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Effects of pH on the Symbiotic Performance of Rhizobium leguminosarum bv.viceae on Faba Bean

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Abstract:

Investigations were carried out on the efffects of the pH range 4.0-8.0 on nodulation and growth of faba bean inoculated with a locally isolated (SVF 01) and an adapted (SU 1397) strains of the faba bean nodulating bacteria (*Rhizobium leguminosarum* bv. *viceae*) in potted soil and in solution culture.

At any pH level, no nodules were detected on plants grown in solution culture. On the other hand, number and dry weight of nodules and the dry weights of shoots and roots increased with increase in pH value from 4.0 to 7.0, but decreased between 7.0 and 8.0 with both bactrerial strians in potted plants. No differences were detected between the two strians in their response to pH level or to aluminium and calcium concentrations in either solution culture or in potted soil.

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The results have covered some of the effects of pH, aluminium and calcium concentrations on the growth of faba bean and the fate of the symbiotic partnership formed by the crop and the bacteria. This is hoped to help in combating the problems they create in the face of crop establishment and the sustainability of production in areas suffering from the adverse effects of such factors.

Introduction:

Soil acidity is an important factor limiting the growth of plants and survival of *Rhizobium* spp in many areas (Bromfield and Jones, 1980). Its main effect is exerted on the survival and the growth of nodulating bacteria rather than on the nodulation process (Howieson and Ewing, 1986).

Acid tolerance is one of the major properties used in the selection of rhizobial strains that are utilized to produce inoculants for use with legumes in acid soils. This involves tolerance to pH and high levels of aluminium associated with low levels of calcium (Wood and Shepherd, 1987). Tolerance of rhizobia to acidity is a consistent and stable strain property (Munns and Keyser, 1981). For isolates of *Rhizobium trifolii*, Lindstrom and Myllyniemi (1987) found that the lowest pH value (critical pH) for growth in acid laboratory media varied between 4.7 and 4.9 *Rhizoium leguminosarum* strain TAL 271 could grow in a wide range of pH and tolerate both moderate acidity and alkalinity, with optimum pH at 5.5 (Helemish and El-Gammal,

1987). Lowendorf *et al.*, (1987) reported that *R. meliloti*, *R. trifolii* and *R.japonicum* were unable to survive for 75 days in soils of pH 5.1, 4.9 and 4.2, respectively.

Strains of rhizobia which are acid tolerant in laboratory media have generally given encouraging responses when inoculated into acid soils, either by exhibiting better survival or by improving nodulation of host plants compared with acid-sensitive strains (Cooper *et al.*, 1985). However, although many research workers reported that screening rhizobia for acid tolerance in laboratory media can be of predictive value, it appears that not all symbiotically acid-tolerant rhizobia were correctly identified by laboratory pre-screening. Improvement may be possible but even so, error is acceptable because artificial media are not like soil (Keyser *et al.*, 1979).

It is well established that acidity inhibits nodulation, but the nature of inhibition is not understood. The observation that nodule bacteria can grow at pH values too low for nodulatoion can be interpreted to mean that acidity does not inhibit nodulation by preventing rhizosphere development (Munns, 1968). The growth and persistance of annual *Medicago* species on moderately acid soils appears to be limited by the inability of known strains of *Rhizobium* to colonize these soils (Howieson and Ewing, 1986). In most cases, reduction in plant growth by acidity corresponds to reduction in nodule numbers, and the reduction in nodule numbers was partly compensated for by increases

in average nodule size (Keyser *et al.*, 1979). Understanding the behaviour of *Rhizobium* in acid soils is, therefore, important for successful nodulation, development of nitrogen fixing symbiosis and ultimately crop yield (Lowendrof *et al.*, 1981).

The objective of this study was to investigate the effects of pH range 4.0-8.0 on nodulation and growth of faba bean, using an adapted and a locally isolated strains of root nodule bacteria.

Materials and Methods:

This study was carried out to investigate the effects of the pH range 4.0-8.0 on nodulation and dry matter yield of *Rhizobium*-inoculated faba bean variety (Agabat). The effects of pH were studied in solution culture using the pH range 4.0 to 8.0, and in potted soil using the pH range 4.0 to 7.4 under glasshouse conditions.

