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Growth response of Medicago sativa to phosphorus fertilization, cycocel and Rhizobium as affected by water regime

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Summary:

A pot experiment was conducted to evaluate the growth response of Medicago sativa to super-phospbate nitrate fertilizer alone or in combination with cycocel and/or Rhizobium when subjected to water regime. Particular emphasis was given to growth parameters, yield and nutrient content as well as sugars, carbohydrates and water use efficiency.

The water regime undertaken for this experiment was to keep the soil moisture always at the field capacity, one irrigation interval (4 days) was tested in a factorial randomised design with three replications.

Application of superphosphate fertilizer alone or combination with cycocel and/or Rhizobium as biofertilizer greatly affected fresh and dry weight of the stressed plant, root-shoot ratio and leaf area as well, in addition water use efficiency and nutrient concentration in plant tissues.

The stimulation effect induced by different treatments was generally more evident in superphosphate fertilizer plus cycocel, while progressive inhibition associated with superphosphate plus cycocel and/or Rhizobium was shown.

Water use efficiency and sugar content were the only parameters recorded noticeable increase with superphosphate fertilizer plus cycocel and/or Rhizobium application.

Introduction:

Water is one of the most limiting factors affecting crop production in semiarid regions, where irrigation has supplemented rainfall for crop production, competition from urban water users has created a need for better understanding of crop water requirement and yield relationships (Labanauskas et al., 1981). Moreover very close relationship has been found to exist between continuous availability of soil moisture and response of plant to fertilizer application. If the soil moisture becomes a limiting factor during any stage of the growing season, the addition of fertilizer under such condition may even adversely affect the yield (Reddy et al., 1980). Those authors also found that on a well fertilized plant, the corn roots developed up to 105 cm in the soil and utilized 8.75 cm^3 more water than utilized by the unfertilized plant.

The adequate supply of fertilizer with suitable irrigation intervals was reported by many authors (Sekhon et al., 1981; Verma and Kalrs 1981, 1983 and Sharma et al., 1984). The last authors reported that the highest seed yield of Lentil with higher nitrogen and phosphorus content were obtained by using of 20 Kg N and 60 Kg P with two irrigation intervals. On the other hand Mohamed et al., (1981) stated that soybean plant responded markedly to N & P application and the maximum yield was produced when N and P were applied together. Furthermore, Saraf and Baitha (1982) found an increase in both dry weight as well as nodule numbers of Lentil plant by the application of phosphorus fertilizer. Hussein et al., (1984) mentioned that 32 Kg P/Fed=4200 m^2 and inoculation of Lentil seeds with specific rhizobia, increased the numbers of pods per plant and nitrogen content. However, rhizobial inoculation seemed to give higher seed yield than the un-inoculated treatments.

The aim of this study was to show that if Medicago sativa plant could establish good growth when subjected to water regime by the application of chemical fertilizer alone or in combination with cycocel and/or Rhizobium as biofertilizer. On these basis, this experiment was conducted to explain these aspects.

Materials and Methods:

Seeds of Medicago sativa cv. Esna were obtained from Agriculture Centre, Ministry of Agriculture, Giza. The experiment was conducted in the experimental garden of the Botany Department, Women's College, Ain Shams University.

Cylindrical plastic pots, 21 cm. diameter and 18 cm. depth were chosen for cultivation. In each pot, 4 Kg of loamy soil was placed. Some physical and chemical soil characters were determined as presented in Table (1), following the methods described by Jackson (1973). Chemical fertilizer, mainly containing supper phosphate-nitrate-potassium, as this type of fertilizer was recommended for optimal results of growth and yield. 0.2 g/pot was applied throughout this experiment, twenty seeds of Medicago sativa plant were sown per pot, after two weeks the seedlings were subjected to the irrigation regime interval (4 days) keeping 5 replicate pots for each treatment, which consist of pots containing cycocel (40 ppm) or Rhizobium (2 gm/pot dried cells). Each of them were applied with superphosphate fertilizer, another treatment containing superphosphate fertilizer plus cycocel and/or Rhizobium was applied. After 90 days from sowing, plants were uprooted, washed and dried at 70°C to constant weight. Growth parameters including, fresh and dry weight of plant, leaf area and root-shoot ratio were recorded.

