Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences
Volume 1 Number 4: 340-342, 2002

Effect of *Rhizobium japonicum* Inoculum Doses (Liquid Culture) on the Growth and Seed Yield of Soybean Crop

[1]F. C. Oad, [2]L. Kumar and [3]J. K. Biswas
[1]Sindh Agriculture University Tandojam, Sindh, Pakistan
[2]Agriculture Research Institute, Tandojam, Sindh, Pakistan
Bangladesh Rice Research Institute, Bangladesh

Abstract: The field experiments were conducted during 2000 at Tandojam, Pakistan to evaluate the effect of *Rhizobium japonicum* inoculum doses of liquid culture on the growth and seed yield of soybean crop. One liter liquid culture medium was prepared by standard procedure by adding 0.5g K_2HPO_4, 0.2g $MgSO_4$, 0.1g NaCl, 0.05g yeast extract, 10.0g Minitol, and 1000ml distilled water. Six *Rhizobium japonicum* doses (10, 15, 20, 25, 30 ml, and untreated control were laid-down in Randomized Complete Block Design. The adequate level of 25 ml with 300 grams sand maize as seed treatment inoculant was found more effective for maximum growth and seed yield as compared to higher and lower doses of *Rhizobium japonicum*. Thus, it was concluded that *Rhizobium japonicum* exhibited the positive change in terms of enhanced growth and seed yield. The satisfactory results would be achieved if, the soybean seed will be treated with 25 ml of *Rhizobium japonicum* inoculum.

Key words: Soybean, *Rhizobium japonicum* inoculum, growth, yield

Introduction

Use of rhizobial cultures in the establishment of legumes has been widely recognized, especially in areas where indigenous nodulation has been found to be inadequate. The benefits by the use of *Rhizobium* inoculants shows that a quite a good deal of money can be saved by the marginal farmers provided they use quality tested inoculants on the farm. Further, it was reported that the legume crops enrich the fertility of the soil, where the atmospheric nitrogen is fixed in the root nodules. The growth, yield of the crop, and soil fertility can be maintained through attempts like:

a Introduction of legume crops as sole and inter-cropping.
b Providing sufficient strain of rhizobia for nitrogen fixation.
c introduction of new legume varieties.
d Evaluation of technology enhancing the newly introduced nitrogen fixers to successfully compete with the strains of nitrogen fixing microorganisms already present in the soil.
e Recommended agronomic practices.
f To evolve simple practices to conserve water on the farm because optimum moisture is needed for successful nodulation and hence biological nitrogen fixation in legumes (Subba Rao, 1988).

The placement of seed inoculant in the soil is usually relatively remote from the location of infection foci on the seeding roots, a circumstance likely to be exacerbated by inefficient natural transport of rhizobia. This is one of several reasons that justify the use of alternative means of inoculation (Brockwell *et al.*, 1980). Granular inoculant applied into the seed row is a well-established and successful commercial practice (Muldoon *et al.*, 1980). Liquid inoculants sprayed into seedbed (Hely *et al.*, 1980), less widely use, are equally successful in promoting nodulation (Hale, 1981). Hydroseeding mixtures containing legume seed and rhizobia are a form of liquid inoculant. Satisfactory survival of rhizobia in those mixes that contain fertilizer depends on a pH value >6.0 (Brown *et al.*, 1983). Survival of rhizobia in such slurries might be improved further by suspending the inoculant in vegetable oil (Kremer *et al.*, 1982).
Post-emergence inoculation of uninoculated legumes was successful in inducing satisfactory nodulation on birdsfoot trefoil (*Lotus corniculatus* L.) and alfalfa (Rogers *et al.*, 1982). and on soybeans (Boonkerd *et al.*, 1984). It seems clear that, for post-emergence inoculation to be successful, soil moisture must be satisfactory. In addition the susceptibility of legume roots is a transient phenomenon (Bhuvaneswari *et al.*, 1981), which suggests that infection foci on a rapidly growing root system might soon become too remote from the site of post-emergence inoculation for adequate infection an nodulation to occur. Interesting work has been done on the means by which soybean compensate for symbiotic deficiencies. Singleton and Stockinger (1983) inoculated soybeans with various mixtures of effective and ineffective strains of *R. japonicum*. As anticipated, they found that the proportion of effective nodules formed increased as the ratio of effective to ineffective bacteria became greater in the inoculant. However, the total volume of effective nodules tissue remained approximately constant throughout. This was regarded as a 'compensatory mechanism' for keeping the amount of effective nodules tissue constant even as the proportion of effective nodules declined.
Thus, the act of inoculation leading to efficient nodulation was a means of conserving soil N, which enhances the growth and yield of the crop. Although inoculation of a prior crop of a non - legume with *R. japonicum* can be used to establish rhizobia in the soil, providing inoculum for a subsequent crop of soybeans (Guar *et al.*, 1980), Kuykendall *et al.* (1982) have demonstrated that, when nodulating and non-nodulating isolines of soybean and other legumes are planted in soil inoculated with *R. japonicum,* best establishment of the bacteria occurs via the nodulating line of soybean. This suggests that rhizobia multiply more prolifically within the nodule than elsewhere, and/ or that 'nodule' rhizobia (recently released from nodules) are better equipped for survival than 'rhizosphere' or 'soil' rhizobia. Once established in soil, *R. japonicum* appears to persist even in the absence of soybean crops (Crozat *et al.*, 1982), although there may be seasonal fluctuations in the size of populations (Mahler and Wollum II, 1982). However, appreciable growth of rhizobia in soil apparently occurs only in the presence of germinating seeds, growing roots and decomposed nodules (Pena-Cabriales and Alexander, 1983). Looking the economic importance of *Rhizobium japonicum* inoculum as seed treatment, the research is attempt for obtaining higher soybean seed production and soil fertility prospects.