Two strains of *Rhizobium leguminosarum* bv. *viceae* were used, namely the adapted strain (SU 1397) and a locally isolated strain (SVF 01). SU 1397 was originally introduced from the NifTAL project, University of Hawaii, USA, and was obtained from the National Laboratory for Legume Inoculant Production, National Research Centre. SVF 01 was isolated from faba bean plants growing at the Demonistration Farm of the Faculty of Agriculture at Shambat. The strain was subsequently authenticated as *Rhizobium leguminosarum* bv. *viceae* and was numbered SVF 01.

Clean undamaged seeds were disinfected by dipping in 95% ethanol for 2 minutes, then immersed for 5 minutes in 0.1% $HgCl_2$ solution, and were finally washed at least 10 times in sterile water. The seeds were allowed to stand in the final change of water for several hours until they were fully imbibed (Vincent, 1970). Seeds were then germinated in sterile petri dishes containing moistened double filter paper for 5 days.

The N-free nutrient solution used was that described by Lie (1969) with the following composition (mg/litre): KH_2PO_4, 80; K_2HPO_4, 250; $MgSO_4.7H_2O$, 250; $CaSO_4.2H_2O$ 250; $NaHPO_4$, 50; $MnSO_4.4H_2O$,1.0; $CuSO_4.5H_2O$, 0.25; $ZnSO_4.7H_2O$, 0.25; H_3BO_3, 0.25 and $NaMoO_4.H_2O$, 0.25.

Solution aliquots were adjusted to the pH levels 4.0, 4.5, 5.0, 5.5 and 6.0 using either 0.1M HCL or 0.1M NaOH. Half a litre of each was dispensed in glass jar assemblies. The jars, together with the solution culture, were autoclaved at 121°C and 1.09 kg cm^{-2} for 15 minutes. The pH of each bottle was checked again and, when needed, re-adjustment was undertaken (Lie, 1968).

Three seedlings were sown per jar and each jar recieved 10 ml of inoculant culture containing 10^7 cells/ml of the required *Rhizobium leguminosarum* strain. The jars were laid in a completely randomized design with four replications. Plants were sampled eight weeks after

sowing. The dry weights of shoots and roots were assessed after oven drying at 85°C for 48 hours. No nodules were found in plant roots growing in solution culture. A similar investigation was carried out during the same period using the pH range 6.0-8.0.

A parallel expriment was carried out using potted soil. The soil used was a mixture of 70% sand and 30% silt. The soil pH was adjusted to different pH levels (4.0, 4.5, 5.0, 5.5 and 6.0) using concentrated HCl and was left to equiliberate. By the end of five days, the pH was measured again to ensure stability and re-adjusted, if required. One treatment was maintained at the original pH of the soil mixture (7.4). A basal nutrient solution containing (mg/pot): K_2HPO_4, 1000; $NaHPO_4$, 80 and $MgSO_4.7H_2O$, 500 was added to each pot containing two kg soil (Mulder and VanVeen, 1960).

Five disinfected seeds were sown per pot. After the seedlings had reached 3-5 cm in length, they were thinned to 3 per pot. The seedlings were then inoculated with eihter of the two strains of *R.leguminosarum* bv. *viceae*. Five ml of 3-days old culture grown in Yeast Extract Mannitol (YEM) broth were used to inoculate each seedling. Daily application of water was followed in order to maintain moisture at approximalty 70% of field capacity. A completely randomized design was adopted. The pots were sampled eight weeks after sowing.

When sampling, roots were gently washed and nodules were picked, counted and weighed after oven drying at 85°C for 48 hours. The dry weights of shoots and roots were also determined.

Results and Discussion:

The results indicated that nodulation and growth of faba bean were affected by hydrogen ion concentration (pH) of the growth medium. In the pH range 4.0-8.0 in solution culture both inoculated and ininculated plants failed to form root nodules even up to eight weeks from sowiing, whereas in potted soil nodules were formed in inoculated plants. the inhibition of nodulation in solution culture could be attributed to inadequate supply of oxygen in such a system where the root system is completely submerged, or that the concentration of nutrients ih the solution culture used (Lie, 1969) might be subraoptimal for nodule formation. Both factors could have exerted an effect as has been noticed by Minchin and Pate (1975) and Sprent (1976) on the requirement for more aeration in solution cultures, the observation of Sprent (1972) on inhibition of nodulation in the presence of subraoptimal concentration of nutrients in solution cultures.

