms [G- and G+ bacteria as well as fungi and yeast] were positively affected due to bio-fertilization and/or organic manuring. The best measurements were obtained by using the treatment no. 8, which included the application of *Azotobacter* + *Azospirillum* + phosphate dissolving bacteria + full dose of K + quarter-dose of N + rock phosphate + sheep dung manure. These results support the emphatic idea for using biofertilization for reducing the costs of mineral fertilization and the problems of environmental pollution.

INTRODUCTION

The Artemisia plants comes from semi-arid areas of the northern hemisphere and are cultivated for their feathery foliage and their fragrance. The unexpanded flower heads of Artemisia (*Artemisia cina* L., Family: Compositae) has long been in official uses in different pharmacopoeias. The drug, as well as, the sesquiterpene lactone and sntonin, obtained from it, have been formerly used as anathematic for *Ascaris lumbricoides* in human and for round worms in swine, dogs and cats (El-Sayed *et al.*, 1988). Also, Rao *et al.* (1997) and Gersh (2001) reported that the essential oil of Artemisia herb, extracted from the shade dried flowering, is recognized as one of the most useful essential oils for formulating natural flavours that are used in cakes, pastries, beverages and tobacco in USA, Europe and Japan. The oil is also used in expensive perfumes and cosmetics composition. Also, they added that *Artemisia annua* is an herb used in China as traditional medicine for the treatment of fever and malaria.

In Egypt, more attention has been made on the cultivation of this herb, especially in the newly reclaimed lands and desert soils, for its importance in therapeutic purposes in our life, as well as for exportation. On the other hand, the essential oil of this herb possessed anti-spasmodic activity (Yashpe *et al.*, 1987), anti-microbial activity (El-Sayed *et al.*, 1988),), anti-fungal activity (Swiader and Krzyzanowska, 1997), anti-bacterial activity against Gram-negative and Gram-positive bacteria (Essawi and Srour, 2000 and Mohamed *et al.*, 2000).

In the light of these importance, this study was designed to investigate the response of Artemisia plants, which commonly found in Egyptian deserts and grown on sandy and calcareous soils, to inoculation with biofertilizers (*Azotobacter* + *Azospirillum* and/or phosphate dissolving bacteria) in different combinations with organic and inorganic fertilizers on plant productivity, oil content, essential oil percentage and oil composition in two different soils (calcareous and sandy soils) in order to improve the growth of this medicinal plant to maximize its content of medicinal substances. Also, the antimicrobial activity of the essential oil, obtained from the best treatment under this study, was determined against some tested microorganisms.

MATERIALS AND METHODS

In this study, a pot experiment was set up in the greenhouse of Soil Fertility and Microbiology Department, Desert Research Center, Ministry of Agriculture and Land Reclamation, Cairo, Egypt, in order to study the response of Artemisia plants to fertilization with different combinations from bioorganic and inorganic fertilizers.

Soil Types:

Two different types of soils were used in this experiment. The first one was calcareous soil from El-Khatataba, while the second was sandy soil from Wadi El-Natrun. The procedures involved in soil collection, airdrying, milling, mixing and soil analyses were employed as mentioned by Gewaily *et al.* (2006). The properties of the tested El-Khatataba and Wadi El-Natrun soils were as following: pH, 7 5 and 7.3; EC, 1.81 and 2.13 ds/m; CaCO3, 30.2 and 1.1%; organic matter , 0.17 and 0.20%; sand , 73.9 and 94.5%; silt, 12.5 and 3.0%; clay , 13.6 and 2.5% in calcareous and sandy soils, respectively.

Organic Amendments:

Sheep dung manure (SD) and rice straw (RS) were brought from the Mariyut Research Station of DRC, Egypt. Preparation of these materials and their analyses were carried out according to Page *et al.* (1982) and recently published by Gewaily *et al.* (2006). These materials were added at the rate of 2% of the soil weight before cultivation.

Mineral Fertilization:

Ammonium sulphate (21.5% N), Calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) were applied according to the recommendation of Abou Zaid (1992) at the rate of 150, 100 and 50kg/fed., respectively. Nitrogen and potassium fertilizers were added in three split applications. The time of application was similar to that employed by Gewaily *et al.* (2006). Also, rock phosphate (RP) was added at the rate of 30 kg/fed., and it was applied prior to planting.

Seedlings used:

Healthy seedlings of Artemisia plants ((*Artemisia cina* L.) were obtained from the Medicinal and Aromatic Plants Department of the DRC, Ministry of Agriculture and Land Reclamation, Cairo, Egypt. They were inoculated and planted in pots and fertilized with mineral, organic and/or biofertilizers according to the applied treatments.

Preparation of Inocula and Cultivation:

Two different isolates of N_2- fixers (*Azotobacter* and *Azospirillum*) and one isolate of phosphate dissolving bacteria were isolated and selected (Allen, 1971) from the soil of the rhizosphere regions of some medicinal and aromatic plants, which commonly grown naturally in Wadi Hagoul region (Gewaily *et al.*, 2006).

Two heavy cell suspensions of efficient isolates of *Azotobacter* and *Azospirillum* were obtained by growing them separately on modified Ashby's medium (Abd El-Malek and Ishac, 1968), and on semi-solid malate medium (Dobereiner, 1978), respectively for 7 days at 28 ± 2°. Also, heavy cell suspension of phosphate dissolving bacteria was prepared by growing on modified Bunt Rovira medium (Abd El-Hafez, 1966) for 4 days at 28 ± 2°.
Suspensions of these isolates containing about 10^8 cell/ml were used as standard inocula. The mixed inoculum was prepared from cell suspension of each isolate at ratio 1:1 for *Azotobacter* + *Azospirillum* and 1:1:1 for *Azotobacter* + *Azospirillum* and phosphate dissolving bacteria. Roots of the seedling of Artemisia plants were washed by water and then immersed into inoculum for 10 minutes and planted immediately in the pots containing either of the two different soils.

