

ASSOCIATIVE EFFECT OF AZOSPIRILUM LIPOFERUM AND
AZOTOBACTER CHROOCOCCUM WITH RHIZOBIUM SPP. ON MINERAL
COMPOSITION AND GROWTH OF CHICK PEA
-6- (CICER ARIETINUM) ON SANDY SOILS.

MEHRESHAN T. EL-MOKADEM, FATMA A. HELEMISH
ZEINAB, Y.M. ABOU BAKR, AND SOAD A. SHETEAWI

BOT. DEPT., WOMEN'S COLLEGE, AIN SHAMS
UNIV. HELIOPDLIS, CAIRO, EGYPT.

Summary

The interactions between Azospirillum lipoferum, Azotobacter chroococum and Rhizobium spp. were assessed on the growth pattern and mineral concentration of chickpea (Cicer arietinum cv. Giza 2) on loamy sand soil and on sandy soil. Seeds were inoculated with Rhizobium and with either Azospirillum or Azotobacter or with mixture of both inoculants. Growth of chickpea was improved by association of Azospirillum and/or Azotobacter with Rhizobium in both soil types used. However, Rhizobium with both inoculants was found to be the most responsive.

Cross sections of chick pea root nodules showed that the two diazotrophs plus Rhizobium improved nodule branching over the Rhizobium inoculated treatments in both soil types.

Generally the values of nutrient concentration in chickpea tissues were higher in plants grown in loamy sand soil than those grown in sandy soil inspite of the plant age and the applied treatment.

Introduction

Several studies in recent years have explored the interactions of bacterial diazotrophs which are able to colonize the root zones of leguminous or nonleguminous plants and subsequently fixing nitrogen either in symbiotic or in associative manner.

There have been reports that mixed cultures of either Azotobacter spp. or Azospirillum spp. and Rhizobium strains when used as inoculants for several legumes increased nodule number, nodule dry weight (Abou Bakr et al. 1987, Burns et al., 1981, Iruthyathas et al, 1983 Rai, 1983) improved grain yield, nitrogenase activity and increased dry matter accumulation in plant parts (Djordjevic et al., 1982, El-Mokadem et al., 1986, Kumar Rao et al., 1976, Singh and Subba Rao, 1979, Plazinski et al., 1984; Sarig et al., 1986).

There is evidence that, equal growth does not necessarily indicate functional equivalence since there may be nutritional and metabolic differences. Furthermore, there are few reports describing alteration in the chemical composition of some crop species by inoculation with Azospirillum or Azotobacter (Kapulnik et al., 1985, Lin et al., 1983, Okon, 1982) who suggested that inoculation with Azospirillum enhanced the uptake of nitrate, phosphate and potassium.

The objective of the present study was to determine the interaction of Azospirillum lipoferum and/or Azotobacter chroococcum and Rhizobium on nodulation, growth and nutrient concentration on growing chick pea (Cicer arietinum CV. Giza 2) in two different soil types (loamy sand soil and sandy soil). Determinations done throughout plant development stages were compared with rhizobia inoculated control plants of similar growth stages and soil type.

Material & Methods

Biological materials:

Chick pea (Cicer arietinum cv. Giza 2) seeds were obtained from Crop Research Institute, Ministry of Agriculture; Azospirillum lipoferum, strain isolated from Fayoum Egyptian soil and identified by Girgis (1985), was cultured in maltate medium, (Dobereiner, 1978), Azotobacter chroococcum R, isolated and identified by Elwan and El-Naggar, (1969) was cultured in nitrogen deficient medium (Elwan and ElNaggar, 1971), Rhizobium sp. local isolate, obtained from Egyptian ministry of Agriculture was cultured in yeast mannitol medium (Vincent, 1970).

Experimental design:

To study the above mentioned interactions, a field plot experiment with (2 x 2 m.) factorial design was carried out on either loamy sand textured soil or sandy soil.

Mean values of some soil characteristics are given in table 1. The physico-chemical properties of soil were determined according to Jackson (1967). Treatments in both soil types were as follows:

- a) Seeds inoculated with Rhizobium spp. (control).
- b) Seeds inoculated with Rhizobium spp. + Azospirillum lipoferum.
- c) Seeds inoculated with Rhizobium spp. + Azotobacter chroococcum.
- d) Seeds inoculated with Rhizobium spp. + mixed culture of Azospirillum and Azotobacter.

Treatments were replicated thrice and were completely randomized.

The average number of cells per ml. varied from 5×10^7 of Rhizobium spp. to 6×10^9 of Azotobacter chroococcum and 12.5×10^8 of Azospirillum lipoferum. Inoculation of seeds were accomplished by moistening seeds with 10% sugar solution and mixing the inoculum with seeds immediately before sowing. Inoculation treatments received an additional application of Azospirillum and Azotobacter 15 days and one month after sowing. Stringent precautions were taken to avoid transfer of inoculant strains between treatments (Young and Mytton, 1983).