At pH 4.0, plant growth was weak and no nodules were formed in either growth culture. However, a gradual increase was observed in both shoot and root dry weights in both potted soil (Fig. 1 and 2) and solution culture (Figs 3 and 4) with the gradual increase in pH value

up to 6.0 in solution culture and 7.0 in potted soil. No consistent differences could be established between plants inoculated with either strain in this respect.

In solution culture, when the pH was raised from 6.0 to 7.0 and further to 8.0, a linear increase was observed in shoot dry weight of plants inoculated with strain SU 1397, while shoot dry weight of plants inoculated with strain SVF 01 were increased at pH 7.0 but suffered a slight reduction at pH 8.0 (Fig. 5). A slight increase was observed in root dry weight of plants inoculated with SU 1397 but not in plants inoculated with SVF 01. However, both were much lower at pH 8.0 (Fig. 6). No nodules were formed below pH 4.5, but nodule number (Fig. 7) and dry weight (Fig. 8) showed a progressive increase with rise in pH value up to 7.4 in potted soil.

The present results have indicated that low pH adversely affected both growth and nodulation of faba bean. Similar adverse effects have been reported with other legumes such as field bean (Schubert *et al.*, 1990), peas (Lie, 1969), trefoil (Kim and Edwards, 1985) and white clover (Cooper *et al.*, 1985), with a usually marked improvement above pH 4.0 Evans *et al.* (1988) demonstrated that in subterranean clover the progressive increase in nodulation with increase in pH value was attributed to increase in the rate at which the clover root nodule bacteria multiplied in soil. This decrease in the rate of multiplication of bacteria at low pH is believed to be created by the inavailability of

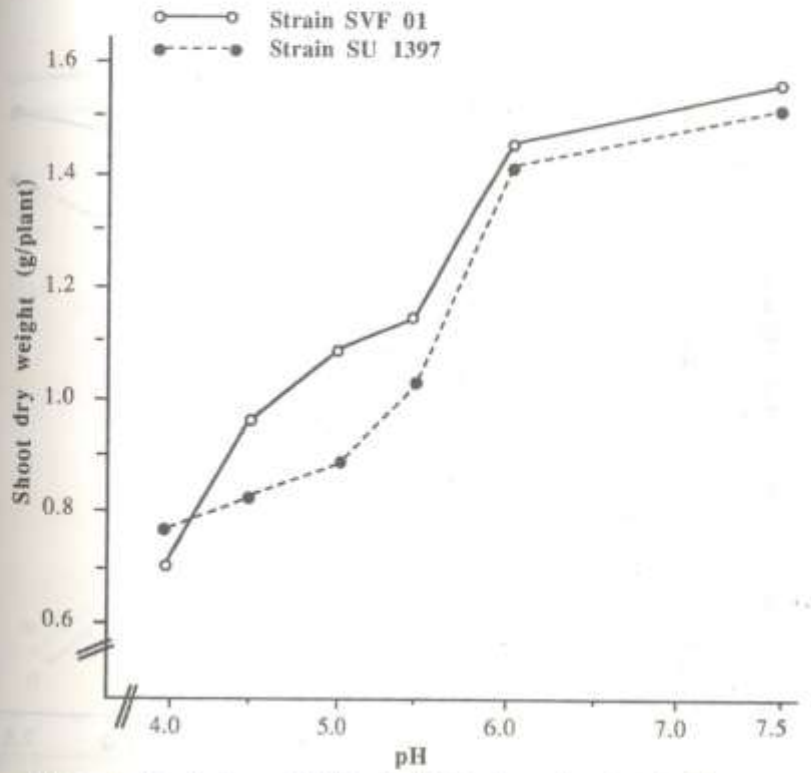

Figure 1: Shoot dry weight/plant of faba bean inoculated with *R. leguminosarum* bv. *viceae* in the pH range 4.0-7.4 in potted soil.