Total carbohydrates and total soluble sugar contents were estimated as g/100 g dry weight of shoot including the leaves. The method used was described by (Dubois et al., 1956). Nitrogen content was determined in dried and finally pulverised plant shoots using H_2SO_4 and $HClO_4$ 1:1 (Jackson, 1973). Mineral determinations in the plants were carried out after digestion of the dried ground material following the method described by Champann and Pratt, (1978).

The obtained data were subjected to analysis of variance (Snedecor and Cochran, 1980).

Results and Discussion:

Growth parameters:

Previous investigation by Abou-Bakr et al., (1993) had found that Medicago-sativa growth was greatly affected when subjected to water stress conditions at different irrigation intervals. An attempt was performed to clarify if stressed Medicago-sativa (4 days interval) could establish good growth by application of chemical fertilizer alone or in combination with cycocel and/or Rhizobium as biofertilizer. Particular emphasis was undertaken to growth behaviour, water use efficiency and nutrient concentration in plant tissue as well. The obtained results revealed that fresh and dry weight of stressed plant (4 days) irrigation interval were enhanced by different treatment application (Figs. 1 & 2). Enhancement of the plant growth was more evident by the application of superphosphate fertilizer plus cycocel as compared to superphosphate alone (control), while noticeable decrease associated with superpbosphate fertilizer in combination with cycocel and/or Rhizobium was shown. Similarly leaf area was affected by different treatments (Fig. 1), being increased by superphosphate fertilizer plus cycocel as compared to the fertilizer alone (control) and decreased by superphosphate fertilizer plus cycocel and/or Rhizobium. Reduction in root-shoot ratio by dry weight may be attributed to reduced root growth which resulted from moisture stress. These findings were in full agreement with that obtained by Mbagwa and Osuigwe (1984) who showed that moisture stress generally reduced root growth of maize and cowpea and thereby decreased root-shoot ratio. On the other hand Reddy et al., (1980) found that if the soil moisture becomes a limiting factor during any stage of the growing season, addition of fertilizer under such conditions may even adversely affect the yield. Furthermore a great reduction on the dry wight of plant which grown under water stress condition was previously studied by many authors (Mbagwa and Osuigwe, 1984; Shouse et al., 1981; Sinclair et al., 1975 and Summerfield et al., 1976), while other workers (Babalola, 1980 and Denmead and Show, 1960) attributed decreased dry weight associated with reduced amounts of water to water stress conditions which invariably resulted to stomatal closure. This leading to the over all effect of reducing the photosynthetic efficiency of the leaves with consequent reduction in yield.

Yield production:

Different treatments had a major effect on yield production. The most severe reduction in yield was found in the treatment of phosphorus fertilizer plus cycocel and/or Rhizobium in which the yield reduction was about 53% of treatment supplying phosphorus fertilizer alone (control). These findings are in complete agreement with (Turk et al., 1980; Shouse et al., 1981 and Hiller et al., 1972), but in disagreement with Summerfield et al., (1976) and Wein et al., (1979). Discrepancies in the experimental findings may be due to the in-determine reproduction nature of certain plant varieties, differing varietal response to water stress at the different growth stage, possible differences in degree of treatment application or other environmental factors not taken into account.

Water use efficiency:

The water use efficiency of the plant (Fig. 5) showed that the treatment for dry matter yield was achieved by the application of phosphorus fertilizer plus cycocel and/or Rhizobium. Other treatment gave poor water use efficiency specially the treatment which supplied with phosphorus fertilizer plus cycocel compared to the treatment receiving phosphorus fertilizer alone. Water use efficiency of stressed plants was relatively low and application of chemical fertilizer (superphosphate) growth promoters (cycocel) and biofertilizer (Rhizobium) had improved it. Kowall and Kassan (1973) reported reduce water use efficiency for maize when soil moisture decreased below its field capacity. Other workers observed that during silking stage for (maize) and flowering and filling stage for (cowpea) water use efficiency was decreased as a result of induced moisture stress.