Materials and Methods

The field experiments were laid-down in Randomized Complete Block design (RCBD) having three replications. The treatments were *Rhizobium japonicum* inoculum (Liquid Culture) doses (10, 15, 20, 25, 30 ml and untreated control). The liquid culture doses were injected in the sand-maize media (300 g/acre) packed in the polyethylene bags. The sand-maize was dried, and re-packed in the powder form. The inoculated sand-maize was mixed with the common table sugar suspension (10%) (50g sugar with 500 ml water) to enhance the survival of rhizobia on soybean seed.

The inoculated seeds were immediately drilled in the soil with single coulter hand drill at the rate of 75 kg ha^{-1}. The distance between the row and plants was maintained at 45 x 15 cm^2. The uniform level of N fertilizer at the rate of 75 kg ha^{-1} was incorporated in all the treated plots. All the cultural practices for area maintenance and growth of the crop were adopted.

Peparation and identification of *Rhizobium japonicum*: The preparation and identification of *Rhizobium japonicum* both were performed through the standard procedure recommended by Subba Rao (1988). The healthy, firm, unbroken and pink in color nodules from the roots of soybean crop were carefully uprooted and washed in the running water to remove adhering soil particles. They were immersed in 0.1% acidified H_2O_2 for 4-5 minutes and washed again in sterile water and then dipped in 705 ethyl alcohol followed by more washing with sterile water. The nodules were crushed in a small aliquot of sterile water with the help of a glass rod. The fluid from crushed nodules was spread on the surface of yeast extract mannitol agar (YEMA) plates with the help of a smooth glass rod. The plates were incubated upto 10 days in the incubator at 26 °C. When the large gummy colonies of bacteria emerged within 4-7 days, the Rhizobium colonies were identified through Gram staining test (Vincent, 1970). The inoculant was stored at 25-30 °C.

Results and Discussion

Physico-chemical properties of the experimental soil: The experimental soil was clay loam in texture, the total nitrogen was low (0.051-0.057%), The Olsen P was 25.24- 30.45 ppm and exchangeable K was optimum (184.23- 193.28 ppm). The pH of the soil ranged between 7.6 to 8.1.

Growth and yield parameters of soybean crop

Plant height: The plant height of the soybean crop was significantly different under liquid culture doses of *R. japonicum* inoculum. The taller plants were exhibited in the plots where higher inoculum doses of *R. japonicum* (25 and 30 ml) were incorporated. However, the inoculum level in the range of 10 to 20 ml for 300 g sand-maize and un-inoculated produced significantly shorter plants. The coefficient of variability recorded was 9.45% which showed that experiment was homogenous (Table 1). The increase in growth mainly plant height may be due to the factor of photosynthesis production which was higher due to increased levels of inoculum. The higher inoculum produced more and healthy nodules which in turn translocated nitrogen from the lower parts to the growing tips and enlarged the height of the plants (Hanway and Weber, 1971).

Pods and seeds per plant: The number of pods and seeds per plant analyzed through Duncans Multiple Range Test showed the superiority of higher doses of inoculum (25 and 30 ml for 300 g sand maize) for recording higher values of both the parameters, whereas the lower values were observed with lower inoculum doses and in un-inoculated (Table 1). Jamro and Larik (1988) also supported the results by reporting that production of photosynthesis decreases during the pod filling stage, and demand for N nutrient appears during this stage. This demand could be satisfied if, the nodules in the plant are vigorous to supply N constantly for growth cycle, which could be achieved through the increased doses of *R. japonicum* inoculum.