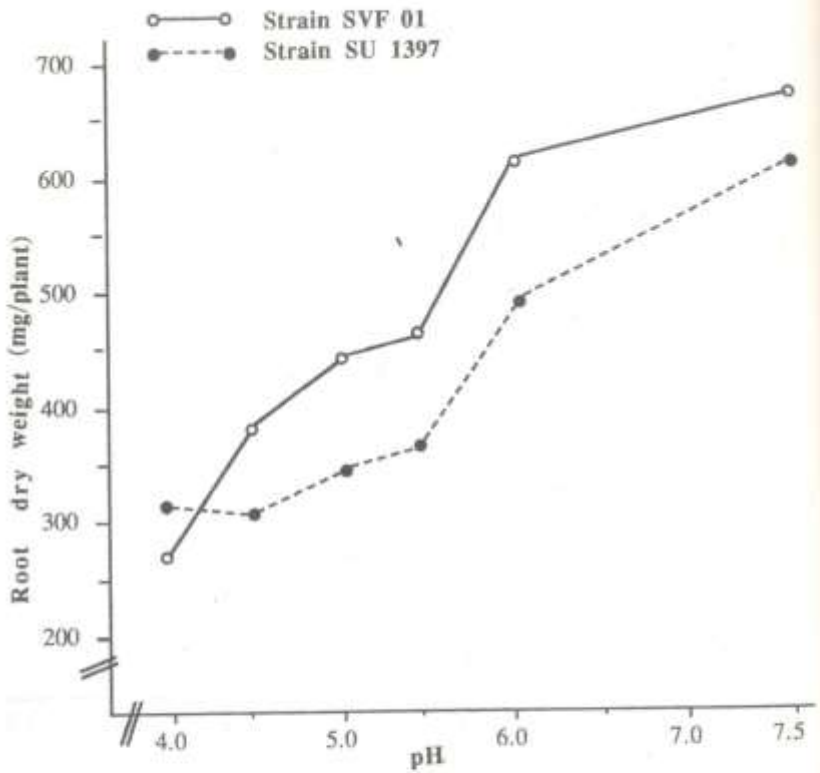

Figure 2: Root dry weight/plant of faba bean inoculated with *R. leguminosarum* bv. *viceae* in the pH range 4.0-7.4 in potted soil.

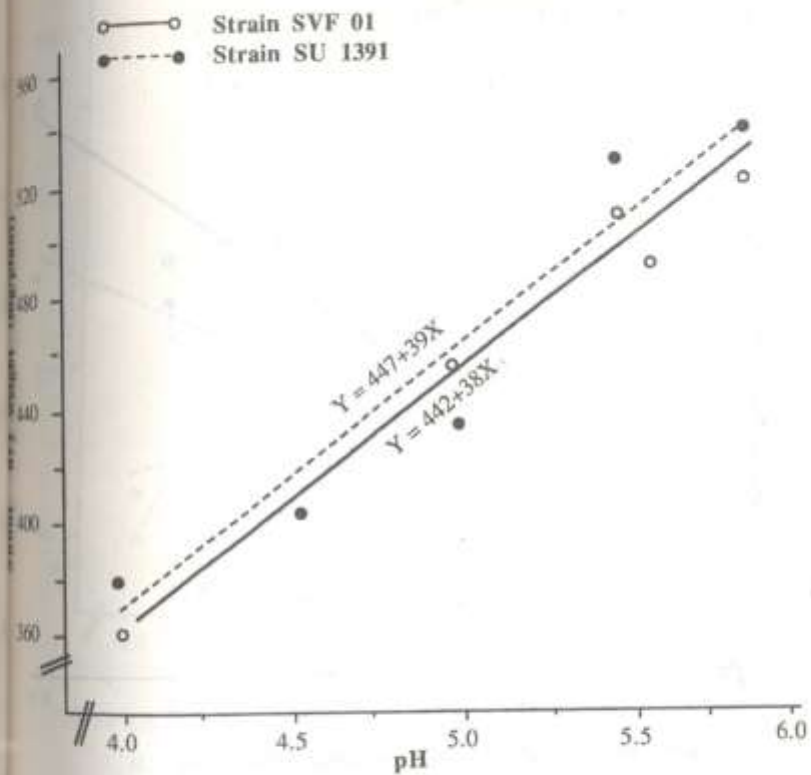

Figure 3 : Shoot dry weight of faba bean inoculated with *R. leguminosarum* bv. *viceae* in the pH range 4.0-6.0 in solution culture.

