

Studies on growth of Rhizobium of Pisum sativum under stress conditions.

F.A.Helemish
Botany Dep., Women's College,
Ain Shams University, Cairo, Egypt.

SUMMARY

One fast growing acid producer Rhizobium strain (local strain) of Pisum sativum was screened for growth behaviour in acid, saline and alkaline broth culture. It grew in yeast extract mannitol broth wide pH range (3.5-10) at varying concentrations of NaCl, Na₂CO₃, Na HCO₃ and CaCl₂.

The growth of this strain increases when NaCl concentrations were raised from 0.2-0.8%, and decreases when CaCl₂ concentrations were raised from 0.2-3.5% and Na₂CO₃ and Na₂HCO₃ from 0.1-1%. The results indicate that growth of Rhizobium infecting Pisum sativum is adversely affected by saline and alkaline conditions. The effect of alkalinity is more drastic than salinity. This in-turn suggests that the ability of Rhizobium to establish a successful symbiotic relationship with Pisum sativum is inhibited by these conditions.

Introduction

Pea, Pisum sativum is an important food in tropical regions of Egypt and Africa. Lands of Egypt are characterised by the presence of excessive soluble salts (about 6% water soluble salts in saline soils). The dominant ions are sodium, calcium, chloride, carbonate and bicarbonate. Most common are sodium and calcium chloride type of salinity.

To obtain satisfactory yields of Pisum sativum in such saline and alkaline soils, varieties of Rhizobium and host genotype

that are tolerant to the stresses associated with these soils are desired. These stresses include high pH and high levels of sodium chloride, calcium chloride, sodium carbonate and bicarbonate.

Some authors studied the effect of salinity on the growth of Rhizobium spp. in saline soils and in broth culture (Upchurch and Elkan 1977, Steinborn and Roughley 1975, Abdel Wahab and Wahran 1979 and Bhardwaj 1972), while others studied the effect of alkalinity on the growth of Rhizobium spp. in alkaline soils and in broth culture (Helemish and El-Gammal 1985, Yadav and Vyas 1971 and Singh et al., 1973). Some strains have been found to be salt sensitive to even 0.1% (Helemish 1985, Abdel Wahab and Zahran 1979), while others have been found to be salt resistance to even 3.0% NaCl (Pallai and Sen 1966, Yadav and Vyas 1973 and Subba Rao et al. 1972).

Optimum pH for rhizobia was neutral or slightly alkaline (Yadav and Vyas 1971) and they were sensitive to acidity (Allen and Allen 1950). According to Pandher and Kahlon (1978), Rhizobium leguminosorum isolated from Pea (Pisum sativum) failed to grow at pH 3.0, maximum growth was attained at pH^{6.5-8.0}. On the other hand pH's above 8.5 was not lethal but did not support growth.

One should aim to find Rhizobium strains host variety combinations which yield well in particular soils and environments in order to exploit the full potential of the symbiotic system.

MATERIAL AND METHODS

Isolation of Rhizobium of Pisum sativum: Pure culture of Rhizobium of Pisum sativum was isolated according to the method described by Allen and Allen (1950), from the roots of Pisum sativum. A big red effective nodule was selected, carefully cleaned with running water from adhering soil particles, the surface was then sterilized in 1:100 HgCl_2 solution for 3-6 minutes and in 95% ethanol for a similar period of time. The nodule was then consecutively transferred to sterile petridishes containing sterile water and continuously agitated to secure sufficient rinsing. The nodule was then removed to a sterile petridish and crushed in one ml sterile distilled water. Streaking using a loopful of the concentrated crushed nodule exudate on the surface of each of 5 plates was carried-out. Yeast extract-mannitol-agar medium was used for such isolation.

