



THE INTERACTIVE EFFECTS OF CaSO_4 AND NaCl ON THE GROWTH AND NODULATION OF PISUM SATIVUM

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Summary:

The interactive effects of CaSO_4 and NaCl on the growth and nodulation of Pisum sativum were examined. Pot experiment was conducted to grow Pisum sativum at four levels of CaSO_4 (0.5, 1.0, 2.5 and 5.0 mM) and NaCl (1, 25, 50 and 100 mM). While NaCl significantly reduced plant growth, addition of CaSO_4 increased plant height leaf number and biomass of salt treated plants. A significant calcium/sodium interaction was not seen for nodule number.

Gradual increase of CaSO_4 concentration from (0.5-2.5 mM) significantly increase nodule number of salt-stressed plants, while significant depression in nodule number at 100 mM NaCl and 0.5 mM CaSO_4 was shown. High levels (1-5) of calcium sulfate clearly promoted the formation of nodules.

The effects of CaSO_4 and NaCl on the content of nitrogen in stem and leaves were completely different. The nitrogen content of stem was depressed at high NaCl level (100 mM) and addition of CaSO_4 did not offset this effect. On the other hand leaves nitrogen content showed considerable increase by salt concentration increase, while CaSO_4 promoted growth (N- content) up to (2.5 mM) then declined. The improvement of Pisum sativum tolerance to salt by addition of CaSO_4 may be attributed to the effect of calcium in maintaining the selective permeability of cell membrane.

Introduction:

Pea (Pisum sativum) is an important food in Egypt. Soils of Egypt are characterized by the presence of excessive soluble salts (Helemish and El-Gammal, 1987). To obtain satisfactory yield of Pisum sativum in such saline soils, varieties of Rhizobium and host genotype that are tolerant to the stresses associated with these soil

are desired (Helcmish, 1986). This author found that the Rhizobium of Pisum sativum (local isolate) grew at wide pH ranges with maximum growth at pH values from (4.5-5.0) She also found that the same strain could be grown in cultures having various concentrations of NaCl with maximum growth at 0.2-0.5% NaCl although growth was low at extremely higher concentration. On the other hand Minchin and Pate (1975) reported very low rates of nitrogen accumulation in Pisum sativum when grown in nutrient solution with the upper nodulated root zone exposed to the air. Such situation easily arise in tropical regions where high salt concentration and high evaporation result in salt deposition on the soil surface (Sprent and Sprent, 1990).

Calcium plays an important role in the functioning of nodules (N_2 -fixation) by many legumes (Banath *et al.*, 1966). Under severe calcium deficiency the amount of N_2 -fixed is limited by restricted host plant growth, Whereas under conditions of mild calcium deficiency impaired nodule function may lead to symptoms of nitrogen deficiency (Edwards, 1977).

High pH as well as low pH generally reduces nodulation. This may come about because of reduced growth and multiplication of rhizobia in soil (Kumar and Garay, 1980 and 1981) on high pH and (Rice *et al.*, 1977 and Mulder *et al.*, 1977) on low pH. Acid soil may also be low in levels of available calcium and also contain levels of aluminium and manganese toxic to the host plant (Munns, 1977 a and b). Liming can alleviate the effect of acid soil, it encourages rhizobial growth and reduce levels of manganese and aluminum (Sprent and Sprent, 1990). Munns and Fox, (1977) observed that the greatest yield increase of legume occurred on a Hawaiiin Oxisol when the soil was limed above pH 5.9. They suggested that this increase was due to the increase in calcium activity. Since above pH 6 exchangeable and soluble aluminum and manganese levels were low and almost unchanging (Hunsen and Munns, 1988 b).

Although several extensive reviews on Pisum sativum have been written in the last ten years, little has been noted concerning its salinity tolerance and the interactive effects of $CaSO_4$ and NaCl on its growth and nodulation.

The purpose of this study was to test whether high calcium levels increase the salt tolerance of Pisum sativum when grown in saline soil at pH 9 by examining the

growth and nodulation response of nodulated Pisum sativum Giza 2 to varying levels of CaSO_4 (0.5 to 5.0 mM) and NaCl (1-100 mM) in pot experiment.