Layout of Pot Experiment and Treatments:

In this experiment, pottery pots of 30 cm diameter were filled with 7 kg of each soil, and 108 pots were used representing the combination of 9 treatments, 2 soils (sandy and calcareous soils), 3 cuts and 6 replicates. These pots were arranged inside the greenhouse in randomized complete block design. The experiment consists of the following treatments:

1. Un-inoculated seedlings + full dose of N, P and K.
2. Un-inoculated seedlings + full dose of N, P and K + 2% sheep dung manure (SD).
3. Un-inoculated seedlings + full dose of N, P and K + 2% rice straw (RS).
4. Inoculation with PDB + full dose of N, P and K and rock phosphate (RP).
5. Inoculation with *Azotobacter* + *Azospirillum* + full dose of P and K.
6. Inoculation with *Azotobacter* + *Azospirillum* + full dose of P and K + quarter-dose of N.
7. Inoculation with *Azotobacter* + *Azospirillum* + full dose of P and K + half-dose of N.
8. Inoculation with *Azotobacter* + *Azospirillum* + PDB + full dose of K + quarter-dose of N +RP + SD.
9. Inoculation with *Azotobacter* + *Azospirillum* + PDB + full dose of K + quarter-dose of N +RP + RS.

The pots were irrigated with tap water to maintain the soil water content at 50% of the W.H.C. in each soil in the pot and throughout the time course of the experiment (12 months). Only one plant was left for each pot.

Each treatment had six replicates for both studied soils. The fertilizers were added to each pot in amounts equal to the recommended dose (dissolved in water), as well as due to the treatments under the study. All the required management practices were carefully employed till the end of the experiment.

Plant Parameters Determination:

Fresh and dry weight of roots and shoots of plant samples were determined, and recorded as g/plant after each cut (after 4, 8 and 12 months) in both of the tested soils. Total nitrogen and phosphorous were determined in plant samples due to modified Kjeldahl method (Chapman and Pratt, 1961), and ascorbic acid method (Watanabe and Olsen, 1965), respectively.

Oil Parameters Determination:

The essential oil percentage in plant samples was determined according to A.O.A.C. (1990). The oil extracted from plant samples of control and the oil of the best treatment of the second cut, which gave the highest essential oil percentage were analyzed using Gas Liquid Chromatography, GLC/PYE, UNI-CAM-PRO-GC, to identify and determine the chemical components of the obtained oils. This analysis was done in the Central Laboratory, Faculty of Agriculture, Cairo University, Egypt.

Antimicrobial Activity of Essential Artemisia Oil:

Antimicrobial activity of the tested oil sample, which gave the highest essential oil percentage, was estimated against four bacterial species namely *Bacillus subtilis*, *B. megaterium* (as G+), *Escherichia coli* and *Pseudomonas fluorescence* (as G-), one mold (*Fusarium oxysporum*) and one yeast (*Saccharomyces cerevisiae*) using the agar-diffusion technique, (filter paper disc 6 mm), due to the method of Maruzzella and Balter (1959). The tested organisms were obtained from Microbiology Unit, Fertility and Soil Microbiology Department, Desert Research Center, Cairo, Egypt.

Nutrient agar, Czpek's Dox and malt extract agar media were prepared as described by Jacobs and Gerstein (1960), Oxoid (1982) and Harrigan and McCane (1966), then inoculated and incubated to test the antimicrobial activity of Artemisia essential oil against the tested organisms (bacteria, mold and yeast), respectively. The inhibition zones (mm) of the microbial growth were measured after 3 days of infection.

All samples and determinations were carried out in triplicates and the data were analyzed statistically according to standard statistical methods. Analysis of variance was calculated and means were differentiated by using L.S.D. (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Shoot fresh and dry weights

Data illustrated in Figures (1A and 1B) indicate that the fresh and dry weights of Artemisia plants increased with application of all treatments including bioorganic and inorganic fertilizers compared with the control treatment, which received full dose of NPK alone. This was evidenced, when the plants were inoculated with tri-mixture inoculants of *Azotobacter* + *Azospirillum* + phosphate dissolving bacteria and the soil received K + 1/4 N + rock phosphate + sheep dung manure (treatment no. 8).

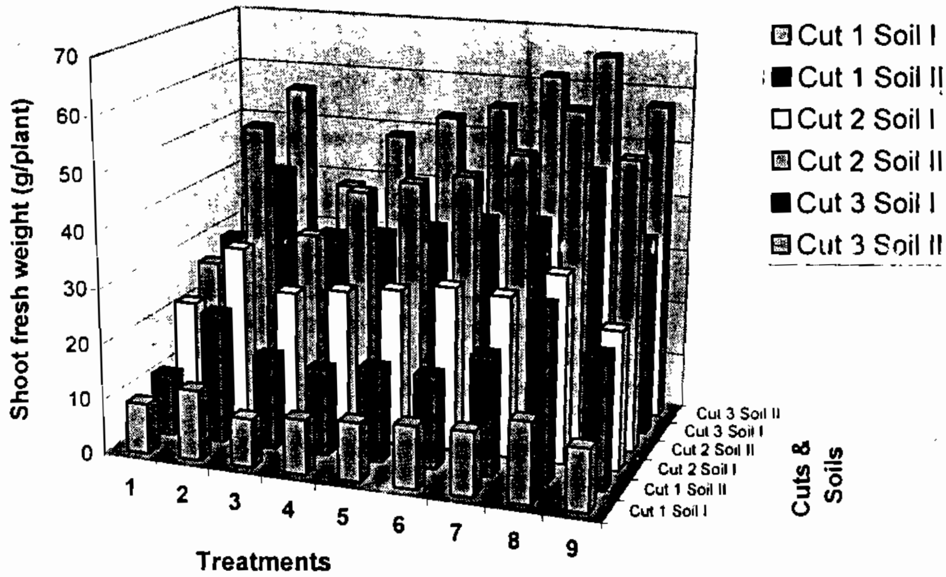

Fig. (1A) Shoot fresh weight (g/plant) of Artemisia plants in three cuts as affected by the applied fertilization treatments in the tested soils.

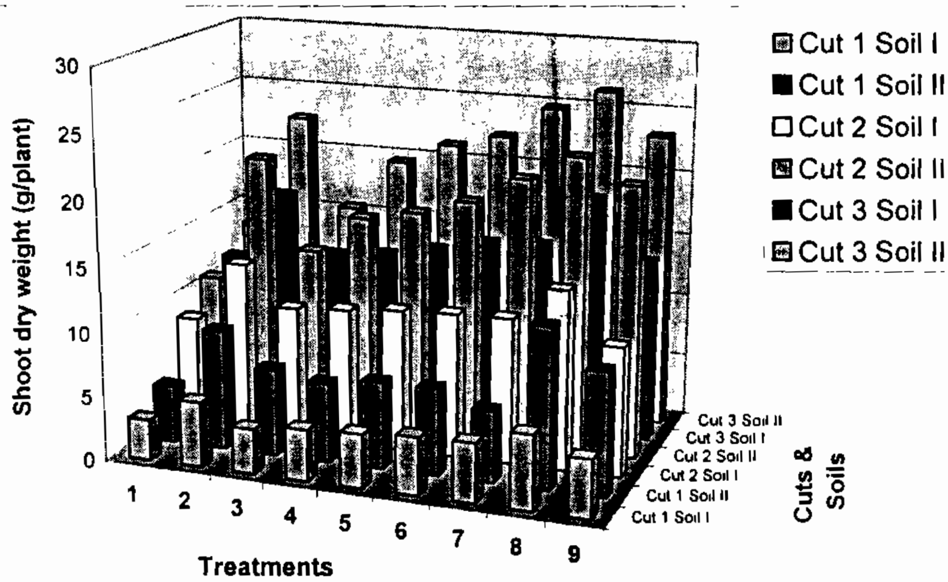

Fig. (1B) Shoot dry weight (g/plant) of Artemisia plants in three cuts as affected by the applied fertilization treatments in the tested soils.