The petri-dishes were then incubated at 30°C for one week after gram staining (-ve) and microscopic testing of some colonies under strict sterilized conditions for each purity, loopfuls from a selected colony were inoculated into slopes of yeast extract-mannitol agar the composition of which is as follows: Mannitol, 10 gm; NaCl, 0.1 gm; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 gm; K_2HPO_4 , 0.5 gm; yeast extract 0.5 gm; CaCl_2 0.1 gm, -ph was adjusted at 7.2, sterilization was carried out at 1.5 atm. for 15 min, 2% agar was added when necessary.

Flasks containing 50 ml liquid medium were inoculated with 2 ml bacterial suspension (about 10^6) prepared from scraping of

2-day old agar cultures, flasks were shaken at 120 rpm./min. at 30°C growth in liquid medium was assessed turbidimetrically using Bausch and Lomb Spectronic 20 at 540 um. every 24 hours. All treatments were replicated twice.

Effect of pH: The effect of pH on the growth of the organism was assessed in liquid medium with the post-sterilisation pH values Viz: 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5 and 10. The pH was adjusted by Tacussel pH meter.

Effect of different salts: The salts tested were NaCl, CaCl₂, sodium carbonate and bicarbonate. NaCl and CaCl₂ were supplied at contrations of 0.0 (control), 0.2, 0.5, 0.8, 1.5, 2.5 and 3.5% (W/V) respectively. Carbonate and bicarbonatge were supplied at concentra-tions of 0.0 (control), 0.1, 0.2, 0.5, 0.8 and 1% (W/V) respectively. All other growth conditions were performed as previously mentioned.

RESULTS

Effect of pH: The results presented in table (I) on growth and change in pH of the medium after 72 hours and after one week incubation clearly show that this strain could be grown at a wide pH range (3.5-10). Subsequently, the pH variations from the initial levels were also noted. This strain was classified as a fast growing acid producer as when it was grown in broth of pH 4.5 and 5.5 decreased to 3.8 and 4.6 within 72 hours.

The decrease in pH was more acute at high initial broth pH 8.5, 9.5 and 10.

Effect of NaCl: Data in Fig. (1) on the growth of Rhizobium of Pisum sativum in broth containing different concentrations of NaCl show that the strain can grow in presence of various concentrations of NaCl. The growth was high at concentration from 0.2-0.8% and low at concentrations from 1.5-3.5% NaCl relative to control.

Effect of CaCl₂: Effect of different concentrations of CaCl₂ on the growth of Rhizobium of Pisum sativum Fig. (2) shows that the strain can grow slowly at concentrations varying from 0.2 to 3.5% CaCl₂, maximum growth was recorded in absence of CaCl₂. At all concentrations the growth decreases and it was lower than control even at 0.2% CaCl₂.

Effect of carbonate and bicarbonate: Effect of different concentrations of sodium carbonate and bicarbonate on the growth of Rhizobium of Pisum sativum Fig. (3) and Fig. (4) indicates that the strain is nontolerant to these salts and even 0.1% was inhibitory to the growth.

Effect of carbonate was much higher than bicarbonate. The growth decreased with the increase of salt concentrations.

DISCUSSION

One of the means of solving salinity and alkalinity problems in saline/alkaline soils is to screen rhizobia and hosts for tolerance to these stress conditions.

The aim of this investigation is to test the behaviour of Rhizobium of Pisum sativum in acid, saline and alkaline broth culture.

The Rhizobium strain of Pisum sativum was classified as fast growing acid producer, since the pH values of 4.5 and 5.5 were decreased to 3.8 and 4.6 respectively within 72 hours of incubation. It grows at a wide range of pH values (3.5-10) with maximum growth at pH values from (4.5-5.5). The results obtained in this investigation were in full agreement with the results obtained by many authors (Rai and Prasad 1984, Helemish and El-Gamma! 1985, Okafor and Alexander 1975 and Yadav and Vyas 1973). Contrary to these results Graham and Parker (1964) found that pH 10 was critical for all rhizobia spp.

Rhizobium strain of Pisum sativum could be grown in presence of various concentrations of NaCl with maximum growth at 0.2-0.5% NaCl although growth was low at extreme higher concentrations.