Materials and methods:

Pot experiment was conducted to evaluate the interactive of CaSO_4 and NaCl at four levels of CaSO_4 (0.5, 1.0, 2.5 and 5.0 mM) and four levels of NaCl (1, 25, 50 and 100 mM) with a total of sixteen treatments on the growth and nodulation of Pisum sativum. CaSO_4 levels were applied ten days after germination, NaCl treatments 30 days after germination (Hunsen and Munns, 1988 a and b).

Seeds of Pisum sativum Giza 2 were obtained from Ministry of Agriculture. Uniform seeds were selected and scarified for 5 minutes in sulfuric acid, rinsed thoroughly and soaked overnight in distilled water. Then seeds were planted in plastic pots 25 cm in diameter with drainage. Once the seedlings emerged they were watered with the levels of CaSO_4 that would be used throughout the experiment. Within seven days of planting, more than 50% of the seeds had germinated. Ten days after planting, the seedlings were inoculated with Rhizobium leguminosarum specific for Pisum sativum. Seedlings were watered with a solution containing 0.5, 1.0, 2.5 or 5.0 mM CaSO_4 . When plants were 25 days old and the roots were nodulated, the experiment was set out in three replications each containing sixteen pots with five plants in each pot, then NaCl treatment (1, 25, 50 and 100 mM) were begun. NaCl was added two times weekly. The salts were added gradually to avoid sudden large decreases in water potential. Growth parameters were recorded at harvest after 65 days of planting. These including plant height, dry matter accumulation of root, shoot, leaves and yield, in addition to nodule and leave numbers. Stem and leaves N was also recorded. Plants were rinsed in water, separated into roots, shoots, leaves and nodules, dried at 70°C for three days and weighed. Number of nodules and leaves of each plant was counted. At the end of the experiment (65 days after planting) oven dried plant samples were ground and thoroughly homogenized in a monliex mill to pass through a 20 mesh screen. For estimation of total nitrogen in shoots and leaves, samples were digested in concentrated H_2SO_4 and measured by microkjeldahl method (Jackson, 1967).

Each value was the mean of three replications. Statistical calculations of the significant levels of the difference among the treatments was carried out using L.S.D. test (Snedecor and Cochran, 1972).

Results:

Plant height (Fig. 1) showed a clear calcium/sodium interaction, sodium chloride depressed growth significantly and CaSO_4 partly offset this effect especially at the higher concentrations of NaCl. At harvest, leaf number (Fig. 2) showed a very significant interaction. Increasing NaCl concentration depressed leaf number especially at higher NaCl levels (100 mM) comparing to lower levels or control (1 mM). Moreover gradual increase of CaSO_4 improved it and maximum increase was attained at (2.5 mM) then declined at (5 mM).

Concerning nodule number (Fig. 3) noticeable increase was shown corresponding to NaCl increase till (50 mM) then decreased at (100 mM). Higher CaSO_4 levels enhanced nodule number by more than 50% up to certain limit (2.5 mM) and depressed it at (5 mM). Increasing NaCl levels decreased root dry weight (Fig. 4) comparing to the control (non-saline treatment or 1 mM NaCl), it was 0.25 g/plant comparing to 0.20, 0.22 and 0.07 at 25, 50 and 100 mM NaCl levels respectively and at lower CaSO_4 levels (0.5 mM), while root dry weight was increased significantly at both higher concentration of CaSO_4 and NaCl. The same trend is shown with respect to stem and leaf dry weight (Fig. 5 and 6). However, at harvest a significant calcium/sodium interaction was seen. Calcium sulfate improved growth of salt stressed plants (Fig. 4, 5 and 6).