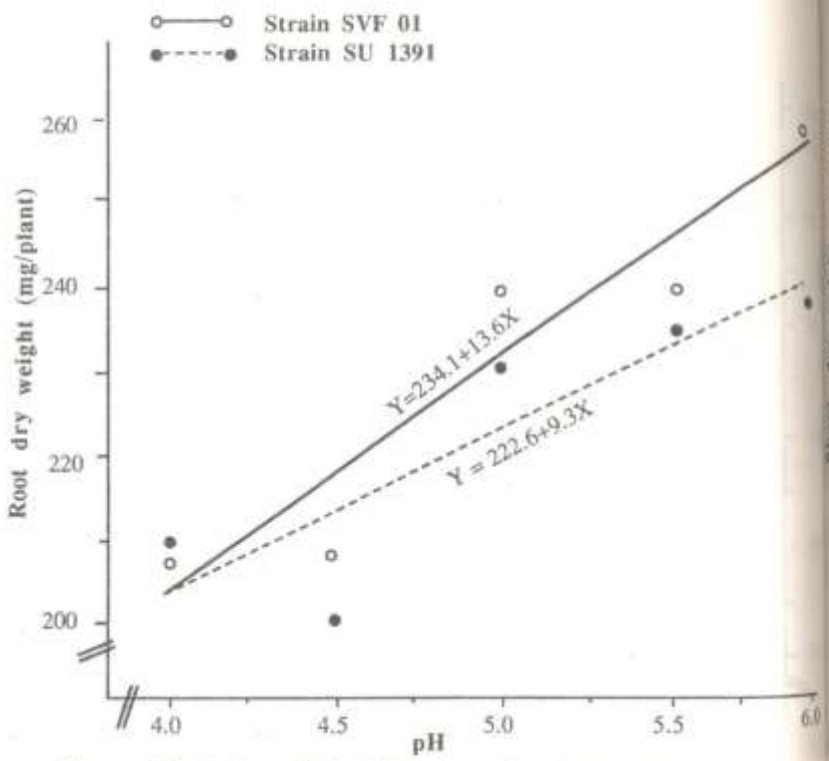

Figure 4:Root dry weight of faba bean inoculated with *R. leguminosarum* bv. *viceae* in the pH range 4.0-6.0 in solution culture.

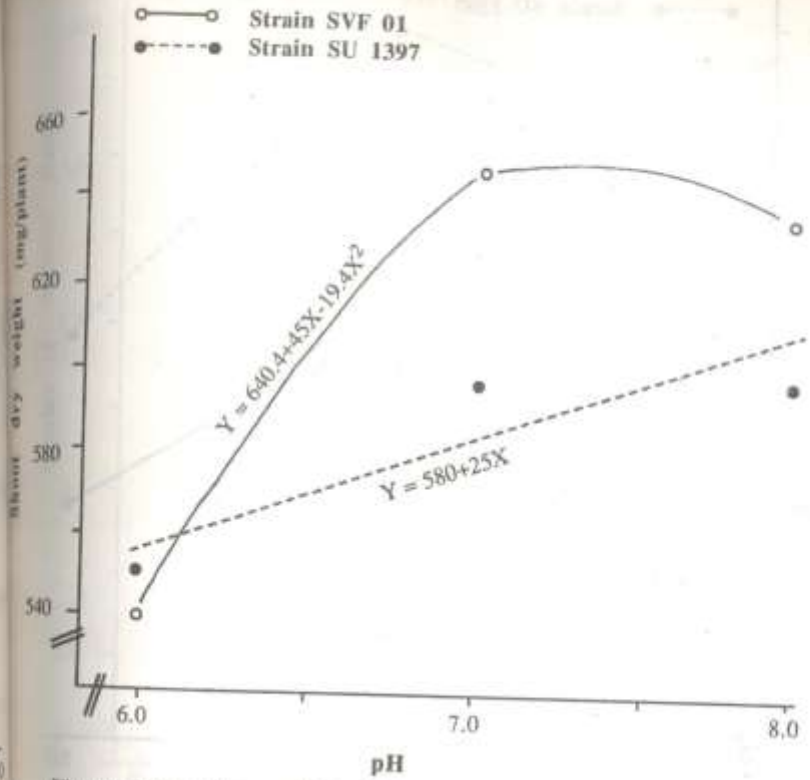

Figure 5:Shoot dry weight of faba bean inoculated with *R. leguminosarum* bv. *viceae* in the pH range 6.0-8.0 in solution culture.