Growth Conditions :

Plants were grown under regular winter conditions and sufficiently irrigated.

Evaluations and assays:

Plant height, number of nodules, dry weight of roots and shoots, number of branches and leaves were recorded in each treatment at 30, 60 and 90 days old. At full maturity pods were collected and seeds were subject to analysis.

For macro and micro nutrient determinations, whole plant samples after harvest were thoroughly washed, dried at 70°C to constant weight, ground and digested in nitric, perchloric and sulfuric acid. Phosphorus was analysed using the vanadatomolybdate colorimetric method (Chapman and Prott, 1978) Ca, Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectrophotometry. K, Na were estimated by flame photometry (Jackson, 1967).

Nitrogen was measured by Kjeldahl method. Protein were estimated by multiplying the total nitrogen content by the factor 6.25 (A.O.A.C 1975). Total carbohydrate constituent were determined according to the method of Malik and Sing (1980).

Preparation of root nodules for observation by light microscope:

Root nodules of 90 days old plants were fixed in Carnoy's, dehydrated, embedded and thin longitudinal sections of the nodule central region were prepared (Drury & Wallingtons, 1967) and examined with light microscope to observe nodule morphology.

Results

Effect of inoculation upon plant growth:

Data given in table 2, reveal that, growth of chickpea was improved by association of Azospirillum lipoferum and/or Azotobacter chroococcum with Rhizobium spp. in both soils used.

It was found that inoculation with Azospirillum or Azotobacter either alone or in combination increased plant dry weight over Rhizobium inoculated control during plant growth (30, 60, & 90 days old). In general, the shoot/root ratio (S/R) of inoculated plants was higher than that of Rhizobium inoculated ones, specially in case of dual inoculation on loamy sand soil, the magnitude of increase ranges from 20% - 87% over their control while differences between treatments were slight in plants grown on sandy soil.

Inoculation with Azospirillum, Azotobacter or a combination of both inoculants produced more leaves/plant than Rhizobium inoculated control plants (Table 2).

The beneficial effect of inoculation were manifested also on the dimensions (height) of the plants; plant height was, in general, proportional to the number of leaves (Table 2). Dual inoculation with both inoculants resulted greater plant height than those inoculated with Rhizobium. Separate inoculation with Azospirillum and Azotobacter produced similar responses in plant height and both treatments produced taller plants than the control. There was little difference between Azospirillum and Aotobacter treatment in plants grown in sandy soil.

Nodulation:

Nodules were formed in all treatments, and mostly of pink interior, suggesting that nitrogen fixation took place. Presence of Azospirillum lipoferum and/or Azotobacter chroococcum caused increase in nodulation (Table 2). The increase in root nodules ranged from 5% to a maximum of 107% (Table 2). In general nodules were bigger and more branched (lobed) in soil inoculated with Azosirillum and Azotobacter. Dual inoculation resulted in the highest response followed by Azospirillum then Azotobacter in both soils used. The internal anatomical response of root nodules to inoculation showed that the two diazotrophs plus Rhizobium

improved nodule branching over the Rhizobium inoculant treatments in both soils (Fig. 1)

Effect of inoculation on nutrient concentration in plant tissues:

From tables 3,4 & 5 it can be seen that, in general the values of nutrient concentrations in chick pea tissues were higher in plants grown in loamy sand soil than those grown in sandy soil inspite of the plant age and the applied treatment.

a) On loamy sand soil, inoculation of Azospirillum and Azotobacter either alone or in combination favours nitrogen and protein accumulation in plant tissues (Table 3), the response was higher with dual inoculation.

The presence of Azospirillum or Azotobacter has shown to have positive influence on phosphorus, potassium, calcium and magnesium content of inoculated plants throughout development.

However, accumulation of sodium in plant tissue was favoured by inoculation at the early stage of growth (30 days old). Azospirillum and Azotobacter inoculation favours the relative uptake of micronutrient like Mn, Zn & Cu in chick pea plants, as compared to Rhizobium treatment. Lower carbohydrates content was shown in inoculated plants.

b) In sandy soil, differences between treatments in terms of nutrient concentration can be contrasted (Table 4).

The presence of Azospirillum or Azotobacter with Rhizobium has shown to have positive influence on nitrogen, protein and phosphorus content of inoculated plants. Inoculation did not significantly affect the concentration of K and Na in plant tissue. However, treatments induced a decrease in the concentration of Ca & Mg in chickpea.

Plant dry matter accumulation was positively correlated with percentage of total carbohydrate and negatively correlated with Ca & Mg. In general micronutrient contents per gm. dry wt. of Zn and Cu were relatively at higher rates at 30 days old then showed a tendency to decline with age till 90 days. Micronutrients were taken up rapidly during the early stage of growth, then the rate decreased.