Effect of NaCl and CaSO_4 on nitrogen content of stem was shown in Fig. (7). Progressive increase of NaCl levels (1-100 mM) had decreased stem N content at all CaSO_4 levels. It was 3.49% for the control (non-saline treatment, 1 mM NaCl) and at 100 mM NaCl, it was 2.99, 2.93, 2.79 and 2.04 at 0.5, 1, 2.5 and 5 mM CaSO_4 , respectively (Fig. 7). With respect to leaf N content, noticeable nitrogen increase was seen by increasing NaCl levels (1-100 mM). Nitrogen content of leaves at 100 mM NaCl was 4.53, 4.96, 4.58 and 4.62% at 0.5, 1, 2.5 and 5 mM CaSO_4 , respectively,

compared to 3.96% of non-saline control (Fig. 8). Effect of NaCl and CaSO₄ on nitrogen content appear inconclusive.

Discussion:

Salinity tolerance of Rhizobium that infect Pisum sativum in nutrient culture have been studied in previous paper by Helmesh, (1986). The present work explain: 1) whether Pisum sativum could establish better growth and nodulation in saline soils as did bacteria in solution culture and 2). The possibility of improving the salinity tolerance of plant grown in these soils in the presence of CaSO₄. On these basis, the present experiment was conducted to evaluate the counteraction of CaSO₄ on NaCl stressed plants and their interactive effects on growth, nodulation and nitrogen content of Pisum sativum in pot experiment.

Sodium chloride inhibited plant height, dry matter biomass and number of leaves and nodules of salt treated plants. This depended on salt concentration, rhizobial strain and the tolerance limit to NaCl (Samir et al., 1991). Increasing salt concentration resulted in a reduction of nodule number, nodule weight and total nitrogen fixation by Glycine wighii (Wilson, 1970). Other authors (Brenstein and Ogata, 1966; Islam and Choulam, 1981 and Lauter et al., 1981) observed similar effect of salinity on nodulation and N₂-fixation of Glycine max, Vicia faba and Cicer arietinum, respectively, Lauter et al., (1987) indicated that the process of nodulation was very sensitive to salinity in Vigna radiata and Soybean. These indications were confirmed by Knobel, (1987) who found that NaCl reduced number of nodules of Vicia faba at early stages of plant development. In addition Samir et al., (1991) found that increasing sodium chloride in irrigation water adversely affected the rate of nodule formation, dry weight of nodules and shoots of Soybean plant. Similar results were obtained by Tu, (1981), who observed that nodulation was completely eliminated at more than or equal to 1.2% NaCl and increasing salinity caused a gradual and decline in Soybean fresh weight and plant height. Kumar and Garg, (1980) studied the effect of salinity and alkalinity on the process of nodulation in pea and they found that salinity and alkalinity or both reduced number and weight of the nodules, furthermore a complete failure of nodulation was

observed at pH 10 with all the studied salinity levels and at pH 9 with only high salinity levels.

In the present study, failure of nodulation in pea was due to higher sodium chloride concentration as well as to soil pH of 9. This in accordance with the findings obtained by Mulder and Veen, (1966) and Burton, (1967) who reported that rhizobia were scanty or absent in saline-alkaline soil. Moreover Bhardwaj, (1975) observed that rhizobial strains of Rhizobium leguminosarum survived a pH of 10 or lower but the multiplications was drastically reduced above a pH of 9.5. Kumar and Garg, (1981) confirmed these finding and attributed the complete failure or drastic reduction in nodulation of salt stressed Pisum sativum plant to the inability of rhizobia to survive and grow under these conditions.

The nodule production with increase in age of plants was low under saline-alkali condition (Kumar and Garg, (1980), however in this experiment growth parameters and nodule formation were examined after 65 days of planting and at soil pH 9. These results are in general agreement with other studies regarding effect of salinity (Lakshmi et al., 1974 and Subba rao et al., 1972) and alkalinity (Habish, 1970 and Lakshmi et al., 1974) on the nodulation of different crops.

Salinity has been reported to reduce rates of division (Gaidamkina, 1967 and Kumar and Garg, 1980). As the production of nodules and their growth essentially involves localized cell division, a reduction in nodulation may well partly be ascribed to this factor.

Depressed growth due to high salinity is attributed to several factors: Water stress, specific ion toxicity and ion imbalance stress of induced nutrient deficiency (Wyn Jones, 1981 and Hanson and Munns, 1988 a and b).