Nitrogen uptake of the plant:

Nitrogen uptake by the stressed plant shown in Fig. (6) indicated that there were variation in nitrogen percent taken up by the plant. The main concentration of N in the plant ranged from 3.45 to 4.7%. The lowest concentration of N was found in plant treatment receiving fertilizer plus Rhizobium and the highest one was treatment supplying with fertilizer plus cycocel. Generally, N uptake was improved by phosphorus fertilizer application plus cycocel more than the other treatments. These results were expected since Rhizobium could not fix nitrogen under water regime or drought condition. These findings might be attributed to the inability of Rhizobium to survive these condition or the inactivation of such strain under water regime. It is known that water stress decreases symbiotic N_2-fixation and growth of legumes (Finn and Brun, 1980 and Dejong and Phillips 1982).

Some data indicated that water stress disrupts interaction between Rhizobium and bost plant directly by altering nodule fine structure which leads to change in either nodule membrane permeability or enzyme activity (Sprent, 1976).

Other evidence suggests that root nodules are affecting indirectly after a decrease in photosynthesis (Finn and Brun, 1980). It is also known that water stress decreases nitrate-reductase activity (Srivastava, 1980) and negatively affects various other aspects of nitrogen metabolism (Hasiao, 1973).

Nutrient uptake of the plant:

Different treatment affected phosphorus concentration in plant tissues (Fig. 7), being high in treatment supplied with phosphorus fertilizer plus Rhizobium and cycocel and low in treatment supplied either with phosphorus alone or in combination with cycocel. Its value was ranged from 0.28% to 0.38%. These concentration were substantial and clearly suggested that the plant was not suffering phosphorus deficiency. Data obtained by other investigators on the effect of phosphorus nutrition in wheat grain and cowpea clearly showed that the values of phosphorus found in Medicago sativa were adequate (Labanauskas et al., 1978 and Labanauskas et al., 1981). These data were contradictory with the obtained data of nitrogen. This manifested that water regime affected nitrogen fixation process but not phosphorus concentration in the plant tissues.

Water regime did not significantly influence the concentration of K in the plant, but there was some variations in K concentration particularly in treatment supplied with phosphorus fertilizer plus cycocel and/or Rhizobium. Concentration of K in plant tissues ranged from 5.8% to 6.8%. The values of K in the plant tissues were substantially higher than found in the wheat seeds in hydroponic or soil studies (Labanauskas et al., 1975, 1978) and cowpea (Labanauskas et al., 1981).

The concentration of Ca were not influenced by water regime. All the treatments were nearly similar except those receiving fertilizer plus cycocel, slight increase was clearly shown. Calcium concentration ranged from 1.19-1.39% (Fig. 9). These values were found higher comparable to those obtained by Labanauskas et al., (1981) on cowpea.

Magnesium concentration in the Medicago sativa ranged from 0.53-0.59% and the concentration levels were not significantly affected by the treatments or by water regime. The concentration levels of Mg in the cowpea seeds in Labanauskas et al., (1981) experiment ranged from 0.18 to 0.19% under different irrigation treatments. The present data were found to be higher than those obtained either by Labanauskas et al., (1976) on wheat or Labanauskas et al., (1981) on cowpea.

Those authors found that concentration levels of Mg in the cowpea seeds were about the same as found in wheat grain from the hydroponic and soil media. Loneragan et al., (1976) had classified N, P and K as mobile elements, Ca as immobile and Mg as intermediate. According to Scott and Brewer (1980), water stress soybean plants do not transpire as much water as well watered plants, therefore lower nutrient element transport occurs in the plant.

Sodium concentration in the plants was not affected by different treatments or by moisture regime. The Na concentrations of about 0.1-0.18% (Fig. 10). In Labanauskas et al., (1981) investigation, Na concentration was lower (0.02%) in cowpea plant subjected to different irrigation treatments. The present data were found high when compared with obtained data by those authors.