Nutrient concentration of seeds:

Effect of Azospirillum & Azotobacter inoculation on nutrient constituents in chickpea seeds in both soils is given in Table, 5. The differences in nutrient concentrations for fully developed chickpea seeds were less pronounced than the differences in plant tissue at the developing stages (Tables 3 & 4), This is an indication that the alteration of nutrients due to inoculation with Azospirillum and/or

Azotobacter may occur probably mainly in vegetative structure or there is every possibility of reachy effect of the alteration of the nutrient constituent of the mature seeds.

Discussion

The experiments conducted indicated that the inoculation of chickpea with Azospirillum lipoferum and/or Azotobacter chroococcum in association with Rhizobium spp. increases the number of nodules, plant height, number of leaves and branches and above and below ground biomass. Plants inoculated with both organisms or Azospirillum grew better than those inoculated with Azotobacter or control plants in loamy sand soil and sandy soil.

This also reflects the involvement of the plant in response to the three interacting bacterial species. However, such growth responses are variable (Table 2) depending on the initial fertility of soil (Lehri et al., 1978 & Subba Rao et al., 1980).

In most experiments there were positive responses of dry matter and/or nitrogen content of chickpea to inoculation of Azospirillum & Azotobacter. The bacterial effect on the S/R ratio found in this study also merit some comments. One of the ways to control the distribution of biomass between shoot and root is based on the feed-

back effect of shoot plant nutrients on the flow of the photosynthetic products to the heterotrophic parts of the system. However another mechanism is also possible to explain the effect of Azospirillum and Azotobacter which are known to synthesize and exude into the medium plant hormones (Tien et al., 1979), which can alter the hormonal balance within the plant, thereby affecting the S/R ratio.

The present results, together with previous reports (Abou Bakr et al., 1987, Burns et al., 1981; Iruthayathas et al., 1983; Rai, 1983) clearly indicate that inoculation of Azospirillum and Azotobacter enhances nodulation and plant growth.

The favourable effect of Azospirillum and/or Azotobacter on agricultural crops is at present attributed to multiple action. They can affect plant growth not only by fixing nitrogen but also by altering microbial balance, suppression of pathogenic microorganism, mobilisation of soil phosphate or by providing metabolites that stimulate plant development (Brown 1974, Cooper 1959, Mishustin & Naumova 1962, Shende, et al. 1975; Brown 1974; Brown and Walker 1970 and Davies et al., 1964).

It can be seen that on inoculation with Azospirillum and Azotobacter, more root hairs become susceptible to rhizobial infection. Okon (1984) and Patriquin et al., (1983)

showed that Azospirillum inoculation shortened time appearance and increased root hair formation in roots of wheat and other grasses. Moreover, perhaps Azospirillum cells produce an excretable compound (S) which create new infection sites (Plazinski & Rolfe 1985). Gross-sections of inoculated nodules showed increased nodule branches (lobes). These effects on nodule morphology may be due to the production of plant growth promoting substances by the colonizing bacteria (Azotobacter and Azospirillum or by the plant as a reaction to colonization. Morphological changes may have a physiological effect on inoculated roots were postulated by Okon & Kapulnik 1986.

One of the objective of this experiment was the evaluation of the role of Azospirillum and Azotobacter in association with Rhizobium on chickpea nutrition in low fertilized loamy sand, and weak sandy soil. Data shown in tables 3, 4 & 5 showed that nutrient concentrations also varied among soil type through plant development. Nutrients were taken up rapidly during the early stages of growth in presence of Azospirillum and Azotobacter. This emphasizes the potential role of both inoculants in increasing the efficiency of mineral assimilation and respectively plant growth. Enhanced nutrient uptake following Azospirillum and Azotobacter inoculation suggests that these rhizosphere bacteria increase the availability of nutrient through altering root surface characteristics involved in nutrient

uptake (Lin et al., 1983 and Helimish et al., 1986). Other mechanisms not involving nitrogen fixation have been cited to explain inoculation responses. Enhanced uptake of nitrate, phosphate and potassium by excised root sections of maize and sorghum inoculated with Azospirillum brasilense have been observed (Kapulnik, et al., 1985, Lin, et al., 1983, and Okon, 1982).

Villas and Döbereiner (1981), suggested that Azospirillum may stimulate nitrate assimilation by plant. Moreover, it has been found that in grasses inoculated with Azospirillum there was an increase in mineral and water uptake by the roots and greater accumulation of dry matter in plant parts (Kapulnik, et al., 1983; Kapulnik, et al., 1981; Sarig, et al., 1984 and Yahalom, et al., 1984) resulting in increase in root and shoot biomass.