High Na⁺ concentration can produce a calcium deficiency in cotton seedlings, supplemental Ca⁺⁺ significantly offset the toxic effect of high NaCl, resulting in improved growth (Kent and Läuchli, 1985). Hanson and Munns, (1988 a) found that growth and nitrogen fixation of Leucaena leucocephala were affected by higher concentration of NaCl and addition of CaSO₄ at different levels offset its deleterious effect. Similar trends were observed in bean plants (LaHaye and Epstein, 1971).

Calcium is important in maintaining the selective permeability of membranes and Na^+ is known to enhance membranes leakage rates (Hanson, 1984 and Leopold and Willing, 1984). The effect of calcium in promoting salinity tolerance is widely recognized (Kent and Lauchli, 1985; LaHaye and Esptein, 1971; Leopold and Willing, 1984) and may be related to the essential role of calcium for membrane integrity. Calcium is thought to bind to the external surface of the negatively charged plasma membranes in complexes with acid group of phospholipid and protein. Through this binding the membrane tightens, so that passive ion fluxes are reduced and the membrane becomes more hydrophobic (Gary-Bobo, 1970 and Hanson and Munns, 1988 b). It is thought that aqueous channels through the membrane close when it is tightened, preventing the movement of hydride ions such as sodium or calcium. Millimolar concentration of calcium are needed for this tightening to occur (Hanson, 1984). It is thought that Na^+ could when present in high enough concentration, displace Ca^{++} of binding sites on the plasma membrane. This disrupting the tightening of the membrane (Gramer *et al.*, 1985). Large yield increase in other leguminous plants have been observed when acid, low calcium soil is limed to a pH of 7.0. It is uncertain whether these increase are due to decrease in hydrogen, aluminum or manganese concentration or to increase in calcium and molybdenum concentration (Hanson and Munns, 1988 b). The Ca requirement might be greater under acid soils conditions than it was in this experiment at alkali pH.

The concentration of N in the leaves was inversely proportional to the leaves dry weight. This might considered to be a dilution effect by other components in the leaves. The main concentration of N in the leaves ranged from 3.72-4.96%. The plants appeared to be adequately supplied with N during the growing season and N was not found to be a limiting factor in leaf production. Nitrogen, being a mobile element, was translocated to the leaves from the maturing plant (Labanauskas *et al.*, 1981).

In conclusion, the selection of a legume host from highly saline or non saline habitats appeared to be the most important factor governing compatible Rhizobium strains to nodulate and fix nitrogen in condition of high soil salinity.

Reduction in nodule number of plants grown in saline soil may ascribed to applications of highly salt levels as well as high soil pH. Salts may affect the symbiosis through effects on the growth and survival of Rhizobium in soil restriction to root colonization, inhibition of process of infection and nodule development or impairment of active nodule functioning (Craig *et al.*, 1991). These effects may be mediated through an effect of salt on the host or through a specific effect on the micro-symbiont itself.

Calcium sulfate counteracted the effect of sodium chloride and improved growth and nodulation of Pisum sativum under soil saline-alkali condition.

References:

- Abd El-Wahab, S. M.; El-Mokadem, M. T.; Helemish, F. A. and Abu El-Nour, M. (1991). The symbiotic performance of Bradyrhizobium, Jaonicum under stress of salinized irrigation water. *Ain Shams Science Bull.* 28 b, 469-488.
- Banath, C. L.; Greenwood, E. A. N. and Loneragan, J. F. (1966). Effect of Calcium deficiency on symbiotic nitrogen fixation. *Plant Physiol.*, 41,760-763.
- Bernstein, L. and Ogata, G. (1966). Effects of salinity on nodulation, nitrogen fixation and growth of soybean and alfalfa. *Agron. J.*, 58, 201-203.
- Bhardwaj, K. K. R. (1975). Survival and symbiotic characteristic of Rhizobium in saline-alkali soils. *Plant and Soil*, 43, 377-386.
- Burton, J. C. (1967). Rhizobium culture and use. *Microbial. Technology.* pp. 1-33. Peppler, H. J. (ed.) Reinhold Publishing Corporation. New York, U.S.A.
- Craig, G. F.; Atkins, C. A. and Bell, D. T. (1991). Effect of salinity on growth of four strains of Rhizobium and their infectivity and effectiveness on two species of Acacia. *Plant and Soil*, 33, 253-262.
- Edward, D. G. (1977). Nutritional factorial limiting nitrogen fixed by Rhizobia. *Biological nitrogen fixation in farming systems of the tropics.* Chichester, U.K.; John Wiley & Sons. pp. 189-204.