This treatment gave the highest values of fresh weights of shoots, namely 14.6 and 31.3 g/plant in the first cut for plants grown on sandy and calcareous soils, respectively. The respective values in the second cut were 33.9 and 60.0 g/plant (fresh weights) and 14.0 and 23.0 g/plant (dry weights) in both tested soils respectively, while they were 47.1 and 65.9 g/plant (fresh weights), and 18.5 and 18.9 g/plant dry weights in the third cut, in both tested soils, respectively.

Generally, it was observed that biofertilizers significantly increased the values of fresh and dry weights of Artemisia shoots as well as the organic fertilizers. Many investigators came to the same general conclusion. Among them are Haggag *et al.* (1994), Barakah *et al.* (1995), El-Khyat and Zaghloul (1999), Abo El-Ala (2002) and Migahed *et al.* (2004).

It is clear also that shoot fresh and dry weights of the tested plants increased in the second and third cuts than those recorded in the first cut. This was true for all applied treatments throughout the experiment. This could be explained on the basis of the growth stages of this herb.

Replacement of either inorganic nitrogen fertilizers by biofertilizers namely mixture of *Azotobacter* + *Azospirillum* or phosphate dissolving bacteria had remarkable effect on shoot fresh and dry weights of Artemisia plants. However, it could not reach those receiving 1/4 the recommended dose of nitrogen together with biofertilization. This emphasizes the necessity for the application of small amounts of inorganic nitrogen to meet the requirement of the plants during the first growth stage.

The obtained results in this work support those mentioned by El-Khyat and Zaghloul (1999). They emphasized the impact of biofertilizers application on medicinal plants for the highest records of growth parameters and seed yield of carawy plants to inoculation with *Azotobacter chroococcum* or *Azospirillum lipoferum*. In the same line, Migahed *et al.* (2004) found that inoculation *Apium graveolens* plant with tri-mixture of *Azotobacter* + *Azospirillum* + phosphate dissolving bacteria in calcareous soil increased plant growth to significant extents comparing with uninoculated ones. Again, the beneficial effects of *Azotobacter* and *Azospirillum* on plant development can be attributed not only to N_2-fixation process (Ishac *et al.*, 1993; and Kader *et al.*, 2000) but also to the production of growth promoting substances such as auxines, gibberellins and cytokinins (Okon, 1984 and Tawfik et al, 2001).

Data show also that omitting superphosphate and inoculating the plants with phosphate dissolving bacteria + rock phosphate + NK (treatment no. 4), the fresh and dry weights of shoots of Artemisia plants were significantly increased in all cuts comparing with the control treatment with full dose of NPK, especially those grown on calcareous soil of El-Khatatba area. These results revealed the impact of inoculating Artemisia plants with phosphate dissolving bacteria, which had a great role in solublizing the inorganic phosphate to be available for the plant growth stages, instead of applying superphosphate to reduce the costs and soil contamination with the excess of inorganic fertilizers. These results are in agreement with those reported by Haggag *et al.* (1999), Barakah *et al.* (1998) and Abo-El-Ala (2002), worked on *Artemisia cina* and stated that amended plant with half dose of N and P fertilizers and inoculated with multi-biofertilzers inoculant including phosphate dissolving bacteria gave the highest weight as compared with control.

Results indicated also that addition of sheep dung manure as organic manure + NPK (treatment no. 2) to both soils raised their productivity over those fertilized with inorganic fertilizers of NPK alone (control treatment no. 1). This treatment ranked second in productivity of the tested plants following treatment no. 8. Also, comparing treatments of the soils with sheep dung as organic manure + NPK (treatment no. 2), and its treatment with rice straw (RS) + NPK (treatment no. 3), significant differences among fresh and dry weight values of Artemisia shoots were observed between the two tested organic materials with high prevalence of the sheep dung application. This difference could be due to wide difference in the C/N ratio of the applied rice straw and sheep dung manures.

Data show also that shoot fresh and dry weights of Artemisia plants grown on El-Khatatba calcareous soil gave higher values than that recorded on plants grown on Wadi El-Natrun sandy soil. This could be attributed to the differences in the physiochemical properties of the tested soils.

Roots Fresh and Dry Weights

The values of fresh and dry weights of Artemisia plants grown on calcareous and sandy soils as affected by application of biofertilizers together with the application of inorganic and organic amendments are presented diagrammatically in Figures (2A and 2B). The first weights are similar to those observed for fresh and dry weights of Artemisia shoots. Significant increases in the values of fresh and dry weights of roots were recorded in response to bio-inoculation with asymbiotic N_2-fixers (*Azotobacter* and *Azospirillum*) and phosphate dissolving bacteria (PDB) in the presence of either inorganic or organic amendments as compared with uninoculated treatments or with the application of mineral fertilizers of N, P and K at full dose alone. This was true in all the three cuts of Artemisia plants.

It is also clear from the same data that inoculating the plants with biofertilizers is promotive of root growth as these biofertilizers include *Azospirillum* bacteria that excretes promoting substances to root development through encouraging and enhancing mineral and water uptake by these roots. Okon (1984) assured the benefits of *Azospirillum* to plant roots by a mechanism related to enhancement of plant roots growth and functioning.

The highest values of fresh and dry weights of roots of the tested plants were recorded when the plants were inoculated with tri-mixture biofertilizers of *Azotobacter* + *Azospirillum* + phosphate dissolving bacteria in the presence of K + 1/4 N + rock phosphate + sheep dung manure (treatment no. 8). This treatment gave the highest figures for the fresh and dry weights of roots of the tested herb, followed by treatment no. 2, in which the soil was treated with NPK + sheep dung manure referring to the importance of organic manure in plant nutrition. On the other hand, the control treatment, without bioinoculation – no. 1, had the lowest values of the fresh and dry weights of Artemisia roots.