Our studies have shown that the presence of Azospirillum and/or Azotobacter could influence the Rhizobium-legume symbiotic interaction. Relationships involving physiological compatibility in the tripartite association are unknown and may match in importance the ability of the three associatives to tolerate independently a common set of environmental and edaphic factors. Extended studies of versatile tripartite associations are appreciated for recommendation of their combined use in practice.

References

- Abou Bakr, Z.Y.M.; Helemish, P.A.; Sheteawi, S.A. and El-Mohadem, M.T. 1987 . Effect of some biofertilizers on Egyptian clover and alfalfa grown in sandy soil. 1st Conf. of Fertilizers Cairo, Egypt. (In press) 13-16 April 1987.
- A.O.A.C. (1975). Official Methods of Analysis of the Association of Official Analytical chemists Washington 3rd edt.
- Brown, M.E. and Walker, N. 1970 . Indolyl-3 acetic acid formation by Azotobacter chroococum. Plant and Soil 32, 250-253.
- Brown, M.E. 1974 . Seed bacterization. Annu. Rev. Phytopathol. 12, 181-197.
- Burns, T.A., Jr., Bishop, P.E., and Israel, D.W. 1981. Enhanced nodulation of leguminous plant roots by mixed cultures of Azotobacter vinelandii and Rhizobium. Plant Soil. 62: 399-412.
- Chapman, H.D. and Pratt, P.E. 1978. Methods of analysis for soils, Plants and waters Univ. of California Div. Agric. Sci., Priced Publication 4034.
- Cooper R. 1959. Bacterial fertilizers in the Soviet Union. Soils Fert. 22, 327-333.
- Davies, J., Gilbert, W. and Corini, I. 1964. Streptomycin, Suppression and the Code. Proc. Nat. Acad. Sci. USA 51, 883-890.

- Drury, R.A.B. and Wallington E.A. 1967. Carleton's histological techniques. Oxford Univ. Press. New York, Toronto.
- Djordjevic, M.A.; Zurkawski, W.; Rolfe, B.G. 1982. Plasmids and stability of symbiotic properties of *Rhizobium trifolii*. J. Bacterial. 151: 560-568.
- Döbereiner, J. 1978. Influence of environmental factors on the occurrence of *S. Lipoferum* in soil and roots. Environmental role of N_2 -fixing blue-green algae and asymbiotic bacteria. Ecol. Bull. (Stockholm) 26: 343-352.
- Elwan, S.H., and El-Naggar M.R. 1969. Effect of streptomycetes spp. on the development of nitrogen fixation 6th Arab Sci. Congress Damascus.
- Elwan, S.H. and El-Naggar, M.R. 1971. Inhibitory effects of some Aspergilli on nitrogen fixation by *Azotobacter chroococcum* in culture and soil, Proc. Egypt. Acad. Sci. Vol. 24, 35.
- El-Mokadem, M.T., Helemish, F.A., and Abou-Bakr, Z.Y.M. 1986. Growth and yield parameter responses of two soybean cultivars to inoculation with *Azospirillum* and *Rhizobium*. The second conference of the A.A.B.N.F Cairo, Egypt 15-19 Dec.
- Girgis, M.G.Z. 1985. studies on free N_2 -fixation in Egyptian soil with special reference to *Azospirillum*. M.Sc. Thesis. Fac. of Agric. Ain Shams Univ.
- Helemish, F.A., Abou-Bakr, Z.Y.M. and El-Mokadem, M.T. 1986. Influence of *Azospirillum* inoculation on growth and nutrient content of Barley, Sunflower and peanut. The second conference of the A.A.B.N.F. Cairo, 15-19 December 1986.

- Iruthayathas, E.E., Gunasekaron, S. and Vlassak, K. 1983. Effect of combined inoculation of Azospirillum and Rhizobium on nodulation and N₂-fixation of winged bean and soybean. Sci. Hartic. 20: 231-240.
- Jackson, D. 1967. Soil Chemical analysis Prentice Hall.
- Kapulnik Y., Sarig S., Nur I, Okon Y, Kigel J. and Henis Y 1981. Yield increases in summer cereal crops of Israeli fields inoculated with Azospirillum. Expl. Agric. 17,179-187.
- Kapulnik Y, Sarig S., Nur I and Okon Y. 1983. effect of Azospirillum inoculation on yield of field grown wheat. Can. J. Microbiol. 29, 895-844.
- Kapulnik Y, Gafny R. and Okon Y., 1985. Effect of Azospirillum spp. inoculation on root development and NO₃-uptake in wheat (Triticum aestivum CV. Miriam) in hydroponic systems. Can. J. Bot. 63, 627-631.
- Kumar Rao, J.V.D.K. and Patil, R.B. 1976. Effect of inoculation with Rhizobium and Azotobacter on nodulation, growth and yield of soybean. Curr. Sci. 45: 523-524.
- Lehri L.K., Bajpai P.D. and Tiwari V.N. 1978. Response to Azotobacter inoculation by wheat crop. Natl. Symp. on Bacterial and Algal mediated Non-symbiotic Nitrogen Fixation p. 16 IARI, New Delhi.
- Lin N., Okon Y. and Hardy RWF 1983. Enhanced mineral uptake by Zea mays and Sorghum bicolor roots inoculated with Azospirillum brasilense. Appl. Environ. Microbiol. 45, 1775-1779.