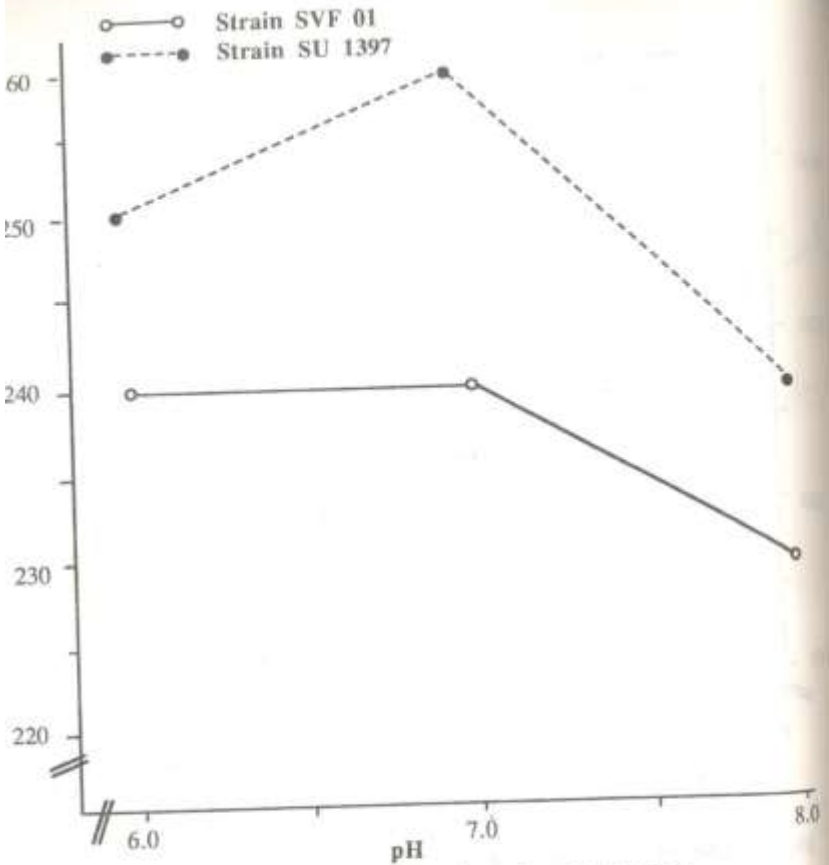

Figure 6:Root dry weight of faba bean inoculated with *R. leguminosarum* bv. *viceae* in the pH range 6.0-8.0 in solution culture.