The concentration of Fe in Medicago sativa plant were affected by the irrigation treatments and by water regime (Fig. 11). Fe concentration ranged from 0.28% to 0.65%. The higher value obtained from treatment supplied with superphosphate fertilization plus cycocel and/or Rhizobium and the lowest value obtained from treatment supplied with superphosphate only. Although there were statistically differences in Fe concentration due to treatments, the plant was well supplied with Fe and no deficiency symptoms appeared at any stage.

Total carbohydrate content %:

Water regime (4 days) did not affect total carbohydrate content. It recorded 3.2% in treatment supplied with phosphate fertilizer alone which acted as control, on the other hand application of other treatments clearly showed decline in carbohydrate content revealed progressive decline with increase in soil moisture stress associated with the elongation of irrigation intervals (Abd El-Rahman et al., 1993; Hodges and Heatherly, 1983; Krizek et al., 1985 and Batanouny et al., 1988). Abou El-Seud (1987) showed that decreasing the applied available water resulted in a continuous decline in total carbohydrate content during the different stress periods. Depletion of total carbohydrate content in treatment supplied with phosphate fertilizer plus cycocel and/or Rhizobium was attributed to the depletion of N_2-fixation process which took place by the bacteria. Some evidence suggested that carbohydrate contents in leguminous plants were affected indirectly after a decrease in photosynthesis (Finn and Brun, 1980). Furthermore Abd El-Rahman et al., (1993) stated that the reduction in carbohydrate accumulation under drought conditions might be referred to decrease in photosynthetic activity, moreover the obtained results may be due to higher resistance of CO_2 diffusion due to narrower or closure of stomatal opening and hence lower chlorophyll and carbohydrate content.

Sugars content %:

Four days water regime affected sugar content in Medicago sativa plant. These findings more found in the treatment supplied with phosphorus fertilizer (control). Contrary, treatments supplied with phosphorus fertilizer plus cycocel and/or Rhizobium greatly promoted sugar content by more than 50%. Abd El-Rahman et al., (1993) mentioned that soluble sugar content demonstrated a gradual and remarkable increase with increase moisture stress associated with increase in irrigation intervals. These findings were noticed only with treatments supplied with fertilizer plus cycocel and/or Rhizobium which were also recorded lower carbohydrate content. This might be attributed to the effect of N_2-fixation process which decrease total carbohydrate content and consequently increased sugar content. It might also be attributed to the presence of cycocel promoters which enhanced sugar content and delayed carbohydrate content. Decrease in carbohydrate content and increase in sugar content in plant is refereed to osmotic adjustment when plants are subjected to increase in moisture stress (Abd El-Rahman et al., 1993). The same authors found also that accumulation of the osmotically active substances such as soluble sugars raises the capacity of roots for water absorption. Furthermore, Hussein et al., (1988) reported that elongation of irrigation intervals resulted in a reduction of total carbohydrate content and a significant increase in soluble sugars content.

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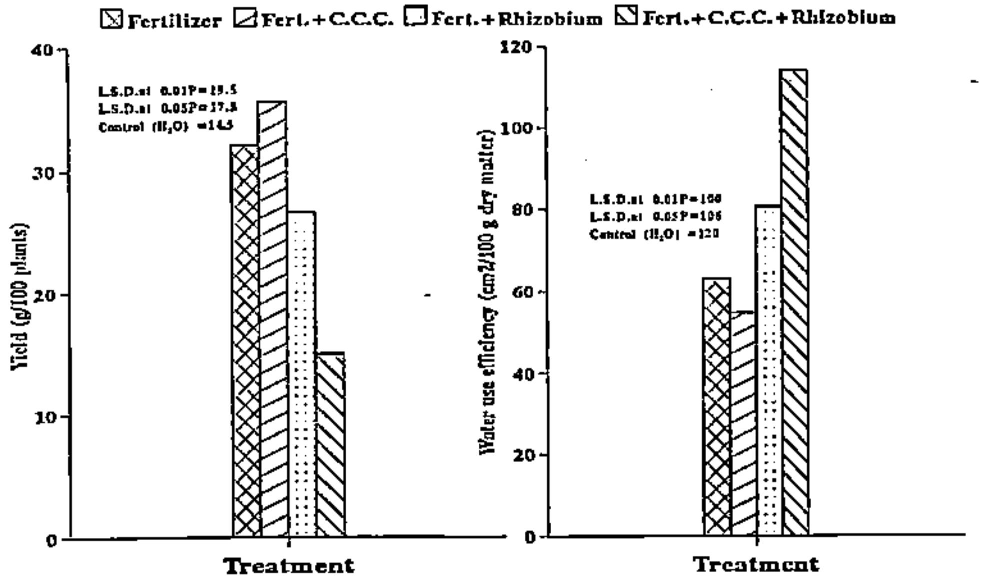