- Malik, C.P., and Singh, M.B. 1980. Plant entomology and Histo-entomology. Kalyani Publishers. New delhi.
- Mishustin E.N. and Naumova A.N. 1962. Bacterial fertilizers, their effectiveness and mode of action, Mikrobiologiya 31, 543-555.
- Okon Y. 1982. Azospirillum: Physiological Properties, mode of association with roots and its application for the benefit of cereal and forage grass crops. Israel J. Bot. 31, 214-220.
- Okon Y. 1984. Response of cereal and forage greasses to inoculation with N₂-fixing bacteria. In Advances in Nitrogen Fixation Research. Eds. C. Veeger and WP Newton. Martinus Nijhaff, Or W. Tunk, Pudoc, Wageningen, The Hague. pp. 303-309.
- Okon Y. and Kapulnik 1986. Development and function of Azospirillum-inoculated roots. Plant and soil 90, 3-16.
- Patriquin D.G., Döbereiner J. and Jain D.K 1983. Sites and processes of Association between diazotrophs and grasses. Can. J. Microbiol. 29, 900-915.
- Plazinski J. Gartner, E.Mc. Iver, Jahnke, R. and Rolfe, B.G. 1984. Effect of Azospirillum on Rhizobium legume symbiosis. In advances in nitrogen fixation research. eds. C. Veeger and W. Newton, Martinus Nijhalff. Dr. W. Junk, Pudoc, Wageningen, the Hague pp. 424.
- Plazinski, J. and Rolfe, B.G., 1985. Influence of Azospirillum strains on the nodulation of clovers by Rhizobium strains Appl. Environ. Microbiol. 49: 984-989.

- Rai, R. 1983. Efficacy of associative N₂-fixation by streptomycin resistant mutants of Azospirillum brasilense with genotypes chickpea Rhizobium strains. J. Agric. Sci. Comb. 100: 75-80.
- Sarig S., Kapulnik Y., Nur I. and Okon Y. 1984. Response of non-irrigated Sorghum bicolor to Azospirillum inoculation. Expl. Agric. 20, 59-66.
- Sarig, S., Kapulnik, Y. and Okon, Y 1986. Effect of Azospirillum inoculation on nitrogen fixation and growth of several winter legumes. Plant & Soil, 90: 335-342.
- Shende S.T., Apte R.G. and Singh T. 1975. Multiple action of Azotobacter. Indian. J. Gen. Plant Breed. 35, 314-315.
- Singh, C.S. and Subba Rao, N.S. 1979. Associative effect of Azospirillum Brasilense with Rhizobium japonicum on nodulation and yield of saybean (Glycine max.) plant and Soil 53: 387-392.
- Subba, Rao N.S. 1980. Crop response to microbiol inoculation In Recent Advances in Biological Nitrogen Fixtion (EDNS subba Rao. pp. 406-420, Edward Arnold, London.
- Tien T.M., Gaskins M.H. and Hubbel D.H. 1979. Plant growth substances produced by Azospirillum brasilense and their effect on growth of pearl millet (Pennisetum americanum L.) Appl. Environ. Microbiol. 37, 1016-1024.

- Vincent, J.M. 1970. A manual for the practical study of root-nodule bacteria. The International Biological programs. Blackwell Sci. Publ. Oxford.
- Villas Baos FCS and Dobereiner J. 1981. Nitrogenase and nitrat reductase in rice plants inoculated with various Azospirillum strains. In Associative N₂-fixation. Eds. PB Vose and AP Ruschel. pp.231-239. CRC Press, Boca Raton, Florida, USA. Vol. II.
- Yahalom E., Kapulnik Y and Okon Y. 1984. Response of *Setaria italica* to inoculation with *Azospirillum brasilense* as compared to *Azotobacter chroococcum* plant and Soil. 82, 77-85.
- Young N.R. and Mytton L.R. 1983. The response of white clover to different hill land reseeding. Grass and Forage Sci. 38, 13-19.