- Gaidamakina, L.F. (1967). Influence of different types of salinization on mitosis in the roots of sunflower and shoot of barley. *Soviet Plant Physiol.*, 14, 625-627.
- Gary-Bobo, C. M. (1970). Effect of Ca^{++} on the water and non-electrolyte permeability of phospholipid membranes. *Nature* 228, 1101-1102.
- Gramer, R. C.; Läuchli, A. and Polito, V. S. (1985). Displacement of Ca^{++} by Na^{+} from the plasmalemma of root cells. *Plant Physiol.* 79, 207-211.
- Habish, H. A. (1970). Effect of certain soil conditions on nodulation of *Accacia* spp. *Plant and Soil*, 33, 1-6.
- Hansen, E. H. and Munns, D. N. (1988 a). Effects of $CaSO_4$ and $NaCl$ on growth and nitrogen fixation of *Leucaena leucocephala*. *Plant and Soil*, 107, 95-99.
- Hansen, E. H. and Munns, D. N. (1988 b). Effects of $CaSO_4$ and $NaCl$ on mineral contents of *Leucaena leucocephala*. *Plant and Soil*, 107, 101-105.
- Hanson, J. B. (1984). The function of calcium in plant nutrition. In *Advances in Plant Nutrition*, Vol. 1. Eds. PB Tinker and A. Läuchli, pp. 149-208. Praeger Publishers, New York.
- Helemish, F. A. (1986). Studies on the growth of *Pisum sativum* under stress conditions. *Annual Review of Women's College, Ain Shams University, Cairo, Egypt.*
- Helemish, F. A. and El-Gammal, S. M. A. (1987). Salt and pH tolerance of *Rhizobium leguminosarum* TAL 271.
- Islam, R. and Ghoulam, W. (1981). Screening of several strains of faba bean *Rhizobium* for tolerance to salinity. *FABIS Newsletter*. 3, 36-37.
- Jackson, M. L. (1967). *Soil chemical analysis*. Prentice Hall.
- Kent, I. M. and Läuchli, A. (1985). Germination and seedling growth of cotton: salinity-calcium interaction. *Plant Cell Env.* 8, 155-159.

- Knobel, W. (1987). Influence of salt stress on the development of nodules of food legumes. In Food Legume Improvement for Asian Farming Systems (Edited by Wallis, E. S.; Blyth, D. E.) Canberra, Australian Centre for International Agricultural Research.
- Kumar, S. and Garg, O. P. (1980). Effect of saline-alkaline conditions on nodulation in pea (*Pisum-sativum* L.). Indian. J. Pl. Physiol. 24, 212-217.
- Kumar, S. and Garg, O. P. (1981). Effect of a shift to saline-alkaline conditions on nodulation, nitrogen fixation and growth in Pea (*Pisum-sativum* L.). Indian. J. Pl. Physiol. 23, 55-60.
- Labanauskas, C. K.; Shouse, P. and Stolzy, L. H. (1981). Effects of water stress at various growth stages on seed yield and nutrient concentration of field-growth cowpeas. Soil Science, 131, No. 4, 249-256.
- Lakshmi-Kumari, M.; Singh, C. S. and Subba Rao, N. S. (1974). Root hair infection and nodulation in lucerne (*Medicago-sativa* L.) as influenced by salinity and alkalinity. Plant and Soil, 40, 261-268.
- Lattaye, P.A. and Epstein, E. (1971). calcium and salt tolerance by bean plants. Physio. Plant 25, 213-218.
- Lauter, D.J; Munns, D. N. and Clarkin, K. L. (1981). Salt response of chickpea as influenced by nitrogen supply. Agron. J. 73, 961-966.
- Leopold, A. C. and Willing, R. P. (1984). Evidence for toxicity effects of salt on membranes. In Salinity Tolerance in Plants: Strategies for Crop Improvement. Eds. R. C. Staples and G. H. Toenhiesen, pp. 67-76. Wiley and Sons, New York.