Fig. (2): Effect of fertilizer, cycocel, Rhizobium and mixture treatments on yield (g/100 pl.) and water use efficiency (cm^3/ 100 g dry matter) of Medicago sativa, grown at (4-day) irrigation intervals (plant age 90 days)

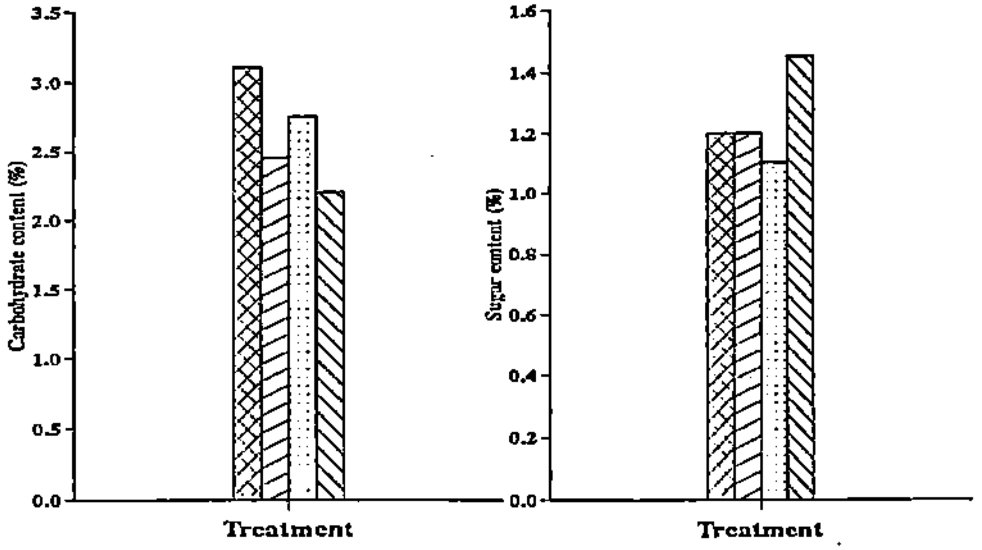

Fig. (4): Effect of fertilizer, cycocel, Rhizobium and mixture treatments on carbohydrate (%) and sugar (%) of Medicago sativa, grown at (4-day) irrigation intervals (plant age 90 days)