Table (1) : Soil analysis and soil water tension

(1)	granuolometric analysis	Sandy soil	loamy sand soil
	Gravel %	10.4%	8.5%
	Coarse sand	50.8	31.2
	Fine sand	38.0	54.6
	silt	6.7	3.3
	clay	4.5	10.9
(2)	<u>Chemical analysis</u>		
	E.C. (μ mhos = S)	2.8×10^{-3}	4.8×10^{-3}
	pH	7.00	7.10
	Organic matter %	0.38	0.67
	Nitrogen %	0.036	0.055
	Chlorides %	0.20	0.10
(3)	<u>Soil water tension</u> (centibar)		
	before irrigation	33.0	30.0
	after irrigation	10.0	10.0

Table (2) * Interaction between Azospirillum lipoferum, Azotobacter chroococcum and Rhizobium spp and their effects on growth and biomass distribution of chickpea (Cicer orientalinum cv. Giza 2)

Treatments	Plant age	S/R ratio dry wt	dry wt (g/ Plant)	plant height (cm)	no. of nodules plant	no. of branches plant	no. of leaves plant	no. of buds/plant	
									Chick pea on loamy soil
Control (Rhizobium)	30 days	3.7	0.14 ± 0.8	17.8 ± 1.8	4.5 ± 0.9	3.0 ± 0.6	4.5 ± 1.0	-	
	60 days	6.9	0.56 ± 0.4	38.3 ± 1.2	7.8 ± 2.0	3.0 ± 0.2	24.8 ± 2.4	21	
	90 days	6.6	1.06 ± 1.6	42.5 ± 1.1	7.6 ± 0.5	4.0 ± 1.0	27.0 ± 6.4	-	
	30 days	5.0	0.17 ± 0.4	20.4 ± 2.4	7.1 ± 1.6	3.3 ± 0.4	8.3 ± 0.4	-	
	60 days	9.9	1.09 ± 0.4	44.0 ± 0.9	10.75 ± 1.4	4.0 ± 0.7	47.0 ± 4.4	32	
	90 days	7.8	3.18 ± 1.2	59.2 ± 1.2	11.0 ± 0.9	7.2 ± 2.7	55.0 ± 7.0	-	
Azotobacter	30 days	4.7	0.18 ± 1.7	22.9 ± 1.1	8.9 ± 1.1	2.9 ± 1.1	8.0 ± 1.2	-	
	60 days	9.8	0.69 ± 1.4	43.9 ± 2.6	9.2 ± 2.1	4.0 ± 0.6	36.7 ± 4.2	32	
	90 days	8.1	2.56 ± 1.3	63.8 ± 1.4	10.8 ± 1.3	5.6 ± 0.8	42 ± 3.9	-	
Azospirillum	30 days	7.0	0.22 ± 1.5	26.0 ± 2.1	9.3 ± 1.0	2.7 ± 0.5	9.6 ± 1.7	-	
	60 days	8.0	1.28 ± 0.1	46.5 ± 2.2	16.8 ± 2.1	5.0 ± 0.8	71.5 ± 9.5	59	
	90 days	12.3	5.86 ± 1.0	75.0 ± 1.8	15.0 ± 2.1	14.0 ± 2.6	97.6 ± 1.0	-	
Chick pea on sandy soil									
Control (Rhizobium)	30 days	3.9	0.18 ± 0.7	22.9 ± 2.2	5.2 ± 0.73	1 ± 0.25	8.8 ± 0.5	-	
	60 days	7.5	0.79 ± 1.5	45.3 ± 4.1	10.6 ± 1.5	3 ± 0.32	39.5 ± 5.7	58	
	90 days	11.0	5.4 ± 1.4	61.0 ± 1.5	15 ± 1.1	12.4 ± 1.7	52.6 ± 10.9	-	
	30 days	4.1	0.25 ± 1.4	22.3 ± 2.9	5.5 ± 0.67	3.42 ± 0.4	8.7 ± 1.5	-	
	60 days	6.9	1.47 ± 1.4	50.5 ± 1.5	18 ± 3.2	4 ± 0.2	55 ± 8.0	82	
	90 days	12.7	6.08 ± 1.6	60.2 ± 1.9	16 ± 3.5	11 ± 3.4	52.2 ± 8.3	-	
Azotobacter	30 days	4.6	0.18 ± 0.9	23.8 ± 2.5	8.1 ± 1.35	2.92 ± 0.2	8.3 ± 0.8	-	
	60 days	4.7	0.63 ± 1.1	51.8 ± 1.9	13.4 ± 2.1	3 ± 0.2	31 ± 4.2	55	
	90 days	15.7	5.08 ± 0.8	67.6 ± 3.3	17.2 ± 3.3	11.2 ± 3.3	77.8 ± 13.4	-	
Azospirillum	30 days	4.0	0.25 ± 0.8	23.2 ± 0.8	10.75 ± 2.7	3.63 ± 0.67	9.3 ± 1.0	-	
	60 days	8.0	1.7 ± 0.9	48.7 ± 1.4	15.7 ± 3.3	4 ± 0.52	60.3 ± 8.0	-	
	90 days	9.9	6.06 ± 1.3	62.6 ± 1.6	18.8 ± 3.1	7 ± 2.2	62.2 ± 9.0	60	

* Means and standard deviation of 30 plants from three experimental field plots

Table (3)*: Interaction between Azospirillum lipoferum, Azotobacter chroococcum and Rhizobium SP. and their effect on some nutrient constituents of chickpea (Cicer arietinum) var. giza 2. Plants grown in Loamy soil.