This is in harmony with the results obtained by Rai and Prasad (1984). However Pillai and Sen (1973) reported that the growth rate of Rhizobium spp. increased with 1% NaCl added to broth media. calcium chloride was inhibitory even at 0.2% the growth was reduced. Steinborn and Roughley (1975) have shown that the growth of both R. trifolii and R. meliloti were decreased by the addition of salts, and CaCl_2 was more toxic than NaCl in broth and peat culture. While Helemish and El-Gamma! (1985) found that Rhizobium leguminosarum TAL 271 strain could tolerate NaCl levels up to 3%, while CaCl_2 was found more toxic.

Sodium carbonate and bicarbonate reduce the growth of Rhizobium of Pisum sativum even at 0.1% concentration. The inhibitory effect of carbonate and bicarbonate was much higher than in chlorides. These results are in harmony with the results obtained by Helemish and El-Gammal (1985) who found that the threshold of tolerance for carbonate and bicarbonate was much lower than in case of chlorides in Rhizobium leguminosarum TAL 271. However, Singh et al., (1973) reported that even 0.1% of either Na_2CO_3 or NaHCO_3 significantly reduced the number of nodules formed by Medicago sativa.

Addition of structural compounds which enable rhizobia to overcome salinity is another way to solve salinity problem. Sawage et al. (1983) found that addition of 10 mM glycine betaine improve the salt tolerance of R. meliloti. However research for new compounds that improve the salts tolerance of rhizobia needs further study.

REFERENCES

1. Abdel Wahab, A.M. and Zahran, H.H. (1979). Salt tolerance of *Rhizobium* species in broth cultures. *Zeitschrift Fur Allgemeine Mikrobiologie* 19: 681-685.
2. Allen, E.K. and Allen, O.N. (1950). Biochemical and Symbiotic properties of rhizobia. *Bact. Rev.* 19:273-330.
3. Rhardwaj, K.K.R. (1972). Note on the growth of *Rhizobium* strains of dhaicha (*Sesbania cannabina* (Retz.) Pers. in a saline-alkali soil. *Indian J. Agric. Sci.* 42: 432-433.
4. Graham, P.H. and Parker, C.A. (1964). Diagnostic features in the characterization of root nodule bacteria of legumes. *Pl. Soil* 20:363-396.
5. F.A. Helemish,† and El-Gamml, S.A. (1985). Salt and pH tolerance of *Rhizobium leguminosarum* T.A.L. 271 (under press).
6. Okafor, N. and Alexander, M. (1975). Primary physiological studies on cowpea rhizobia. *Soil Biol. Biochem.* 7:405-406.
7. Pillai, R.N. and Sen, A. (1973). Salt tolerance of *Rhizobium* from *Dolichos lablab*. *Zentral bl. Bakteriologie. Abt. Parasitenkd. Infektionsk. Hys.* 2: 128:538-542.
8. Pillai, R.N. and Sen, A. (1966). Salt tolerance of *R. trifolii*. *Indian J. Agric. Sci.* 36: 80-84.
9. Pander, M.S. and Kahlon, S.S. (1978). pH and salt tolerance of *Rhizobium leguminos* isolates from Pea (*Pisum sativum*). *Indian J. Microbiol.* 18 (2): 81 - 84.