Seed weight per plant and per hectare: The seed weight on per plant and per hectare basis both were higher linearly with the increased doses of *R. japonicum* inoculum and the seed yield declined as the inoculum doses decreased from 25 ml (Table 1). This may due to the reason that in certain field soils, mineralization of organic N nitrification may provide levels of NO^{-}_{3} that satisfy the N requirements of young soybeans, but inhibit nodulation. When the NO^{-}_{3} is exhausted, the plants may enter a N-deficient phase, during which sufficient nodules are being formed to compensate for loss of soil N. If this period is prolonged, yield may be reduced. Herridge *et al*. (1984), working with one such soil, found that increasing the rates of inoculant applied into the seedbed led to more extensive distribution, numerically and in depth, of rhizophere, improved nodulation and nitrogen fixation, and larger residual populations in the soil after harvest. They argued that concentrations of NO^{-}_{3} in soil water were not uniform, that those parts of the root system exposed to low concentrations of NO^{-}_{3} would nodulate first, and that these conditions would most likely be satisfied when large populations of rhizobia were extensively distributed through the soil by means of heavy inoculation. Turner *et al*. (1984) further reported that more nitrogen was fixed where soil N had been depleted by a prior cereal crop. The proportion of plant N derived from atmospheric nitrogen increased with time as soil N was further depleted and reached > 80% between 98 and 114 days with the highest rate of inoculation.

The number of rhizobia present in the soil, their effectiveness, and their often superior ability to compete with an inoculant strain, determine the success of the inoculum. Inoculated soybeans sown into soils containing appreciable levels of *R. japonicum* do not usually result in significant differences in seed yield. Generally, only 5 to 10% of the nodules are formed by the inoculant applied to the seed even when the rhizobia are supplied at numbers manifold higher than usual. Even when 50% of the nodules result from inoculation, an inoculum response may not occur (Trinick, 1985). In the literature both the success and failure of *Rhizobium* in the field have been reported by Hamdi (1976), Hera (1976), Ham *et al.*

Table 1: Effect of *Rhizobium japonicum* inoculum doses (liquid culture) on growth and yield parameters of soybean

Rhizobium japonicum inoculum doses (ml) (liquid culture)	Plant height (cm)	Pods/ plant	Seeds/ pod	Seed weight/ plant (g)	Seed yield/ hectare (tons)
Control (un-treated)	30.22b	05.00e	01.51c	22.23e	1.24e
10	41.83b	09.07d	01.73bc	27.84d	1.43d
15	42.83b	16.40c	02.31abc	35.85c	1.63c
20	42.64b	20.25b	02.63ab	42.93b	1.84b
25	63.56a	25.45a	03.17a	50.56a	2.41a
30	63.93a	25.55a	03.23a	50.78a	2.39a
LSD (5%)	16.32	02.244	01.035	03.519	0.115
CV(%)	09.45	03.620	11.690	02.520	1.950

Means in the column followed by similar letter are not significantly different at 5% level of probability.

(1976), Subba Rao (1977) and Suba Rao and Talak (1977). Failure to obtain the desired response may be due to:

a The response of native strain, which could not be displaced by the introduced effective strains.
b The presence of antagonists of rhizobia which minimize the number of rhizobia in the rhizosphere.
c The availability of soil condition which limit symbiosis caused by acidity, alkalinity, other factors relating to soil structure, application of pesticides and high nitrate to soil. However, the responses of the inoculation may be in terms of growth, yield and its favorable residual nitrogenous effect for the next crop (Subba Rao, 1988).

References

Bhuvaneswari, T.V., A.A. Bhagwat and W.d. Bauer, 1981. Transient susceptibility of root cells in four common legumes to nodulation by rhizobia. Plant Physiol., 68:1144-1149.

Boonkerd, N., C. Arunsri, W. Rungrattanakasin and Y. Vasuvat, 1984. Effects of postemergence inoculation on field grown soybean, pp: 327. In: C. Veeger and W.E. Newton (eds.) Advances in Nitrogen Fixation Research. Nijhoff/Junk, The Hague.

Brockwell, J., R.R. Gault, D.L. Chase, F.W. Hely, M. Zorin and E.J. Corbin, 1980. An appraisal of practical alternatives to legume seed inoculation: field experiments on seed bed inoculation with solid and liquid inoculants. Aust. J. Agric. Res., 31: 47-60.

Brown, M. R., D.D. Wolf, R.D. Morse and J.L. Neal, 1983. Viability of *Rhizobium* in fertilizer slurries used for hydroseeding. J. Environ. Qual., 12:388-390.

Crozat, Y., J.C. Cleyet- Marel, J.J. Giraud and M. Obaton, 1982. Survival rates of *Rhizobium japonicum* populations introduced into different soils. Soil Biol. Biochem., 14:401-405.

Guar, Y.D., A.N. Sen and N.S. Subba Rao, 1980. Improved legume-*Rhizobium* symbiosis by inoculating preceding cereal crop with *Rhizobium* Plant Soil, 54:313-316.