Date	Loamy soil	Treatment	mg / g dry wt										
			Nitrogen	protein	total carbohy.	P	K	Ca	Mg	Na	Mn	Zn	Cu
30 days	Control	Azospirillum	35.7	223.7	86.0	2.0	24.8	5.4	2.5	1.1	43.2	38.7	17
		Azotobacter	43.1	269.4	61.8	4.3	51.0	9.0	3.7	1.9	106.8	87.0	22
		Azospirillum + Azotobacter	40.9	255.6	92.5	5.2	49.5	8.1	3.3	1.7	68.4	73.8	18
		Azospirillum + Azotobacter	45.8	286.3	41.3	4.6	52.5	9.3	3.7	1.9	73.8	70.5	28
		Control	29.1	181.9	40.0	2.8	27.0	18.8	4.2	0.9	127.8	68.1	25
		Azospirillum	29.9	186.9	67.5	4.6	46.5	17.5	4.6	1.4	104.4	60.3	21
60 days	Control	Azotobacter	22.7	141.9	55.5	5.3	30.0	27.5	4.5	1.0	155.4	72.0	38
		Azospirillum + Azotobacter	36.1	200.6	57.9	5.5	26.2	18.8	4.5	0.8	131.4	83.4	26
		Control	23.8	149.8	86.6	3.2	30.0	18.8	4.4	0.8	77.4	76.2	7
		Azospirillum	28.1	175.6	74.5	3.7	34.5	15.0	4.2	0.8	81.0	99.0	12
		Azotobacter	27.0	168.8	75.0	3.5	36.5	13.8	4.3	0.8	83.4	81.0	9
		Azospirillum + Azotobacter	29.5	184.8	62.0	3.9	36.8	15.0	4.5	0.6	75.0	97.2	9
90 days	Control	Azospirillum	23.8	149.8	86.6	3.2	30.0	18.8	4.4	0.8	77.4	76.2	7
		Azotobacter	27.0	168.8	75.0	3.5	36.5	13.8	4.3	0.8	83.4	81.0	9
		Azospirillum + Azotobacter	29.5	184.8	62.0	3.9	36.8	15.0	4.5	0.6	75.0	97.2	9
		Control	23.8	149.8	86.6	3.2	30.0	18.8	4.4	0.8	77.4	76.2	7
		Azospirillum	28.1	175.6	74.5	3.7	34.5	15.0	4.2	0.8	81.0	99.0	12
		Azotobacter	27.0	168.8	75.0	3.5	36.5	13.8	4.3	0.8	83.4	81.0	9

* Values are means of three determinations.

Women's Coll. Ann. Rev.
VOL. 15 (1990).

Table (4)*: Interaction between Azospirillum lipoferum, Azotobacter chroococcum and Rhizobium SP. and their effect on some nutrient constituents of chickpea (Cicaf arjellium) Var. giza 2. Plants grown in sandy soil:

Date	Treatment	Nitrogen	protein	mg / g dry wt. . .							Mg / g dry wt		
				total carbohy	P	K	Ca	Mg	Na	Mn	Zn	Cu	
30 days	Control (Rhizobium)	35.6	222.5	51.9	2.7	47.3	11.4	4.7	1.5	93.0	66.9	17	
	Azospirillum	35.3	220.6	48.0	4.0	48.8	9.9	3.9	1.4	74.7	75.6	23	
	Azotobacter	34.2	213.8	45.2	3.9	50.3	8.7	3.7	1.4	63.6	81.3	37	
	Azospirillum + Azotobacter	37.5	234.3	58.5	3.5	43.5	10.5	4.4	1.5	78.0	70.0	20	
	Control (Rhizobium)	23.0	143.8	66.7	4.6	36.0	23.8	5.4	1.1	165.6	97.5	38	
	Azospirillum	29.6	185.0	63.1	4.3	45.0	15.0	2.8	0.9	80.4	71.1	18	
60 days	Azotobacter	26.6	166.3	57.4	3.7	39.8	16.3	3.7	0.9	68.4	78.0	20	
	Azospirillum + Azotobacter	24.1	150.6	65.9	3.9	37.5	15.0	5.8	0.8	79.2	75.6	21	
	Control (Rhizobium)	33.7	210.6	78.8	4.1	39.0	20.0	3.8	0.9	73.8	86.7	10	
	Azospirillum	25.1	156.9	42.0	3.4	33.0	15.0	3.7	0.9	63.0	81.0	9	
	Azotobacter	26.5	165.6	81.3	3.5	36.8	15.0	3.4	0.8	60.6	92.7	10	
	Azospirillum + Azotobacter	33.1	206.9	85.3	3.8	34.5	15.0	3.4	0.7	57.0	72.0	6	
90 days	Control (Rhizobium)	33.7	210.6	78.8	4.1	39.0	20.0	3.8	0.9	73.8	86.7	10	
	Azospirillum	25.1	156.9	42.0	3.4	33.0	15.0	3.7	0.9	63.0	81.0	9	
	Azotobacter	26.5	165.6	81.3	3.5	36.8	15.0	3.4	0.8	60.6	92.7	10	
	Azospirillum + Azotobacter	33.1	206.9	85.3	3.8	34.5	15.0	3.4	0.7	57.0	72.0	6	