10. Rai, R. and Prasad, V. (1984). Studies on growth and symbiotic nitrogen fixation of *Rhizobium* of *Vigna radiata* under stress conditions. *J. agric. Sci., Camb.* 102: 399-404.
11. Sawage, D., Hemeline, J. and Lartier, F. (1983). Glycine betaine and other structurally related compounds improve the salt tolerance of *Rhizobium meliloti* plant. *Sci. Lett.* 31: (213) 291-302.
12. Subba Rao, N.S. Lakshmi-Kumari, M., Singh, C.S. and Magt, S.P. (1972). Nodulation of Lucerne (*Medicago sativa* L.) under the influence of sodium chloride. *Ind. J. Agric. Sci.* 42: 384-386.
13. Steinborn, J. and Roughley, R.J. (1975). Toxicity of sodium and chloride ions to *Rhizobium* spp. in broth and peat cultures. *J. Appl. Bacteriol.* 39:133-138.
14. Singh, C.S. Lakshmi-Kumari, M., Biswas, A., Subba Rao, N.S. (1973). Effect of carbonate and bicarbonate of sodium on growth of rhizobia and nodulation in Lucerne (*Medicago sativa*). *Indian J. Microbiol.* 13: 125-128.
15. Upchurch, R.G. and Elkan, G.H. (1977). Comparison of Colony morphology, salt tolerance and effectiveness in *Rhizobium Japonicum*. *Cand. J. Microbiol.* 23 (9): 1118-1122.
16. Yadav, N.K. and Vyas, S.R. (1971). Note on the response of root-nodule rhizobia to saline, alkaline and acid conditions. *Indian J. Agric. Sci.* 41(12): 1123-1125.
17. Yadav, N.K. and Vyas, S.R. (1973). Salt and pH tolerance of Rhizobia. *Folia Microbiol., Praha* 18:242-247.