Hale, C.N., 1981. Methods of white clover inoculation-their effect on competition for nodule formation between naturalized and inoculated strains of *Rhizobium Trifolii*. Newzealand J. Exp. Agric., 9:169-172.

Ham, G.E., R.J. Lawn and W.A. Burn, 1976. Influence of inoculation, nitrogen fertilizer and photosynthetic sources-sink manipulations of field grown soybeans, pp: 239-253. *In*: Symbiotic Nitrogen Fixation in Plants. Eds. P.S. Nutman, Cambridge Univ. Press.

Hamdi, V.D., 1976. Field and green house experiments on the response of legumes in Egypt to inoculation and fertilizers, pp: 289-298. *In*: Symbiotic Nitrogen Fixation in Plants. Eds. P.S. Nutman, Cambridge Univ. Press.

Hanway, T.J. and C.R. Weber, 1971. Dry matter accumulation in soybean *Glycine max L.* plants influenced by N and K fertilization. Agron. J., 63:263-266.

Hely, F.W., R.J. hutchings and M. Zorin, 1980. Methods of rhizobial inoculation and sowing techniques for *Trifolium subterraneum* L. establishment in a harsh winter environment. Aust. J. Agric. Res., 31:307-712.

Hera, C., 1976. Effect of inoculation and fertilizer application on the growth of soybean in Rumania, pp: 269-279. *In*: Symbiotic Nitrogen Fixation in Plants. Eds. P.S. Nutman, Cambridge Univ. Press.

Herridge, D.F., R.J. Roughley and J. Brockwell, 1984. Effects of rhizobia and soil nitrate on the establishment and function of the soybean symbiosis in the field. Aust. J. Agric. Res., 35:149-161.

Jamro, G.H. and A.S. Larik, 1988. Influence of nitrogenous fertilizer on nodulation, leghaemoglobin content and biological nitrogen fixation in *Phaseolus vulgaris* L. Genet. Agric., 42: 51-58.

Kremer, R.J., J. Polo and H.L. Peterson, 1982. effect of suspending agent and temperature on survival of *Rhizobium* in fertilizer. Soil Sci. Soc. Am. J., 46:539-542.

Kuykendall, L.D., T.E. Devine and D.B. Cregan, 1982. Positive role of nodulation on the establishment of *Rhizobium japonicum* in subsequent crops of soybean. Curr. Microbiol., 7:79-81.

Mahler, R.L. and A.G. Wollum II, 1982. seasonal fluctuations of *Rhizobium japonicum* under a variety field conditions in North Carolina. Soil Sci., 134:317-324.

Muldoon, J. F., D.J. Hume and W.D. Beversdorf, 1980. Effects of seed and soil applied *Rhizobium japonicum* inoculants on soybeans in Ontario. Can. J. Plant Sci., 60:399-409.

Pena-Cabriales, J.J. and M. Alexander, 1983. Growth of *Rhizobium* in unamended soil. Soil Sci. Soc. Am. J., 47:81-84.

Rogers, D.D., R.D. Warren Jr. and D.S. Chamblee, 1982. Remedial postemergence legume inoculation with *Rhizobium*. Agron. J., 47:613-619.

Singleton, P.W. and K.R. Stockinger, 1983. Compensation against ineffective nodulation in soybean. Crop. Sci., 23:69-72.

Subba Rao, N.S., 1988. Rhizobium Inoculant. In: Biofertilizers in Agriculture. 2nd Edition, pp: 16-76. ISBN-81-204-0125-5. Published by Mohan Primlani, Oxford & IBH Publishing Co. Pvt. Ltd. 66 Janpath, New Delhi.

Subba Rao, N.S., 1977. Soil microorganisms and plant growth. Oxford & IBH Publishing Co., New Delhi.

Suba Rao, N.S. and K.V.B.R. Talak, 1977. Rhizobial cultures-their role in pulse production, pp: 31-34. *In*: Souvenir Bulletin, Directorate of Pulses Development, Govt. of India, Lucknow.

Trinick, M.J., 1985. Rhizobium strain competition for host nodulation, pp: 911-917. *In*: World soybean Research Conference III. ISBN-0-8133-0091-6 Edited by Richard Shibles.

Turner, G.L., R.R. Hault and F.J. Bergersen, 1984. Measurements of natural abundance of 12 N in a soybean crop. pp: 7-8. *In*: I.R. Kennedy and L. Copeland (eds.) AIAS Occ. Publ. No.2 Aust. Inst. Agric. Sci., Sudney.

Vincent, J.M., 1970. A Manual for the practice study of the root nodule bateria, IBH Hand Book No. 15, Blackwell Scientific Publications, Oxford.