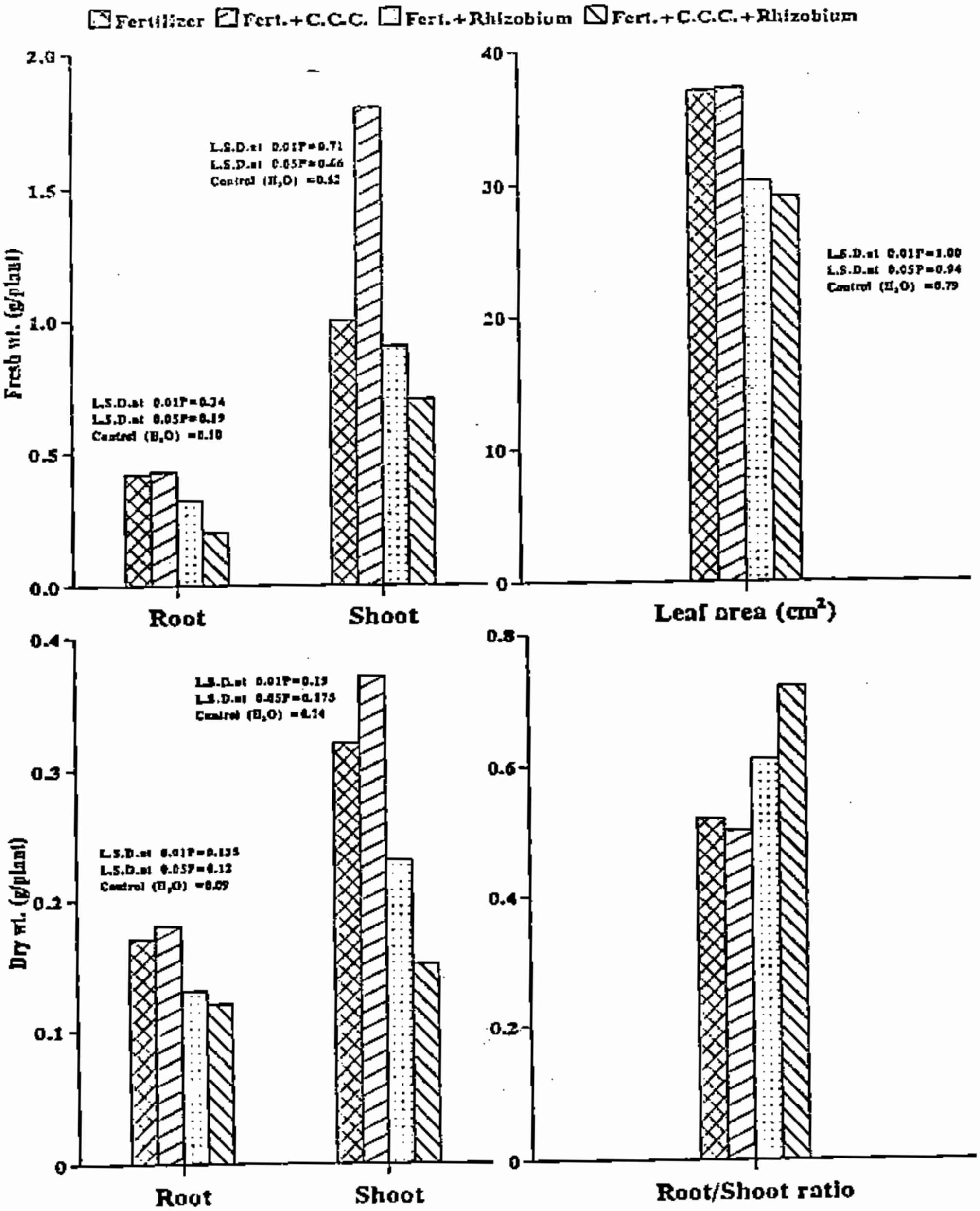

Fig. (1): Effect of fertilizer, cycocel, Rhizobium and mixture treatments on fresh and dry weight (g/pl.), leaf area (cm^2) and root-shoot ratio of <u>Medicago</u> <u>sativa</u>, grown at (4-day) irrigation intervals (plant age 90 days)