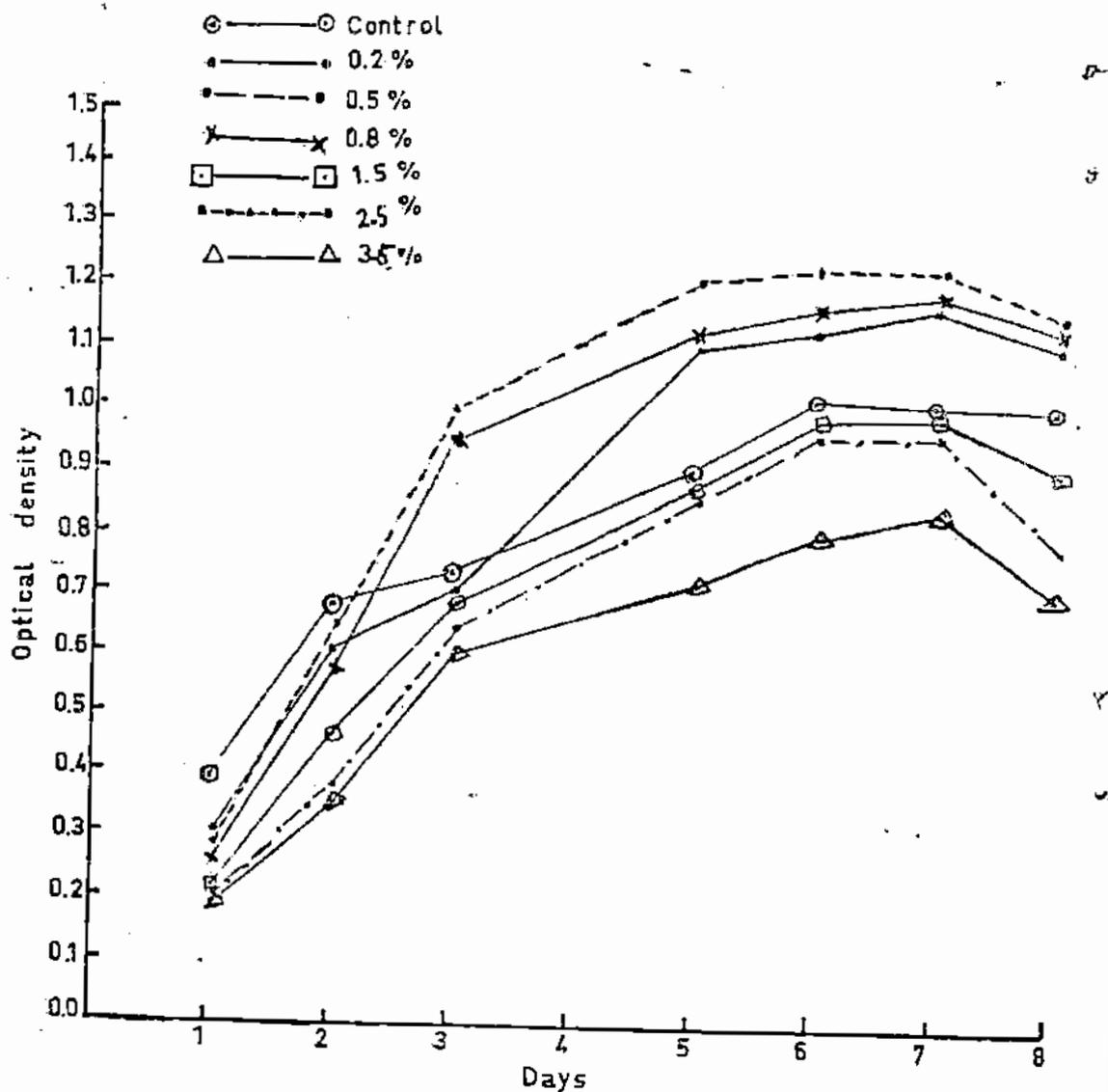


Fig. (1). Growth of *Rhizobium* under different level of NaCl.