- Michin, F. R. and Pate, J. S. (1975). Effect of water, aeration and salt regime on nitrogen fixation in a nodulated legume definition of an optimum root environment. *J. Exp. Bot.*, 26, 60-69.
- Mulder, E. G. and Veen, W. L. Van. (1966). Effect of pH and organic compounds on nitrogen fixation by red clover. *Plant and Soil*, 13, 91-113.
- Mulder, E. G.; Lie, T. A. and Houwers, A. (1977). The importance of legumes under temperature conditions. In *A Treatise on Dinitrogen Fixation. IV Agronomy and Ecology* (eds. R. W. F. Hardy and A. H. Gibson), John Wiley and Sons, New York, pp. 221-42.
- Munns, D. N. (1977 a). Soil acidity and related factors. In *Exploring the legume Rhizobium Symbiosis in Tropical Agriculture* (eds. J. M. Vincent, A. S. Whitney and J. Bose), Miscellaneous publications, College of Agriculture, University of Hawai'i, 145, pp. 211-36.
- Munns, D. N. (1977 b). Mineral nutrition and the legumsymbiosis. In *A Treatise on Dinitrogen Fixation. Section IV Agronomy and ecology* (eds. R. W. F. Hardy and A. H. Gibson, Wiley and Sons, New York, pp. 353-91.
- Munns, D. N. and Fox R. L. (1977). Comparative lime requirements of tropical and temperate legumes. *Plant and Soil* 46, 533-548.
- Rice, W. A.; Penney, D. C. and Nyborg, M. (1977). Effects of soil acidity on rhizobia numbers, nodulation and fixation by alfalfa and red clover. *Can J. Soil Sci.* 57, 197-203.
- Snedcor, G. W. and Cochran, W. G. (1980). *Statistical methods*. Seventh edit. Iowa State Univ. Press, Ames, Iowa, U. S. A.

Sprent, J. I. and Sprent, P. (1990). Nitrogen Fixing Organisms "Pure and applied aspects". Chapman and Hall. London, New York.

Subba Rao, N. S.; Lakshmi-Kumari, M.; Singh, C. S. and Magu, S. P. (1972). Nodulation of Lucerne (*Medicago-sativa* L.) under the influence of sodium chloride. Indian J. Agri. Sci., 42, 384-386.

Tu, J. C. C. (1981). Effect of salinity on Rhizobium root hair interaction, nodulation and growth of soybean. Ca. J. of Sci. 61(2), 231-239.

Wison, J. R. (1970). Response of salinity in Glycine VI. Some effects of a range of short-term salt stresses on the growth, nodulation and nitrogen fixation of Glycine wightii. Aust. J. of Agric. Res. 21, 571-582.

Wyn Jones, R. G. (1981). Salt tolerance. In *Physiological Processes Limiting Plant Productivity*, Ed. C. B. Johnson, pp. 271-292. Butterworths, London.

Fig. 1:

Effects of CaSO_4 & NaCl
on plant height

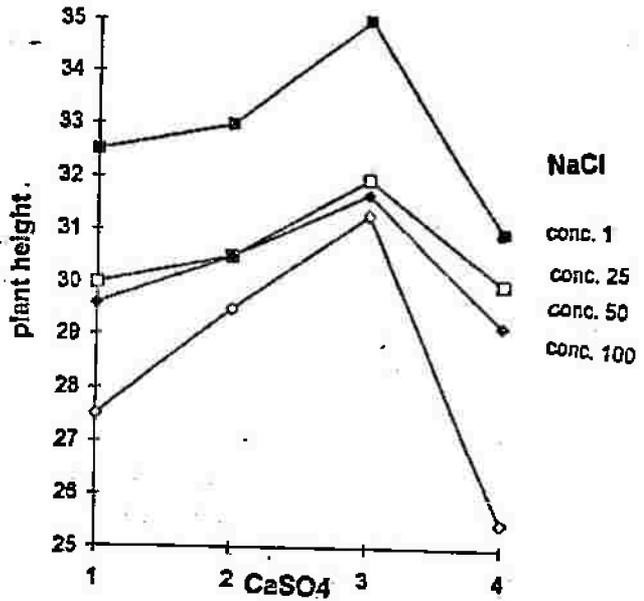


Fig. 2:

Effects of CaSO_4 & NaCl
on leave numbers

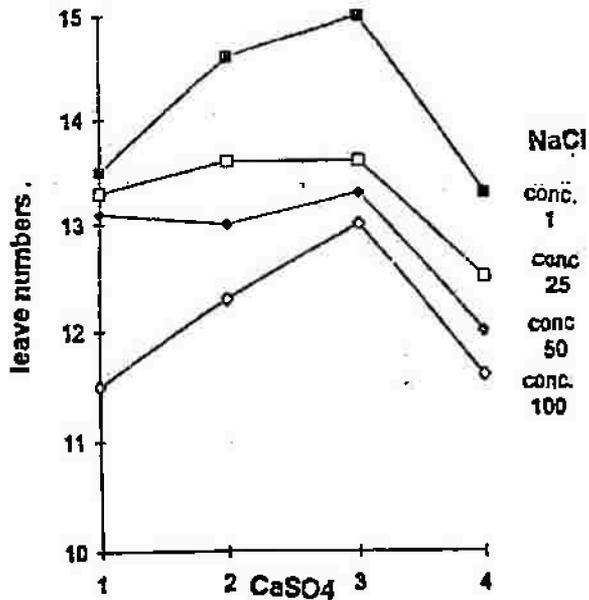


Fig. 3 :

**Effects of CaSO₄ & NaCl
on nodule numbers**

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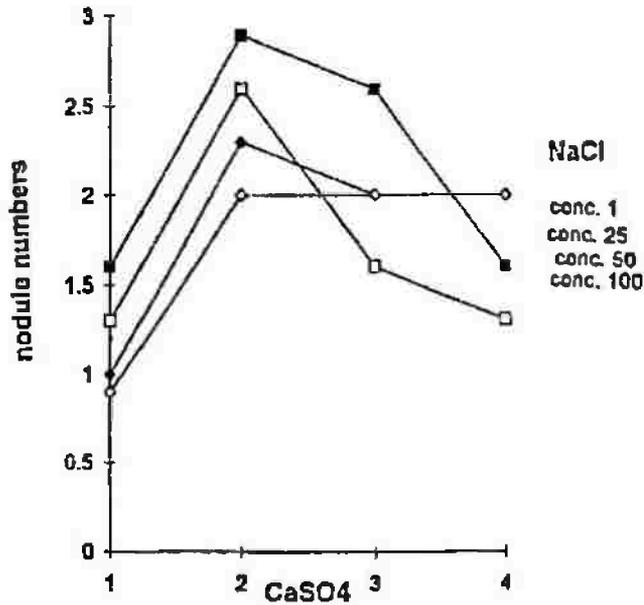


Fig. 4 :

**Effects of CaSO₄ & NaCl on
dry weight of root**

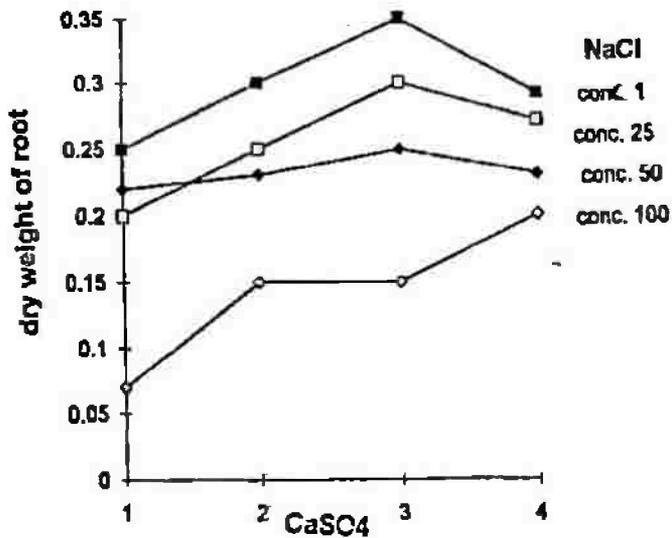


Fig. 5:

Effects of CaSO₄ & NaCl on dry weight of stem

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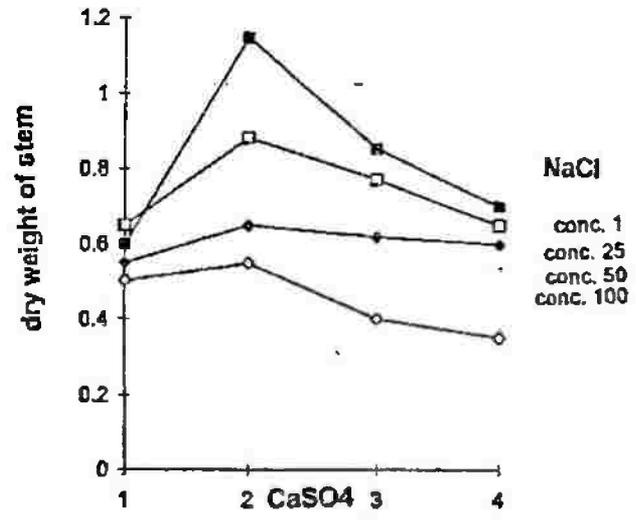


Fig.6 :

Effects of CaSO₄ & NaCl on dry weight of leaves

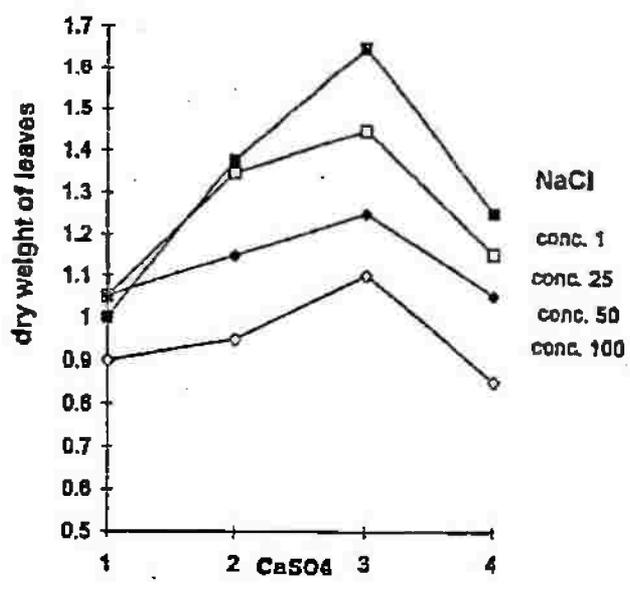


Fig. 7.

**Effects of CaSO₄ & NaCl
on Nitrogen content of
stem**

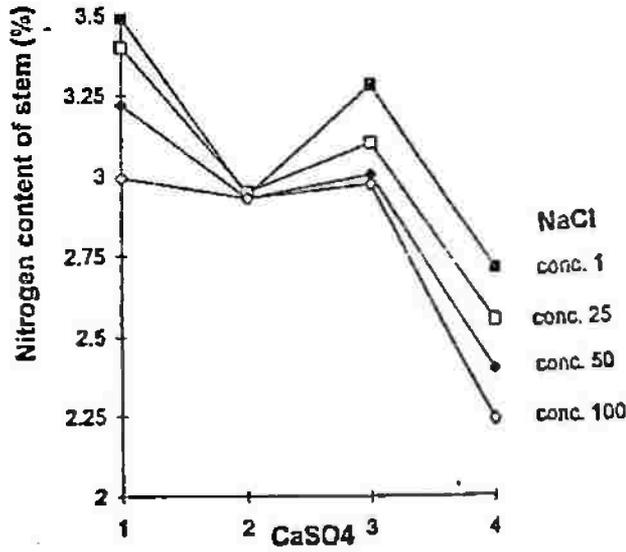


Fig. 8:

**Effects of CaSO₄ &
NaCl on Nitrogen
content of leaves**