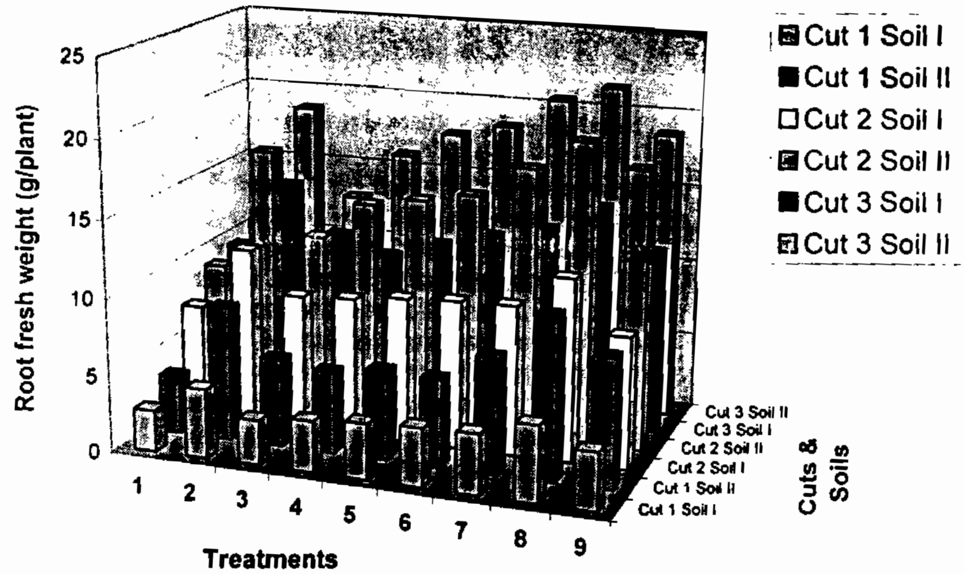

Fig. (2A) Root fresh weight (g/plant) of Artemisia plants in three cuts as affected by the applied fertilization treatments in the tested soils.

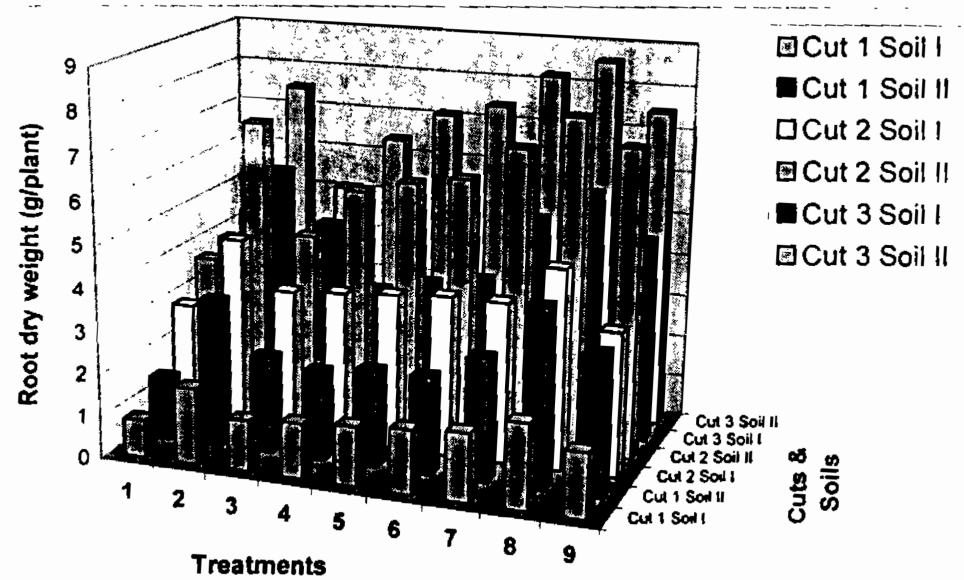

Fig. (2B) Root dry weight (g/plant) of Artemisia plants in three cuts as affected by the applied fertilization treatments in the tested soils.

Again, the treatment no. 8 demonstrated that the fresh and dry weight values of roots in sandy soil were 4.0 and 2.0 g/plant, 11.8 and 4.7 g/plant and 14.5 and 5.8 g/plant in the first, second and third cuts, respectively. While in calcareous soil, however, the respective values were 10.5 and 4.2 g/plant, 19.5 and 7.8 g/plant and 21.5 and 8.6 g/plant in the first, second and third cuts, respectively.

Data show also that the values of roots fresh and dry weights of Artemisia plants grown on calcareous soil were as double as, in general, those grown on sandy soil in response to many of the applied treatment. This assures the role of the soil as nutritional reservoir and the organic matter content of medicinal plants and the micro-environment for specific microbes transforming different elements. Clark and Duncan (1993) and Senigagliesi and Ferrari (1993) reported that organic matter plays a fundamental role in structural stability of the soil.

Total Nitrogen Content in Shoots

Data presented in Figure (3) demonstrate that, in treatment no.8, the nitrogen values of Artemisia shoots grown on sandy soil were 144, 526 and 917 mg/plant for the first, second and third cuts, respectively. The respective values for those grown on calcareous soil were 323, 801 and 1151 mg/plant. However, the respective values of the control (NPK only without bio-inoculation) were 42, 148 and 242 mg/plant for those grown on sandy soil, and 84, 227 and 386 mg/plant for those grown on calcareous soil.

Results also revealed that application of organic fertilizers in the presence of NPK dose increased the values of nitrogen content as compared with control treatment (NPK only). However, sheep dung treatment was superior to treatment with rice straw in the two studied soils. The high nitrogen content of sheep dung (2.12%), compared with the low nitrogen content in rice straw (0.9%), could be quite satisfactory for the growth of Artemisia plants resulting in such high increase in the nitrogen value.

Inoculation with N_2-fixing bacteria + PK (treatment no. 5) without inorganic N ferlilzer significantly increased the nitrogen content in shoots of Artemisia plant grown on both soils compared with the control, which received NPK only. However, application of 1/4 or 1/2 dose of N in combination with biofertilizers increased the values of nitrogen content. This refers to the usefulness of partially replacing inorganic nitrogen by asymbiotic N_2-fixers to reduce the costs of medicinal plants fertilization and decrease soil pollution with the excess nitrate-N as well. In this concern, Saleh *et al.* (1998) in their work on datura plant found that dual inoculation with asymbiotic N2-fixers and VAM in treatment supplemented with full dose of rock phosphate significantly increased the dry matter, nitrogen content and alkaloids, in datura plants. However, Badawi (2000) who found that the experiment on roselle plant in North Sinai using full dose of NPK + biofertilizers (*Azotobacter* + *Azospirillum*) gave the highest seeds and sepals yields.