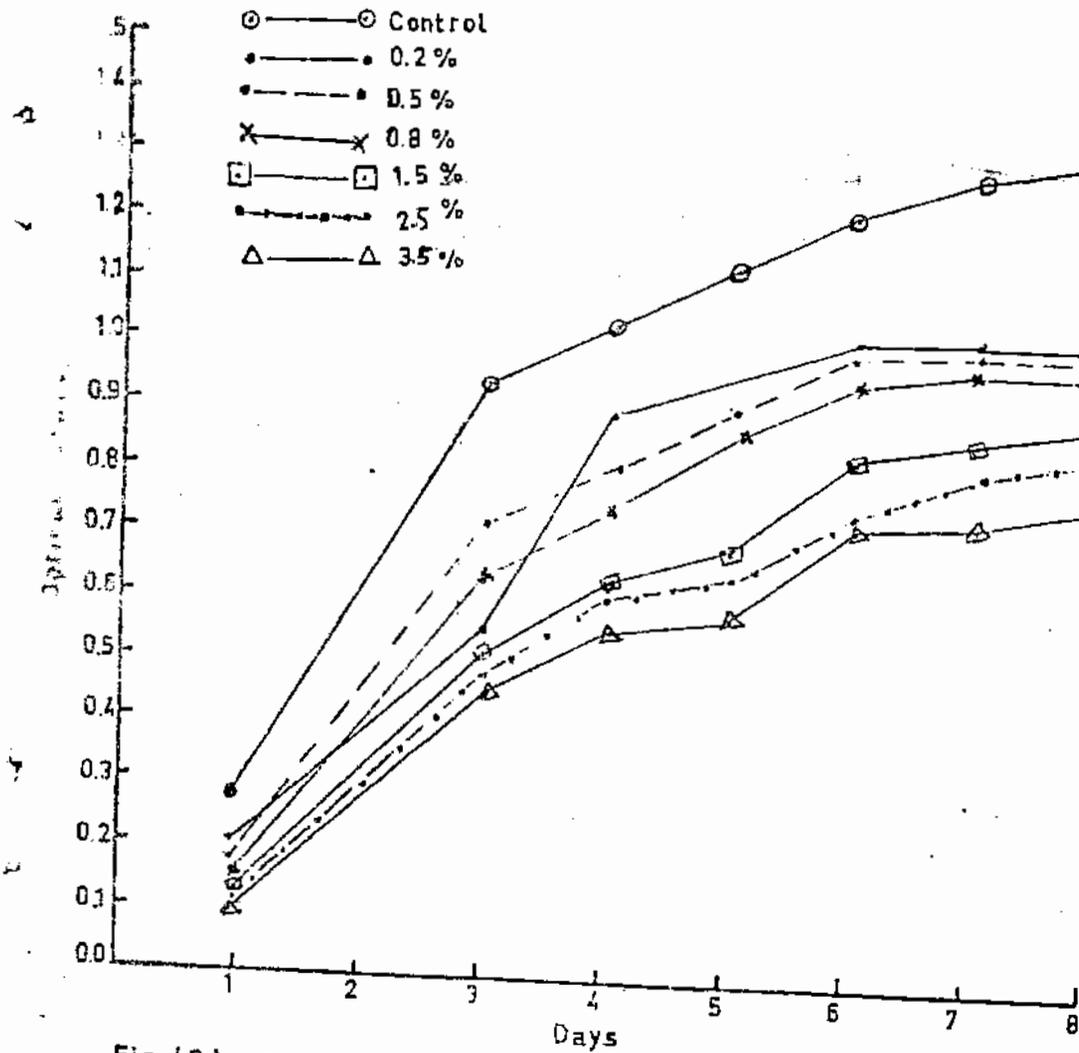


Fig. (2): Growth of Rhizobium under different level of CaCl_2