* Values are means of three determinations.

Table (5) Interaction between Azospirillum lipoferum, Azotobacter chroococcum and Rhizobium spp and their effect on some nutrient constituents in the seeds of chickpea (Cicer arietinum) var giza 2

Soil type	Treatment	mg/ g dry wt.										Mg/ g dry wt.				
		Nitrogen	Protein	Total carbohy-	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu			
Loamy soil	Control(Rhizodium.)	32.0	200.0	294	5.7	16.5	1.6	1.5	0.7	85.8	27.0	67.5	6			
	Azospirillum	32.6	203.8	200.5	5.5	15.5	2.2	1.5	0.7	92.3	36.6	65.1	3			
	Azotobacter	30.4	190.0	286.8	4.8	13.5	1.5	1.3	0.6	48.1	28.2	54.9	6			
	Azospirillum + Azotobacter	33.4	210.0	239.3	5.6	15.0	1.8	1.3	0.6	53.3	28.2	64.5	5			
Sandy soil	Control (Rhizobium.)	27.8	173.8	271.1	4.9	17.3	1.8	1.8	0.5	57.2	30.0	60.6	1			
	Azospirillum	32.6	203.8	215.0	5.5	15.0	1.5	1.4	0.5	44.2	25.8	68.7	8			
	Azotobacter	25.1	156.9	205.3	4.8	15.0	1.5	1.7	0.5	49.4	19.8	58.5	3			
	Azospirillum + Azotobacter	28.6	178.8	273.8	5.2	12.0	1.6	1.0	0.7	58.2	24.6	60.0	1			

* Values are means of three determinations

Women's Coll. Ann. Rev.
Vol. 15 (1990).

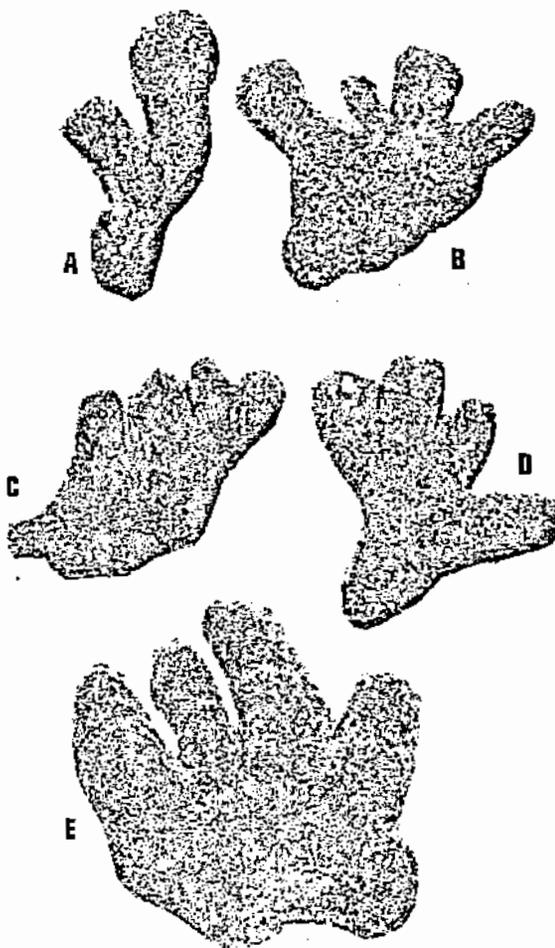


Fig. 1. Cross section of chick pea nodules X 5
 A) Control, sandy soil B) Azotobacter, sandy soil.
 C) Control, loamy soil D) Azospirillum, loamy soil.
 E) Azospirillum + Azotobacter, loamy soil.

Women's Coll. Ann. Rev.
 Vol. 15 (1990).