Generally, the data show also that total nitrogen content of Artemisia plants grown on calcareous soil increased significantly than that of those in sandy type. The mean values of nitrogen content in the first one were 169, 494 and 709 mg/plant for the three cuts, respectively. While, the respective values in the second one were 94, 314 and 501 mg/plant.

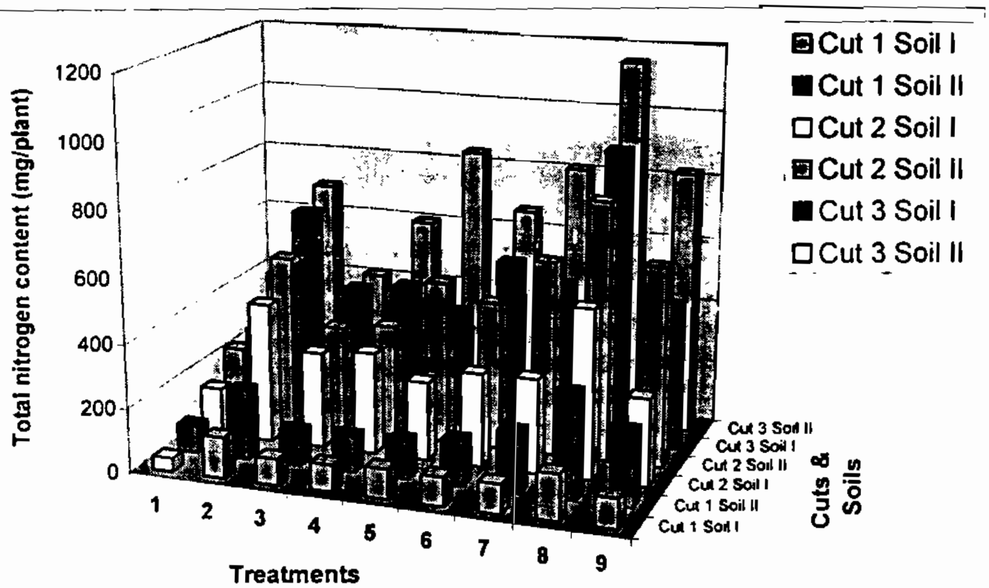

Fig. (3) Total nitrogen content (mg/plant) of Artemisia plants in three cuts as affected by the applied fertilization treatments in the tested soils.

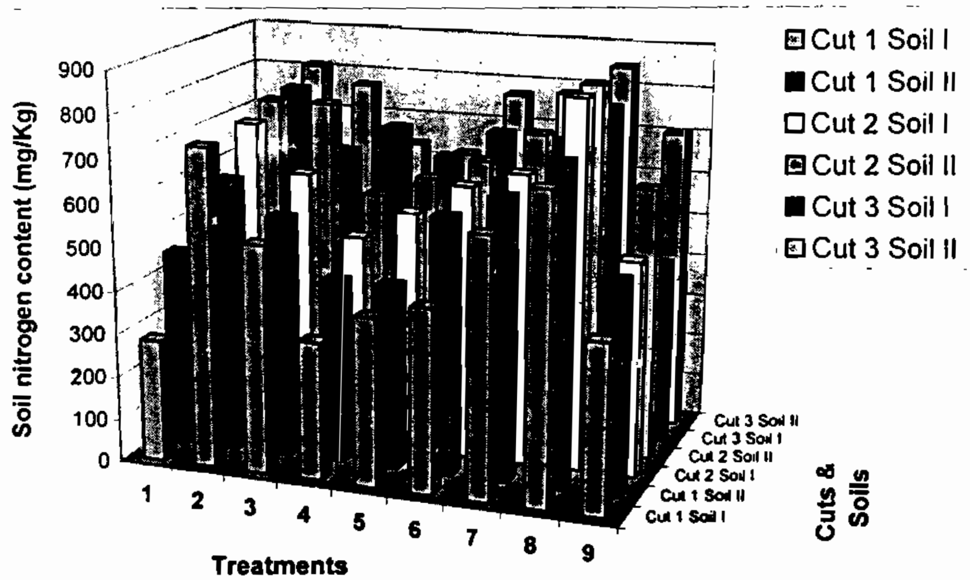

Fig. (4) Total nitrogen content (mg/Kg dry soil) under Artemisia plants in three cuts as affected by the applied fertilization treatments in the tested soils.

This is due to the differences in the physicochemical properties and fertility status of the two tested soils.

Soil Nitrogen Content

Generally, data in Figure (4) show that soil nitrogen content increased in both soils as a result of treatment with biofertilizers and/or organic manure as compared with the control treatment. However, the magnitude of increase varied according to soil types as well as to the applied treatment. Calcareous soil recorded higher average values than the sandy soil being 553, 685 and 730 for the first soil and 487, 605 and 639 mgN/Kg dry soil for the second soil in the three cuts, respectively. Addition of sheep dung + NPK to the sandy soil (treatment no. 2) raised the values to more than double of those with the control, where it gave 738, 740 and 776 mgN/Kg dry soil after the first, second and third cuts, respectively. For calcareous soil, however, the respective values were 639, 770 and 810 mg N/Kg dry soil. The use of biofertilizers (*Azotobacter* + *Azospirillum*) instead of inorganic nitrogen (treatment no. 5) brought about little increases in soil nitrogen of both soils. But, when half the N dose was added to the previous treatment, treatment no. 7, a remarkable increase in soil nitrogen was observed in both soils under study after three cuts.

In the present work, treatment with phosphate dissolving bacteria (PDB) together with the N2-fixers (*Azotobacter* + *Azospirillum*) and in the presence of rock phosphate and organic manure (SD) + 1/4 N + K (treatment no. 8) raised the values of soil nitrogen to reach their peak in both studied soils comparing with the control treatment (NPK only). The soil nitrogen values with the last treatment were 707, 849 and 780 mg N/kg dry sandy soil in the three cuts, respectively. The respective values in calcareous soil were 735, 855 and 850 mg N / kg dry soil. The aforementioned values were almost equal to those amended with sheep dung + NPK. This means that using sheep dung as organic manure could have an additional increases in soil nitrogen that were equal to the amount added by treating the soil with treatment no. 8. These results are in agreement with those reported by Ishac *et al.* (1986) and Fayez (1990). They attributed the increase in soil nitrogen to inoculating the soil with *Azotobacter* + *Azospirillum* owing to their great fixation of atmospheric nitrogen reflecting the increases in soil nitrogen and plant growth.