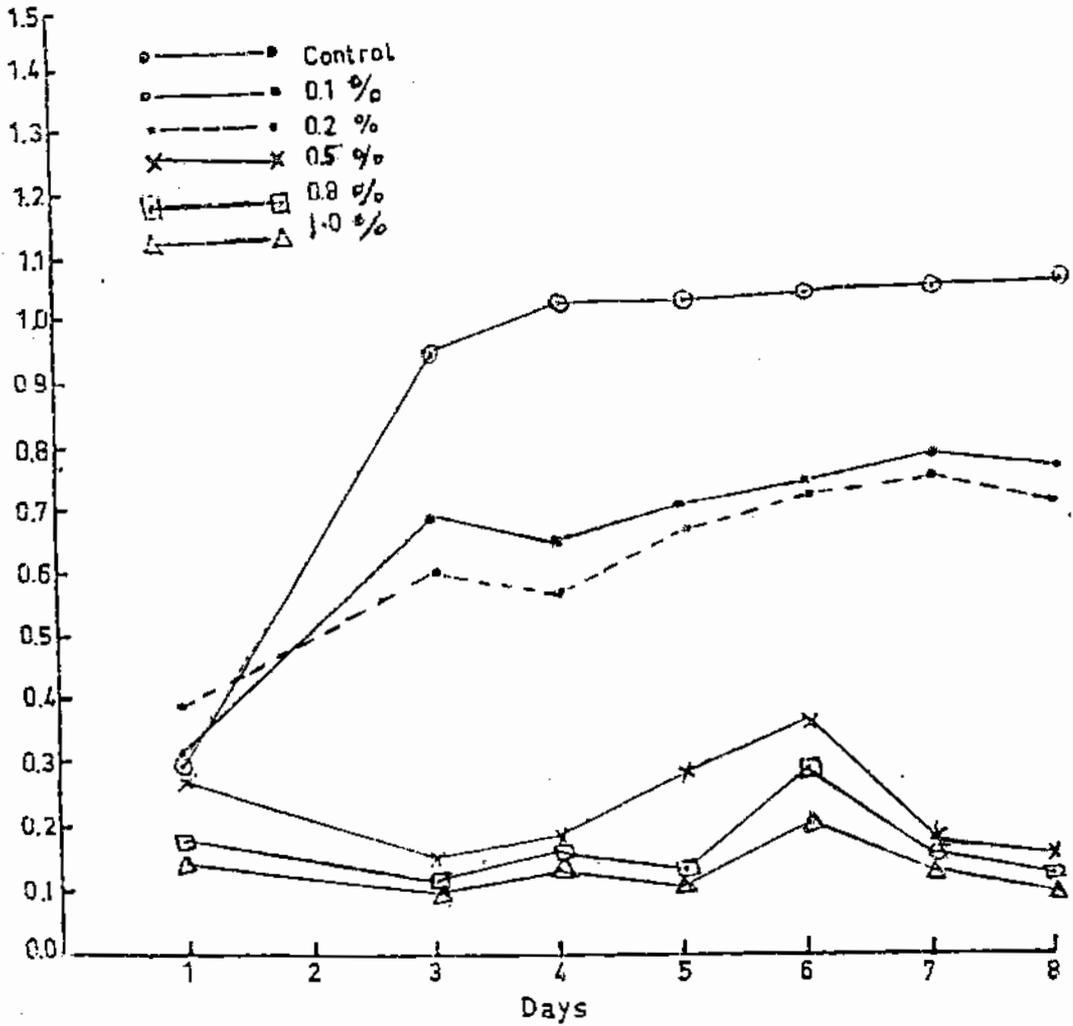


Fig. (3): Growth of Rhizobium under different level of carbonate.