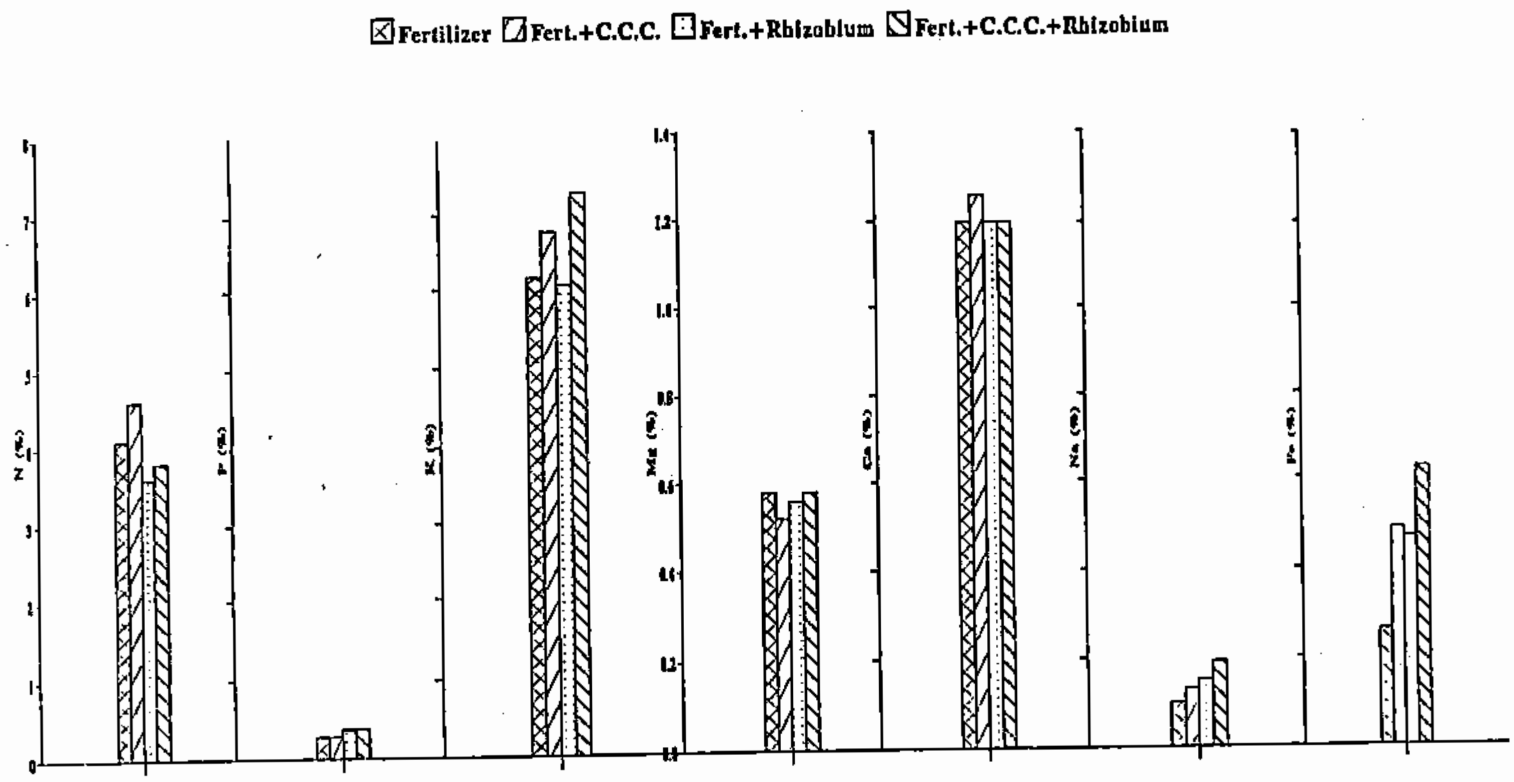

Fig. (3): Effect of fertilizer, cycocel, Rhizobium and mixture treatment on nutrient uptake (%) of Medicago sativa, grown at (4-day) irrigation intervals (plant age 90 days)

Table (1): Mechanical and chemical properties of the soil used in the experiments.

<u>Mechanical Analysis:</u>		
Coarse and fine sand	%	36.4
Silt	%	30.0
Clay	%	31.6
Soil texture		Loamy soil
Field capacity	%	21.00
<u>Chemical Analysis:</u>		
$CaCO_3$	%	2.46
pH	1:2.5	8.31
E.C.	mmhos/cm	0.66
Organic matter	%	1.05
Extraction	(mg/100g soil)	
N	%	1.05
P		3.51
K^+		41.48
Na^+		48.60
Ca^{++}		62.0
Mg^{++}		32.55
$CO_3^=$		—
HCO_3^-		68.32
CL^-		91.0
$SO_4^=$	ppm	22.14
Available microelements	ppm	
Fe		7.54
Mn		38.85
An		3.5
Cu		3.36

Table (2): Climatic conditions during the growth period.

	Air temperature °C		Relative humidity %
	Mean minimum	Mean maximum	
January	10.0	18.8	62
February	10.6	20.0	56
March	12.5	23.2	54
April	16.5	28.5	46
May	18.4	31.8	48
June	20.8	34.4	52
july	23.5	35.7	56