Total Phosphorous Content in Shoots

Data presented in Figure (5) indicate that phosphorous contents in Artemisia plants grown on calcareous soil of El Khatatba were higher than those recorded in plants grown on the sandy soil of Wadi El-Natrun. This was true in the first, second and third cuts, being in the mean values of 18.7, 73.8 and 69.5 mg/plant for the first one, and 10.8, 42.2 and 45.4 mg/plant for the second soil in the first, second and third cuts, respectively.

In addition, inoculation of the tested plants with phosphate-dissolving bacteria (PDB) + rock phosphate (treatment no. 4) significantly increased the values of plant phosphorus content than those receiving sheep dung or rice straw + NPK (treatments no. 2&3). This has been observed in the three cuts and also in the plants grown on calcareous and sandy soils. Also, replacement of inorganic nitrogen totally by inoculating Artemisia plants with a mixture of *Azotobacter* + *Azospirillum* (treatment no. 5) significantly increased the values of plant phosphorus content as compared with the control treatment, which received full dose of NPK. The values did not reach those obtained when 1/4 or 1/2 the dose of N was applied to the soil indicating the necessity of addition of part of plant N requirement as inorganic nitrogen especially in the first plant growth stages.

In the same line, treatment no. 8, in which the soil received organic and biofertilizers + K + RS +1/4 N + SD, gave the highest phosphorus figures among the different treatments under investigation, ensuring the beneficial effect of replacing inorganic phosphorus and nitrogen fertilizers with biofertilizers to reduce the costs and decrease soil pollution with inorganic fertilizers. These results confirm those obtained by Abou-Aly and Gomaa (2002). They found that inoculation of coriander plants with *Azotobacter chrococcum* + *Azospirillum brasilense,* combined with *Glomus mossede* gave significant increases in N, P and K contents in the plants.

In general conclusion, the obtained results emphasize the importance of the use of biofertilizers, phosphate dissolving bacteria, rock phosphate and sheep dung manure to increase the total phosphorus content in the tested Artemisia plants.

Essential oil content (%)

Results in Figure (6) demonstrate that there were a significant increased values of the essential oil content of Artemisia plants, not only due to different bio-inoculation treatments as compared with the control treatments, but also due to the soil type used in this study. In addition, treatment no.8 in both soils with K + 1/4N + rock phosphate + sheep dung and inoculating the plants with tri-mixture of *Azotobacter* + *Azospirillum* + phosphate dissolving bacteria gave highest oil percentage as compared with control treatment, which received NPK only. These results are in consistence with those reported by Neelima and Janardhanan (1996), since they stated that dual inoculation of VAM + *Azospirillum brasilense* increased the growth, yield and oil content of palmorosa significantly over the uninoculated control.

Again, the treatment no. 8 gave the highest results being 1.31, 1.36 and 1.25% for the plants of Artemisia in the first, second and third cuts, respectively, in case of plants grown on Wadi El-Natrun sandy soil. While, the respective values were 1.46, 1.51 and 1.41% in case of El-Khatatba calcareous soil, respectively. Data also show that when sheep dung was applied to the soil receiving NPK (treatment no. 2), a significant increase in the plant oil percentage was also observed as compared with the plants of the control treatment, NPK only. Their recorded values were between those treated with biofertilizers + K + 1/4 N + rock phosphate + sheep dung manure (treatment no. 8) and the control treatment, no. 1. The same observation was also recorded when comparing the results of treatments no. 8 and no. 9 when rice straw replaced by sheep dung. This was most probably due to the wide C/N ratio of the rice straw (77) compared with (10) for sheep dung, affecting the plant growth and consequently reflected on plant components especially oil percentage in such medicinal plants.

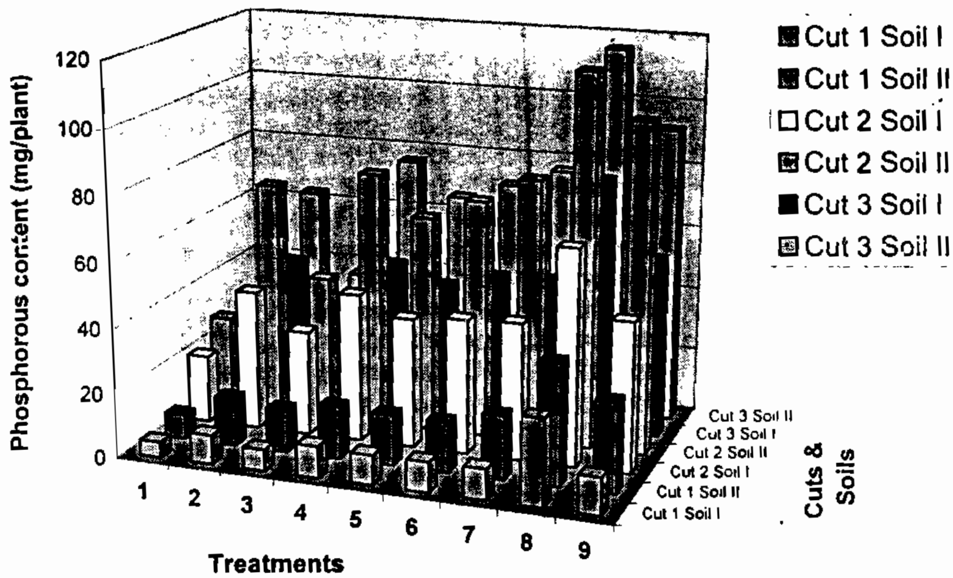

Fig. (5) Phosphorus content (mg/plant) of Artemisia plants in three cuts as affected by the applied fertilization treatments in the tested soils.