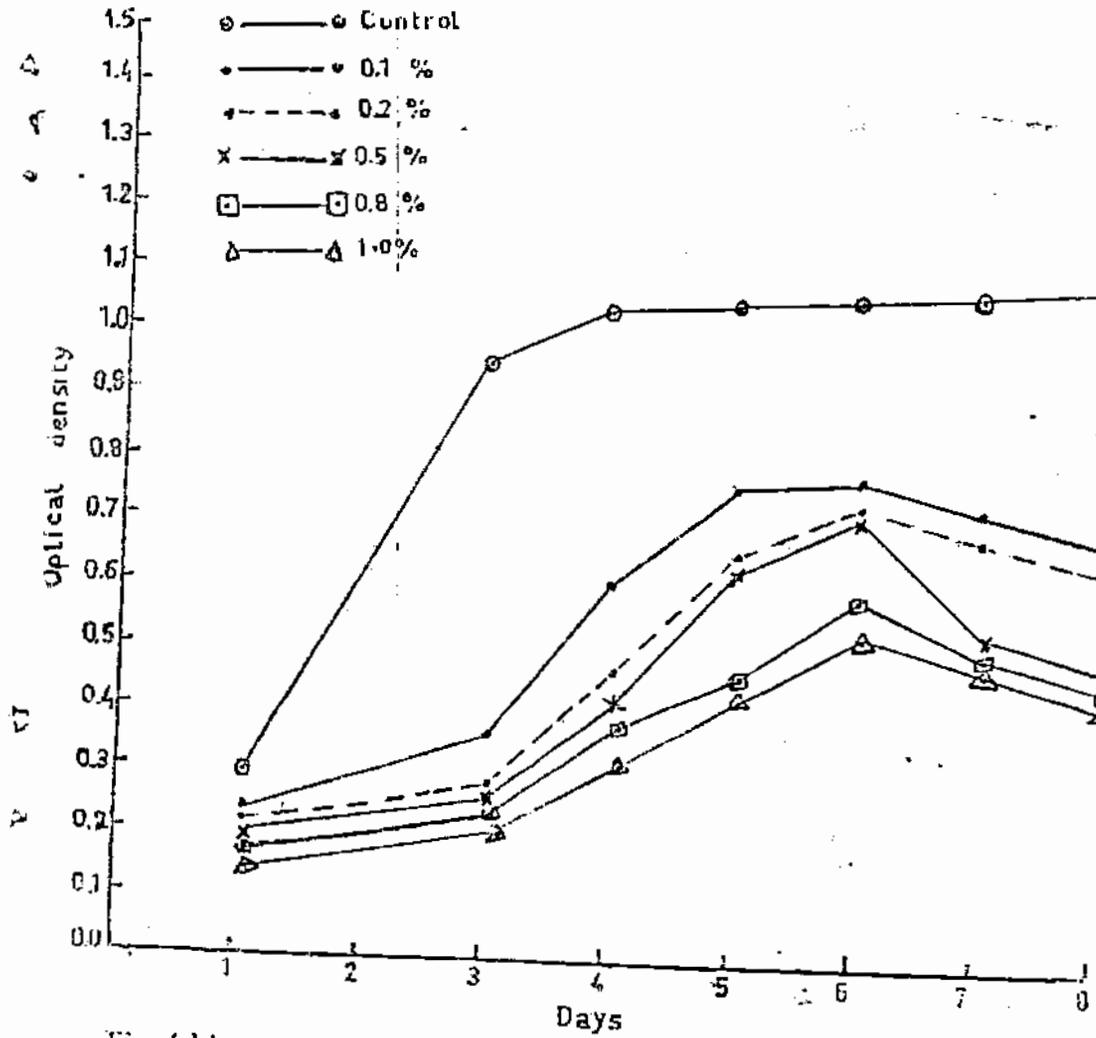


Fig (4). Growth of Rhizobium under different level of bicarbonate.

Table I: Effect of pH on growth of Rhizobium and change in initial broth pH after 72 hrs and after one week of incubation.

Initial pH value	O.D. after 72 hours	pH after 72 hrs.	O.D after one week	pH after one week
2.5	0.07	2.2	0.07	2.2
3.5	0.40	3.2	0.58	3.2
4.5	0.80	3.8	0.80	3.8
5.5	0.96	4.6	0.84	4.6
6.5	0.68	5.6	0.68	5.6
7.5	0.59	5.8	0.68	5.8
8.5	0.47	6.8	0.52	6.8
9.5	0.45	6.2	0.52	6.2
10	0.42	6.2	0.43	6.2

دراسات على نمو ريزويم البسلة تحت ظروف غير ملائمة

فأما عبد الهادي حليش

قسم النبات - كلية البنات - جامعة عين شمس - جمهورية مصر العربية - القاهرة

مخلص

يهدف هذا البحث الى دراسة نمو ريزويم البسلة تحت ظروف غير ملائمة من

الحموضة والملوحة والقلوية في بيئه العرق السائلة بفرض الحصول على سلالات تستطيع

النمو في الاجرء الحاضيه والملحيه والقلويه وذلك لانتشار هذه الاراضى في جمهوريه

مصر العربيه وخصوصا في المناطق المستصلحة التى تتميز بوجود نسبة عاليه من الاملاح

القابله للذوبان في الماء . هذا وقد اوضحت النتائج ما يأتى /

١ - استطاع ريزويم البسلة النمو على مدى واسع من الرقم الايدروجينى (٣.٥ - ١١.٥)

في بيئه العرق السائله وكان اكثر نمه عند رقم ايدروجينى ٥.٥ .

٢ - اعتبرت هذه السلاله سريعه النمو ومنتجه للحامض لان الرقم الايدروجينى تنسد

نقص بعد ٢٢ ساعه من النمو وبعد ان كان ٤.٥ - ٥.٥ اصبح ٣.٨ - ٤.٦ على

التوالى وكان النقص اكثر ما يمكن عند الرقم الايدروجينى المرتفع و (٥.٨ - ٥.٩ ،

١٠) .

٣ - استطاع ريزويم البسلة النمو على بيئه العرق السائله المحتويه على تركيبات متدرجه

من كلوريد الصوديوم من ٢ر٠ - ٨ر٠ % وكان اكثر نمو عند تركيبات تراوحت بين

٢ر٠ - ٥ر٠ % بينما كانت التركيزات العاليه شبطه للنمو .

٤ - لم تستطع هذه السلاله النمو على بيئه العرق السائله المحتويه على تركيبات متدرجه

من كلوريد الكالسيوم حيث كان اقل تركيز ٢ر٠ % له تاثير شبطه .

٥ - اعتبرت كربونات وبيكربونات الصوديوم ذات تاثير شبطه على نمو ريزويم البسلة وذلك

لنقص النمو حتى عند تركيز ١ر٠ % .