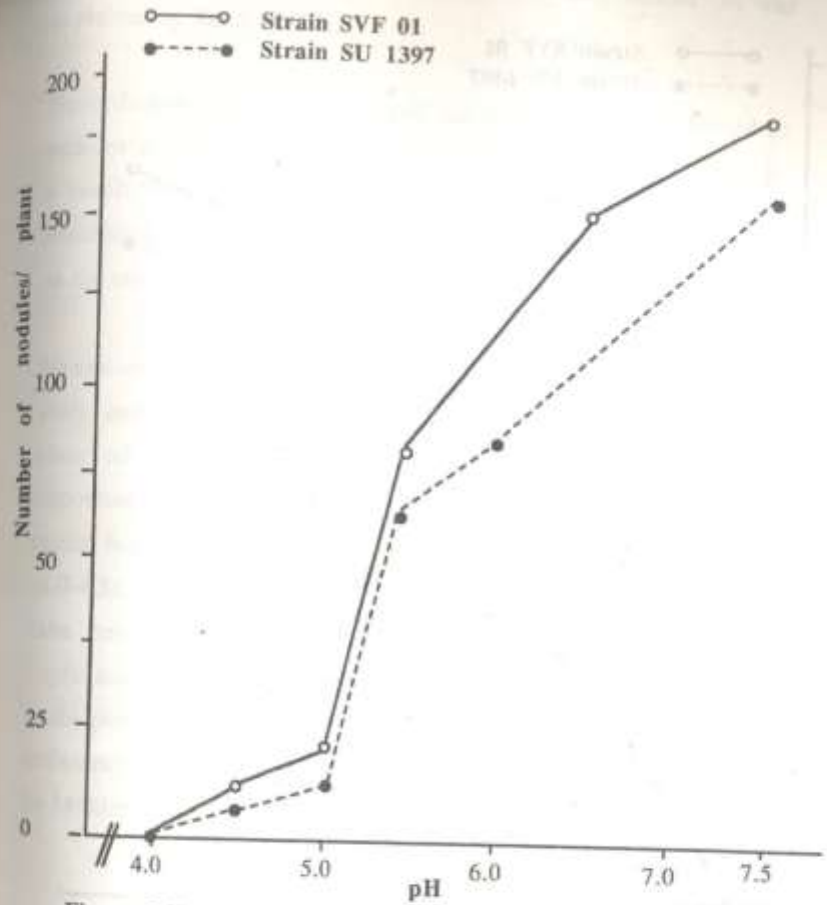

Figure 7:Number of nodules/plant of faba bean inoculated with *R. leguminosarum* bv. *viceae* in the pH range 4.0-7.0 in potted soil.

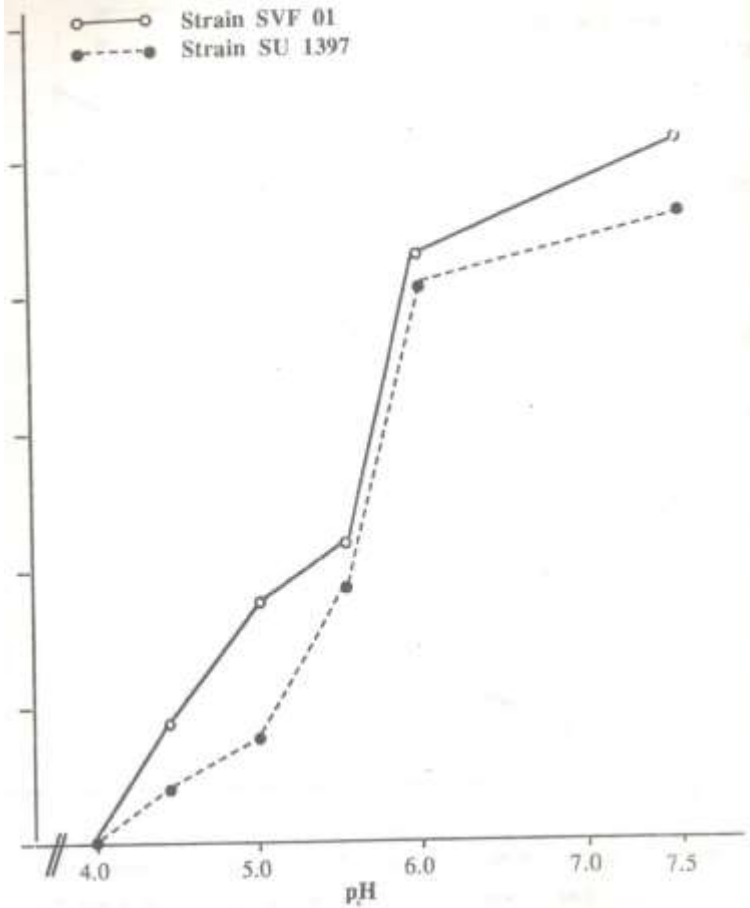

Figure 8: Nodules dry weight/plant of faba bean inoculated with *R. leguminosarum* bv. *viceae* in the pH range 4.0-7.4 in potted soil.

many ions due to interplay of various "infertility factors" as was explained by Smith (1982).

Nevertheless, Lie (1969) showed that pea plants were devoid of nodules at pH 4.5 in the presence of large numbers of rhizobia, a result which indicates that the adverse effect of low pH on *Rhizobium* was on its ability to invade roots (invasiveness) rather than on the rate of growth and multiplication.

No assessment of bacterial numbers has been carried out in the present study, and any of the two possible hypotheses could have caused the observed absence of nodulation at low pH. However, the more important aspect is how this is reflected on plant growth? The present results have shown that plant growth was very weak at low pH value (4.0-4.5). This study was not intended to analyse the factors affecting faba bean nutrition and growth at low pH, but one possible explanation for such weak growth is inadequacy of nitorgen supplies to the plant in the absence of nodulation at low pH. Similar nitrogen deficiency at low pH in non-nodulated red clover plants was observed by Mulder and Van Veen (1960).

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