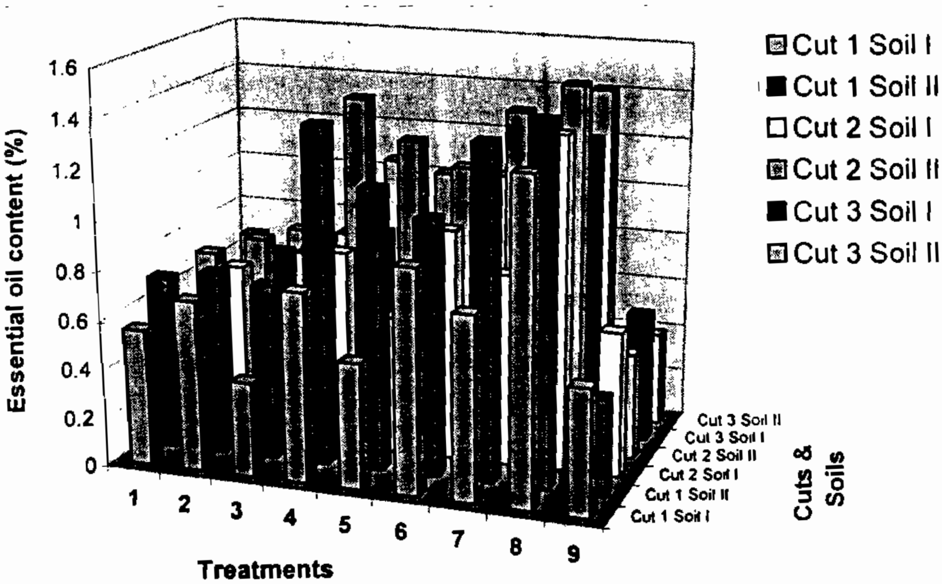

Fig. (6) Essential oil content (%) of Artemisia plants in three cuts as affected by the applied fertilization treatments in the tested soils.

These general results were similar to those observed in the previously determined parameters in this study, indicating the usefulness of biofertilizers application in replace of inorganic nitrogen and phosphorous fertilizers. In addition, these results support the data reported by those of Ibrahim (2000) who found that inoculation of sweet fennel plants with *Azotobacter* + *Azospirillum* in the presence of NPK gave the tallest plants, more umbles per plant and higher seed and oil yields. Abou-Aly and Gomaa (2002) obtained also similar results with coriander (*Coriandrum sativum*) concerning the increase of its oil content due to biofertilizer application using *Azotobacter chrococcum* or *Azospirillum brasilense* combined with *B. megaterium* var. phosphaticum. In addition, Migahed *et al.* (2004) found that mixed inoculants of *Azotobacter* + *Azospirillum* + PDB increased oil yield of *Apium graveolens* seeds grown on calcareous soil as much as three fold when compared with the uninoculated control.

Essential Oil Components

Data in Table (1) show the GLC profile analysis of the essential oil extracted from two representative samples of Artemisia plants grown on sandy soil of Wadi El-Natrun as well as plants grown on calcareous soil. The first samples were taken from Artemisia plants grown on the best treatment (no. 8) of the second cut, which gave the highest essential oil percentage, and the second one taken from the control plants (treatment no. 1). Data revealed that the profile analysis exhibits 5 different characteristic components, namely α-pinene, β-pinene, thujone, myrecen and cineol at different concentrations. The detected components represent the main important active substances characterized the essential oil of Artemisia plants. Their concentration differed, to some extent, in the essential oil of the best treatment (no. 8) as compared with the control treatment (no. 1). For example, cineol concentration in the oil extracted from plants grown on sandy soil were 45.52% and 40.40% for treatment plants and control. While the respective values in the oil of the plants grown on calcareous soil were 54.28% and 51.79% for the treated plants and control, respectively. This refers to higher concentration of some oil components due to biofertilization and organic and inorganic fertilizer application as compared with the oil extracted from plants of the control. There were some differences in α-pinene and β-pinene and thujone that have been detected in oil extracted from Artemisia plants grown on sandy soil, but not detected in the oil of plants grown on calcareous soil. Also, the concentration of cineol component of oil extracted from plants grown on calcareous soil exceeded cineol in plants grown on sandy soil.

Table (1) Gas liquid chromatography analysis of the essential oil (%) of Artemisia plant shoots.

	Sandy Soil		Calcareous Soil	
	Control	Best treatment	Control	Best treatment
α-pinene	0.12	0.11	Traces	Traces
β-pinene	0.10	0.12	0.06	Traces
Thujone	Traces	0.48	Traces	Traces
Myrecen	19.05	18.04	13.62	15.19
Cineol	40.40	45.52	51.79	54.28

The essential oil of the treatment, which received biofertilizers (Az. +Azosp. +PDB) + organic and inorganic amendments (treatment no. 8) as compared with the control, which received NPK only.

These results confirm those obtained by Stefania Nin *et al.* (1995), since they reported that the essential oil of *Artemisia absinthium* analyzed by GC were 90 components occurred on trace amounts. Eight components, which have reported to have antibacterial activity (α- and β- thujone, terpinen, linalool, nerol, geraniol, α-pinene and 1,8-cineol). However, Milka *et al.* (1997) reported that the chemical composition of essential oil of 16 samples of *Artemisia lecebiana* analyzed by GC and GC/MS, the main components were 1,8-cineole (1.8 – 45.5), α-thujone (2.5 – 47.8%), β- thujone (0.0 - 34.5%), camphor (0.0 – 45.7%), borneol (0.0 – 16%), cis-sabino (0.0 – 25.6%), dovanone (0.0 - 38.3%) and β-cubebere (2.6 – 20.8%).

Antimicrobial Activity

Data in Table (2) demonstrate the diameter of inhibition zones due to the antimicrobial activity of the essential oil extracted from Artemisia plants under cultivation on sandy and calcareous soils against some representative G(-) and G(+) bacteria, as well as against certain fungi.

Presented data show that the oil extracted from plants treated with the tested nine treatments due to different amendments significantly showed high values of inhibition zones as compared with the control. Also, treatment no. 8, in which the tested soils were treated with biofertilizers (*Azotobacter* + *Azospirillum* + phosphate dissolving bacteria) + K + 1/4N + rock phosphate + sheep dung manure gave the best results concerning the oil potency against *E. coli* (21 and 19 mm), *Pseudomonas fluorescence* (27 and 28 mm), *B. subtilis* (26 and 25 mm), *B. megatherium* (32 and 34 mm), *Fusarium oxysporum* (27 and 23 mm) and *Saccharomyces cerevisiae* (23 and 21 mm) in both sandy and calcareous soils, respectively. This could be attributed to the highest concentration of the active components of the essential oil due to biofertilization or organic manure application affecting the property of oil such as crude alcoholic extracts like α-pinene, β-pinene, thujone, myrecen and cineol, which characterize the essential oil of Artemisia plant as indicated in the profile of the oil components of the treated plants. These results confirm those obtained by Yashpe *et al.* (1987) in their study on the antimicrobial and antispasmodic activity of *Artemisia herba*. They found that all the oils showed antibacterial activity especially against *Pseudomonas aerogenosa* and *Serratia marcesens*. In addition, Stefania Nin *et al.* (1995) reported that the essential oil of Artemisia absinthium analyzed by GLC had eight components, which have reported to have antimicrobial activity (α- and β- thujone, terpinen, linalool, nerol, geraniol, α-pinene and 1,8-cineol).

The highest inhibition zones were obtained from essential oil extracted from Artemisia plants treated with biofertilization and/or organic and inorganic fertilizers (treatments no. 7 and no. 8). Also, the tested G(+) bacteria, *B. megaterium* showed highest sensitivity (35 mm) of the oil extracted from plants grown on calcareous soil of El-Khatatba and treated with biofertilizers (*Azotobacter* + *Azospirillum* + P + K + 1/2N, treatment no. 7).

Table (2) Antimicrobial action of Artemisia essential oil against bacteria, fungi and yeast calculated as the diameter of inhibition zone (mm) as affected by the applied treatments of fertilization in the two tested soils.

Treatments	Gram negative bacteria						Gram positive bacteria						Fungi			Yeast		
	Escherichia coli			*Pseudomonas fluorescence*			*Bacillus subtilis*			*B. megaterium*			*Fusarium oxysporum*			*Saccharomyces cerevisiae*		
	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean	Soil I	Soil II	Mean
1	14	13	14	14	11	13	14	14	14	15	11	13	13	13	13	14	14	14
2	15	13	14	15	12	14	16	14	15	17	12	15	16	13	15	15	13	14
3	15	14	15	15	12	14	16	15	16	16	12	14	15	14	15	16	14	15
4	16	15	16	17	13	15	22	23	23	16	13	15	14	13	14	18	16	17
5	16	14	15	17	14	16	23	23	23	17	14	16	19	12	16	18	17	18
6	18	17	18	15	13	14	23	21	22	19	21	20	20	16	18	19	17	18
7	18	18	18	21	29	25	25	18	22	27	35	31	20	17	19	21	20	21
8	21	19	20	27	28	28	26	25	26	32	34	33	27	23	25	23	21	22
9	19	18	19	27	26	26	25	22	24	27	21	24	25	22	24	22	21	22
Mean	17	16		19	18		21	19		21	19		19	16		18	17	
$LSD_{(5\%)}$																		
Soils			1			1			1			1			1			1
Treatments			1			2			2			2			2			2
Interaction			2			3			3			3			2			2

Soil I = Wadi El-Natrun soil Soil II = El-Khatatba soil

It seem that biofertilization supported the medicinal plants with the required macro- and micro-nutrients, which reflected on the quality and quantity of the oil component and raising the percent of the active components of the Artemisia oil as bactericidal and/or fingicidal substances. Data revealed that the potency of the Artemisia essential oil against G(-) represented by *E. coli* and *Pseudomonas fluorescence* was higher than those obtained against G(+) bacteria (*B. subtilis* and *B. megaterium*) reffering to the higher sensitivity of G(-) than G(+) to such Artemisia oil. These results are in harmony with those obtained by Yadava and Saini (1991), who studied the antimicrobial efficiency of essential oil of *Marjoram hortensis* and found significant activity against *B. anthracis*, *Staph. aureus*, *E. coli*, *Proteus vulagaris*, *Aspergillu niger* and *Asp. fumigatus*.

Generally, it can be concluded that the extreme condition prevailing our desert soils such as sandy soil of Wadi El-Natrun and calcareous soil of El-Khatatba necessitate the introduction of important microorganisms especially N_2-fixers and P-dissolvers together with organic manure. In this work, biofertilization of medicinal plants represented by Artemisia plants and/or organic manure application under cultivation in sandy and calcareous soils of our desert enhanced the vegetative growth, nitrogen content, phosphorous content, and essential oil content. Again, the antimicrobial activity of such oil against G(-) and G(+) bacteria, as well as fungi and yeast were also positively affected due to biofertilization and/or organic manuring.

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إستجابة نباتات الشيح البلدى (*Artemisia cina* L.) للتسميد الحيوى والعضوى والمعدنى تحت ظروف الزراعة فى الاراضى الجيرية والرملية

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فى هذه الدراسة تم تصميم تجربة أصص فى الصوبة الزراعية لدراسة إستجابة نباتات الشيح البلدى للتسميد بتسع معاملات مختلفة وهى عبارة عن توالیف من التسميد الحيوى بإستخدام البكتيريا المثبتة للنيتروجين الجوى (أزوتوباكتر + أزوسبيريللم) والبكتيريا المذيبة للفوسفات فى وجود صور مختلفة من الأسمدة العضوية والمعدنية والزراعة فى تربة رملية من وادى النطرون وتربة جيرية من منطقة الخطاطبة كمثال للاراضى الشائع وجودها فى الأراضى الصحراوية المصرية.

وتشير نتائج هذة الدراسة بان نباتات الشيح البلدى المختبرة تستجيب بصورة معنوية لتأثير التسميد الحيوى المستخدم والذى يكون واضح التأثير فى قيم كل من الأوزان الطازجة والجافة للجذور والمجموع الخضرى لهذا العشب – محتواها من النيتروجين والفوسفور ومحتوى الزيت الطيار وجودتة بتلك النباتات كما تشير بذلك قيم المواد الفعالة الهامة (سينيول – ميريسين) المميزة لهذا النبات الطبى.

أيضا اظهر الزيت الطيار لهذا النبات تأثير موجب ضد نشاط بعض الميكروبات المختبرة من البكتيريا الموجبة والسالبة لجرام بالإضافة للفطر والخميرة وذلك كنتيجة للإستجابة للتسميد الحيوى مع أو بدون التسميد العضوى. وكانت أفضل معاملة والتى أظهرت أعلى القيم فى هذا الخصوص هى المعاملة رقم ٨ والتى تضمنت إستخدام البكيتريا المثبتة للنيتروجين (أزوتوباكتر + أزوسبيريللم) + البكتيريا المذيبة للفوسفات + جرعة كاملة من البوتاسيوم + ١/٤ جرعة من النيتروجين + صخر الفوسفات + سماد الغنم. وهذة النتائج تدعم الفكرة المؤكدة على إستخدام الأسمدة الحيوية لخفض تكاليف إستخدام التسميد المعدنى ولخفض المشاكل الناتجة عن التلوث البيئى.